Northwest Territories Environment and Natural Resources

April 22, 2014

The Government of the Northwest Territories (GNWT) Department of Environment and Natural Resources (ENR) would like to submit the following supplemental information, to both MVEIRB and MVLWB, for the De Beers Snap Lake Mine Environmental Assessment (EA1314-002) and regulatory review. ENR requests that all of the attached information be considered by MVEIRB throughout the Environmental Assessment process.

ENR notes that this information is being supplied at this time to aid the proponent in its response to Information Request #7, noted on April 15th during the technical session. As per Information Request #7, ENR requests that the proponent provide rationale and clarity on why several of the below guidelines and primary references were not included in its derivation of Site Specific Water Quality Objectives (SSWQO's) for Total Dissolved Solids, chloride and other EA parameters.

Examples of Other Relevant Jurisdictions[1] with TDS or Chloride Regulations:

1. United States Environmental Protection Agency (US EPA) Aquatic Life Criteria Table for chloride. Available online at:
   http://water.epa.gov/scitech/swguidance/standards/criteria/current/index.cfm#altable
   Note: Acute Chloride (Cl) Criteria of 860 mg/L and Chronic Criteria of 230 mg/L. Also in the US EPA Office of Water 1986. Quality Criteria for Water (Gold Book).

2. British Columbia Ministry of Environment (BC MOE) CAPP Freshwater Salinity Working Group and the Salt Technology Advisory Sub-Committee of the British Columbia Upstream Petroleum Committee. *A Review of the Toxicological Literature for Salt- 2002 to 2007* (attached).
   Note: BC MOE recommends a Cl concentration of 150 mg/L.

3. BC MOE Ambient water quality guidelines for sulphate- (attached).

4. State of Pennsylvania, Chapter 93. Water Quality Standards. Available online at:
   http://www.pacode.com/secure/data/025/chapter93/chap93toc.html
   Note: TDS limit of 500 mg/L as a monthly average; maximum grab 750 mg/L.

5. Alaska Department of Environmental Conservation (DoEC), Water Quality Standards. 18 ACC 70. 2009 (attached).
   Note: Golder identifies a seasonal limit of 1000mg/L for the Teck Resources Red Dog Mine; however, this is mine is located in close proximity to the ocean (Chukchi Sea) where

[1] ENR notes that the Snap Lake Environmental Monitoring Agency has submitted for the Boards consideration a summary of applicable alternate jurisdictions for the regulation of TDS discharges in the United States.

ecosystems may be more adapted to saline influences and a lower value applies during environmentally sensitive periods. For comparison, at an inland mine (Gold Creek), the Alaska DoEC has set TDS at 300mg/L. In addition the TDS limit is set in Alaska depending on the receiving waterbody. Alaska may limit the concentration of chloride to 200 mg/L as per the US EPA aquatic life criteria.

6. Health Canada- Drinking Water Quality Guidelines (attached).
Note: The Aesthetic Objective is 500 mg/L for TDS.

Amendment -relevant Scientific Journal Articles (attached within GNWT IR response):

1. Weber-Scannel P.K and Duffy L.K. 2007. Effects of Total Dissolved Solids on Aquatic Organisms: A Review of Literature and Recommendation for Salmonid Species. *American Journal of Environmental Sciences.*

2. Mount et al. 1997. Statistical Models to Predict the Toxicity of Major Ions to Ceriodaphnia Dubia, Daphnia Magna and Pimephales Promelas (Fathead Minnows). *Environmental Toxicology and Chemistry*.

3. Brix K.V et al. 2009. The effects of total dissolved solids on egg fertilization and water hardening in two salmonids- Arctic Grayling (*Thymallus arcticus*) and Dolly Varden (*Salvenlinus malma*). *Aquatic Toxicology*.

4. Hallock R.J. and Hallock L.L. 1993. Detailed Study of Irrigation Drainage in and near Wildlife Management Areas, West-central Nevada. United States Geological Survey.

5. Bodkin et al. 2007. Limiting Total Dissolved Solids to Protect Aquatic Life. *Journal Of Soil and Water Conservation*.

6. Carmargo et al. 2005. Nitrate Toxicity to aquatic animals: a review with new data for freshwater invertebrates. *Chemosphere* Volume 58.

7. Cormier et al. 2013. Assessing causation of the extirpation of stream macroinvertebrates by a mixture of ions. *Environmental Toxicology and Chemistry.*

8. Cormier et al. 2013b Relationship of land use and elevated ionic strength in Appalachian watersheds. *Environmental Toxicology and Chemistry.*

9. DeMarch. 1988. Acute Toxicity of Binary Mixtures of Five Cations (Cu2+, Cd2+, Zn2+, Mg2+ and K+) to the freshwater amphipod gammarus lacustris (Sars): Alternative Descriptive Models. *Canadian Journal of Fisheries Aquatic Science.*

10. Kunz et al. 2013. Use of reconstituted waters to evaluate effects of elevated major ions associated with mountaintop coal mining on freshwater invertebrates. *Environmental Toxicology and Chemistry.*

11. Pond and North. 2013. Application of a benthic observed/expected-type model for assessing Central Appalachian streams influenced by regional stressors in West Virginia and Kentucky. *Environmental Monitoring and Assessment*.

12. Sorenson et al. 1977. Suspended and dissolved solids effects on freshwater biota: A review. US EPA document number EPA-600/3-77-042.

13. Suter and Cormier. 2013. A Method for assessing the potential for confounding applied to ionic strength in central Appalachian streams. *Environmental Toxicology and Chemistry*, Vol 32.

Other Amendment-relevant Articles for the Proponents Response to IR#7 (not attached):

14. Banack et al. 2012. Toxicity of fluoride to a variety of aquatic species and evaluation of toxicity modifying factors. In Harkness J, van Aggelen G, Kennedy CJ, Jarvis RA, Burridge LE (eds), Proceedings of the 39th Annual Aquatic Toxicity Workshop: September 30 to October 3, 2012, Sun Peaks, BC, Canada. Fisheries and Oceans Canada, St. Andrews Biological Station, St. Andrews, NB, Canada, p. 54.

15. Borgmann. 1996. Systematic analysis of aqueous ion requirement of *Hyallea Azteca*- A standard artificial medium including the essential bromide ion. *Environmental Contaminants Toxicology*, 30:356-363.

16. Brannock et al. 2002 Salt and Salmon: The effects of hard water ions on fertilization. Aquatic Science Meeting. *American Society of Limnology and Oceanography* Feb 11-15

17. Cowgill and Milazzo. 1991 The sensitivity of Two Cladocerans to Water Quality Variables: Salinity <467 mg NaCl/L and Hardness <200 mg $CaCO_3$/L. *Environmental Contaminants and Toxicology.*

18. Evans and Prepas. 1996. Potential effects of climate change on ion chemistry and phytoplankton communities in prairie saline lakes. *Limnology Oceanography.*

19. Zalizniak et al. 2006. Is all salinity the same? The effect of ionic compositions on the salinity tolerance of five species of freshwater invertebrates. *Marine and Feshwate*r Research 57:75-82.

20. EVS Environment Consultants. 1998. Effects of TDS on fertilization and viability of rainbow trout and chum salmon embryos. Revised Final Draft EVS Project No. 9/302-28. Prepared for Cominco Alaska.

21. Ivey et al. 2013. Sensitivity of freshwater mussels at two life stages to acute or chronic effects of NaCl and KCl. SETAC poster. Society of Environmental Toxicology and Chemistry.

ELSEVIER

Chemosphere 58 (2005) 1255–1267

CHEMOSPHERE

www.elsevier.com/locate/chemosphere

Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates

Julio A. Camargo *, Alvaro Alonso, Annabella Salamanca

Departamento Interuniversitario de Ecología, Edificio de Ciencias, Universidad de Alcalá, E-28871 Alcalá de Henares, Madrid, Spain

Received 9 December 2003; received in revised form 19 October 2004; accepted 25 October 2004

Abstract

Published data on nitrate (NO_3^-) toxicity to freshwater and marine animals are reviewed. New data on nitrate toxicity to the freshwater invertebrates *Eulimnogammarus toletanus*, *Echinogammarus echinosetosus* and *Hydropsyche exocellata* are also presented. The main toxic action of nitrate is due to the conversion of oxygen-carrying pigments to forms that are incapable of carrying oxygen. Nitrate toxicity to aquatic animals increases with increasing nitrate concentrations and exposure times. In contrast, nitrate toxicity may decrease with increasing body size, water salinity, and environmental adaptation. Freshwater animals appear to be more sensitive to nitrate than marine animals. A nitrate concentration of 10 mg NO_3-N/l (USA federal maximum level for drinking water) can adversely affect, at least during long-term exposures, freshwater invertebrates (*E. toletanus*, *E. echinosetosus*, *Cheumatopsyche pettiti*, *Hydropsyche occidentalis*), fishes (*Oncorhynchus mykiss*, *Oncorhynchus tshawytscha*, *Salmo clarki*), and amphibians (*Pseudacris triseriata*, *Rana pipiens*, *Rana temporaria*, *Bufo bufo*). Safe levels below this nitrate concentration are recommended to protect sensitive freshwater animals from nitrate pollution. Furthermore, a maximum level of 2 mg NO_3-N/l would be appropriate for protecting the most sensitive freshwater species. In the case of marine animals, a maximum level of 20 mg NO_3-N/l may in general be acceptable. However, early developmental stages of some marine invertebrates, that are well adapted to low nitrate concentrations, may be so susceptible to nitrate as sensitive freshwater invertebrates.

Keywords: Nitrate toxicity; Aquatic invertebrates; Fishes; Amphibians; Review

1. Introduction

In the aquatic environment, the most common ionic (reactive) forms of inorganic nitrogen are ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-). These ions may be present naturally in aquatic ecosystems as a result of atmospheric deposition, surface and groundwater runoff, dissolution of nitrogen-rich geological deposits, N_2 fixation by certain prokaryotes (cyanobacteria, particularly), and biological degradation of organic matter (Spencer, 1975; Kinne, 1984; Gleick, 1993; Wetzel, 2001; Rabalais, 2002). Ammonium tends to be oxidized to nitrate in a two-step process ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$) by aerobic chemoautotrophic bacteria (*Nitrosomonas* and *Nitrobacter*, primarily), even if levels of dissolved oxygen decline to a value as low as 1.0 mg O_2/l (Sharma and Ahlert, 1977; Stumm and Morgan, 1996; Wetzel, 2001). In consequence, concentrations of nitrate in

* Corresponding author. Tel.: +34 91 885 49 27; fax: +34 91 885 49 29.

E-mail address: julio.camargo@uah.es (J.A. Camargo).

0045-6535/$ - see front matter
doi:10.1016/j.chemosphere.2004.10.044

freshwater and marine ecosystems usually are higher than those of ammonium and nitrite (Spencer, 1975; Kinne, 1984; Gleick, 1993; Wetzel, 2001; Rabalais, 2002). Nitrate (but also ammonium and nitrite) may however be removed from water by aquatic plants, algae and bacteria which assimilate it as a source of nitrogen (Nixon, 1995; Smith et al., 1999; Wetzel, 2001). Furthermore, when concentrations of dissolved oxygen decrease to minimum values, facultative anaerobic bacteria (e.g., *Pseudomonas*, *Micrococcus*, *Bacillus*, *Achromobacter*) can utilize nitrate as a terminal acceptor of electrons, resulting in the ultimate formation of N_2 (Austin, 1988; Wetzel, 2001).

During the past two centuries, the human species has substantially altered the global nitrogen cycle, increasing both the availability and the mobility of nitrogen over large regions of Earth (Vitousek et al., 1997; Carpenter et al., 1998; Galloway and Cowling, 2002). Consequently, in addition to natural sources, inorganic nitrogen (NH_4^+, NO_2^-, NO_3^-) can nowadays enter aquatic ecosystems via anthropogenic sources such as animal farming, urban and agricultural runoff, industrial wastes, and sewage effluents (including effluents from sewage treatment plants that are not performing tertiary treatments) (Meybeck et al., 1989; Conrad, 1990; Bouchard et al., 1992; Welch and Lindell, 1992; Gleick, 1993; Vitousek et al., 1997; Carpenter et al., 1998; Smith et al., 1999; Wetzel, 2001; Rabalais, 2002). Moreover, the atmospheric deposition of inorganic nitrogen (mainly in the form of NO_3^-) has dramatically increased because of the extensive use of nitrogen fertilisers and the huge combustion of fossil fuels (Vitousek et al., 1997; Carpenter et al., 1998; Moomaw, 2002; Boumans et al., 2004). As a result, concentrations of nitrate in ground and surface waters are increasing around the world, causing one of the most prevalent environmental problems responsible for water quality degradation on a worldwide scale (Meybeck et al., 1989; Conrad, 1990; Bouchard et al., 1992; Welch and Lindell, 1992; Gleick, 1993; Nixon, 1995; Smith et al., 1999; Wetzel, 2001; Rabalais, 2002; Smith, 2003). Nitrate concentrations may actually exceed values as high as 25 mg NO_3-N/l in surface waters and 100 mg NO_3-N/l in ground waters (Bogardi et al., 1991; Goodrich et al., 1991; Gleick, 1993; Ministry of Agriculture, Fisheries and Food, 1993; Steinheimer et al., 1998). On the other hand, in marine aquaria and aquaculture systems, where water is recirculating with good oxygenation, nitrate concentrations can approach values of 500 mg NO_3-N/l (De Graaf, 1964; Pierce et al., 1993).

In spite of the current worldwide environmental concern about increasing nitrate concentrations in ground and surface waters, comparatively few studies have been conducted to assess nitrate toxicity to aquatic animals, probably because it has been traditionally assumed that other occurring inorganic nitrogen compounds, such as ammonia (the unionized form of NH_4^+) and nitrite, are more toxic (Russo, 1985; Meade and Watts, 1995; Wetzel, 2001; Alonso and Camargo, 2003). In fact, although safe levels of ammonia have been well established for fishes and aquatic invertebrates (Alabaster and Lloyd, 1982; US Environmental Protection Agency, 1986), no safe level of nitrate has been established for aquatic animals (US Environmental Protection Agency, 1986; Scott and Crunkilton, 2000). It however is worth mentioning that an acceptable level of nitrate for seawater culture was considered to be less than 20 mg NO_3-N/l (Spotte, 1979).

The main toxic action of nitrate on aquatic animals is due to the conversion of oxygen-carrying pigments (e.g., hemoglobin, hemocyanin) to forms that are incapable of carrying oxygen (e.g., methemoglobin) (Grabda et al., 1974; Conrad, 1990; Jensen, 1996; Scott and Crunkilton, 2000; Cheng and Chen, 2002). Nevertheless, owing to the low branchial permeability to nitrate, the NO_3^- uptake in aquatic animals seems to be more limited than the uptake of NH_4^+ and NO_2^-, contributing to the relatively low toxicity of nitrate (Russo, 1985; Meade and Watts, 1995; Jensen, 1996; Stormer et al., 1996; Cheng and Chen, 2002; Alonso and Camargo, 2003).

Elevated nitrate concentrations in drinking waters have serious risks for humans. Ingested nitrates may cause methemoglobinemia in infants through their conversion to nitrites (under anaerobic conditions in the gut) and the subsequent blockade of the oxygen-carrying capacity of hemoglobin (Sandstedt, 1990; Amdur et al., 1991; Wolfe and Patz, 2002). In addition, ingested nitrates have a potential role in developing cancers of the digestive tract through their contribution to the formation of nitrosamines, which are among the most potent of the known carcinogens in mammals (Harte et al., 1991; Nash, 1993). To prevent these deleterious effects of nitrate on human health, drinking water quality criteria have been established: the USA federal maximum contaminant level is 10 mg NO_3-N/l (US Environmental Protection Agency, 1986; Nash, 1993; Scott and Crunkilton, 2000).

The chief purpose of this paper is to review published scientific literature on the toxic effects of nitrate (NO_3^-) on freshwater and marine animals (invertebrates, fishes and amphibians) to establish preliminary safe levels of nitrate for aquatic life. To better compare toxicity data from different authors, all concentrations and levels of nitrate were expressed as mg NO_3-N/l. Additionally, we present new data on the short-term toxicity of nitrate to three species of freshwater invertebrates that are relatively common in rivers and streams of Central Spain: *Eulimnogammarus toletanus* Pinkster & Stock (Gammaridae, Amphipoda, Crustacea), *Echinogammarus echinosetosus* Pinkster (Gammaridae, Amphipoda, Crustacea), and *Hydropsyche exocellata* Dufóur (Hydropsychidae, Trichoptera, Insecta). Individuals of *E. toletanus* and

E. echinosetosus are shredder and detritivorous animals that feed on coarse particulate organic matter. Caddisfly larvae of *H. exocellata* are filter-feeders that construct fixed silk retreat-nets to strain food particles from the current. These species were chosen because the information on nitrate toxicity to freshwater invertebrates, particularly to freshwater amphipods, was very limited.

2. Materials and methods

Adults of *Eulimnogammarus toletanus* (average size of 8.5 mm in length) and *Echinogammarus echinosetosus* (average size of 11.2 mm in length), and last instar larvae of *Hydropsyche exocellata* (>1 mm head capsule width), were obtained from relatively unpolluted reaches of the Henares River (Central Spain). Invertebrates were transported to the laboratory using plastic containers with river water. No animal died during transportation. In the laboratory, invertebrates were deposited into three glass aquaria (one for each species) and acclimated to water quality conditions for seven days prior to the beginning of toxicity bioassays. During acclimation, amphipods were fed with macerated poplar leaves from the Henares river, and caddisfly larvae were fed with fine particulate dried fish food.

Invertebrate species were tested separately. Three static (with water renovation) short-term toxicity bioassays were conducted in triplicate for five days using small glass aquaria, each containing one litre of bottled drinking water (with no chlorine). A control and 5-6 different nominal nitrate concentrations were used per bioassay, with 10 animals per concentration/aquarium (including control). Test nitrate concentrations ranged from 5 to 160 mg NO_3-N/l for *E. echinosetosus*, from 15 to 480 mg NO_3-N/l for *E. toletanus*, and from 20 to 640 mg NO_3-N/l for *H. exocellata*. In all cases, nitrate solutions were made from sodium nitrate ($NaNO_3$, Merck, Germany). These nitrate solutions, together with water in control aquaria, were daily renewed. Invertebrates were not fed during bioassays to prevent changes in nitrate concentrations. Water oxygenation and turbulence were produced with air pumps and airstones. Average water quality conditions during bioassays were: 7.7 mg O_2/l for dissolved oxygen, 17.9 °C for temperature, 7.8 for pH, and 293 mg $CaCO_3$/l for total hardness. In the case of *H*. exocellata, and following previous recommendations by Camargo and Ward (1992), PVC pieces were added to quaria to facilitate net-building by net-spinning caddisfly larvae. Mortality was recorded every day, dead animals being removed.

Statistical analyses were performed using the multifactor probit analysis (MPA) software (US Environmental Protection Agency, 1991; Lee et al., 1995). The MPA methodology solves the concentration-time-response equation simultaneously via the iterative reweighed least squares technique (multiple linear regression). The dependent variable is the probit of the proportion responding to each concentration, and the independent variables are exposure time and toxicant concentration. After evaluating several MPA models regarding the heterogeneity factor (chi-squared variable divided by degrees of freedom), a parallel-regression-line model was selected as the best one. 48, 72, 96 and 120 h LC_{10} and LC_{50} values were calculated for each test species. In addition, 120 h $LC_{0.01}$ values (lethal concentrations for 0.01% response after 120 h of exposure) were estimated for each test species as short-term safe levels of nitrate.

3. Toxicity to aquatic invertebrates

Nitrate toxicity to aquatic invertebrates increases with increasing nitrate concentrations and exposure times (Camargo and Ward, 1992, 1995; Scott and Crunkilton, 2000; Tsai and Chen, 2002; Alonso and Camargo, 2003). Conversely, nitrate toxicity decreases with increasing body size and water salinity (Camargo and Ward, 1992, 1995; Tsai and Chen, 2002). In general, freshwater invertebrates appear to be more sensitive to nitrate toxicity than marine invertebrates as a probable consequence of the ameliorating effect of water salinity on the tolerance of aquatic invertebrates to nitrate ions. However, early life stages of some marine invertebrates may be very sensitive to nitrate toxicity (Muir et al., 1991).

Camargo and Ward (1992), studying the short-term toxicity of $NaNO_3$ to the Nearctic net-spinning caddisflies *Cheumatopsyche pettiti* and *Hydropsyche occidentalis*, calculated 72, 96 and 120 h LC_{50} values of nitrate-nitrogen for early and last instar larvae of these two hydropsychid species (Table 1). In both cases, early instar larvae appeared to be more sensitive to nitrate toxicity than last instar larvae. Additionally, Camargo and Ward (1995) estimated short-term safe levels (120 h $LC_{0.01}$ values) of 6.7 and 9.6 mg NO_3-N/l for early and last instar larvae of *C. pettiti*, and 4.5 and 6.5 mg NO_3-N/l for early and last instar larvae of *H. occidentalis* (Table 1).

Meade and Watts (1995) examined the toxic effects of $NaNO_3$ on the survival and metabolic rate (oxygen consumption) in juvenile individuals (9–13 mm total length) of the Australian freshwater crayfish *Cherax quadricarinatus*. After 5 days, no mortality was observed in crayfish exposed to a nominal nitrate concentration of 1000 mg NO_3-N/l. Furthermore, no significant difference was observed in oxygen consumption between control (0 mg NO_3-N/l) and experimental (1000 mg NO_3-N/l) individuals (Table 1).

Jensen (1996) studied the uptake and physiological effects of nitrate ions (from $NaNO_3$) in the freshwater crayfish *Astacus astacus*. The nitrate uptake was minor

Table 1
Comparative toxicity of nitrate-nitrogen (NO_3-N) to aquatic invertebrates

Species	Developmental stage	Aquatic medium	Toxicological parameter (mg NO_3-N/l)	References
Cheumatopsyche pettiti	Early instar larvae	Freshwater	191 (72 h LC_{50})	Camargo and Ward (1992)
	Early instar larvae	Freshwater	113.5 (96 h LC_{50})	Camargo and Ward (1992)
	Early instar larvae	Freshwater	106.5 (120 h LC_{50})	Camargo and Ward (1992)
	Early instar larvae	Freshwater	6.7 (120 h $LC_{0.01}$)	Camargo and Ward (1995)
	Last instar larvae	Freshwater	210 (72 h LC_{50})	Camargo and Ward (1992)
	Last instar larvae	Freshwater	165.5 (96 h LC_{50})	Camargo and Ward (1992)
	Last instar larvae	Freshwater	119 (120 h LC_{50})	Camargo and Ward (1992)
	Last instar larvae	Freshwater	9.6 (120 h $LC_{0.01}$)	Camargo and Ward (1995)
Hydropsyche occidentalis	Early instar larvae	Freshwater	148.5 (72 h LC_{50})	Camargo and Ward (1992)
	Early instar larvae	Freshwater	97.3 (96 h LC_{50})	Camargo and Ward (1992)
	Early instar larvae	Freshwater	65.5 (120 h LC_{50})	Camargo and Ward (1992)
	Early instar larvae	Freshwater	4.5 (120 h $LC_{0.01}$)	Camargo and Ward (1995)
	Last instar larvae	Freshwater	183.5 (72 h LC_{50})	Camargo and Ward (1992)
	Last instar larvae	Freshwater	109 (96 h LC_{50})	Camargo and Ward (1992)
	Last instar larvae	Freshwater	77.2 (120 h LC_{50})	Camargo and Ward (1992)
	Last instar larvae	Freshwater	6.5 (120 h $LC_{0.01}$)	Camargo and Ward (1995)
Cherax quadricarinatus	Juveniles (9–13 mm)	Freshwater	1000 (5 d NOAEL)	Meade and Watts (1995)
Astacus astacus	Juveniles	Freshwater	14 (7 d NOAEL)	Jensen (1996)
Ceriodaphnia dubia	Neonates (<24 h)	Freshwater	374 (48 h LC_{50})	Scott and Crunkilton (2000)
	Neonates (<24 h)	Freshwater	7.1–56.5 (7 d NOEC)	Scott and Crunkilton (2000)
	Neonates (<24 h)	Freshwater	14.1–113 (7d LOEC)	Scott and Crunkilton (2000)
Daphnia magna	Neonates (<48 h)	Freshwater	462 (48 h LC_{50})	Scott and Crunkilton (2000)
Potamopyrgus antipodarum	Adults (2.6–3.8 mm)	Freshwater	2009 (24 h LC_{50})	Alonso and Camargo (2003)
	Adults (2.6–3.8 mm)	Freshwater	1128 (24 h LC_{10})	Alonso and Camargo (2003)
	Adults (2.6–3.8 mm)	Freshwater	1297 (48 h LC_{50})	Alonso and Camargo (2003)
	Adults (2.6–3.8 mm)	Freshwater	728 (48 h LC_{10})	Alonso and Camargo (2003)
	Adults (2.6–3.8 mm)	Freshwater	1121 (72 h LC_{50})	Alonso and Camargo (2003)
	Adults (2.6–3.8 mm)	Freshwater	629 (72 h LC_{10})	Alonso and Camargo (2003)
	Adults (2.6–3.8 mm)	Freshwater	1042 (96 h LC_{50})	Alonso and Camargo (2003)
	Adults (2.6–3.8 mm)	Freshwater	585 (96 h LC_{10})	Alonso and Camargo (2003)
	Adults (2.6–3.8 mm)	Freshwater	195 (96 h $LC_{0.01}$)	Alonso and Camargo (2003)
Crassostrea virginica	Juveniles	Seawater	3794 (96 h LC_{50})	Epifano and Srna (1975)
Penaeus spp.	Juveniles	Seawater (28‰)	3400 (48 h LC_{50})	Wickins (1976)
Haliotis tuberculata	Juveniles (12–14.4 g)	Seawater (34‰)	250 (15 d safe level)	Basuyaux and Mathieu (1999)
Paracentrotus lividus	Juveniles (2.7–5.9 g)	Seawater (34‰)	100 (15 d safe level)	Basuyaux and Mathieu (1999)
Penaeus monodon	Protozoea (I stage)	Seawater (32‰)	0.226 (31–37% mortality 40 h)	Muir et al. (1991)
	Protozoea (I stage)	Seawater (32‰)	2.26 (35–43% mortality 40 h)	Muir et al. (1991)
	Protozoea (I stage)	Seawater (32‰)	22.6 (37–58% mortality 40 h)	Muir et al. (1991)
	Juveniles (22–35 mm)	Seawater (15‰)	2876 (48 h LC_{50})	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (15‰)	1723 (72 h LC_{50})	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (15‰)	1449 (96 h LC_{50})	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (25‰)	3894 (48 h LC_{50})	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (25‰)	2506 (72 h LC_{50})	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (25‰)	1575 (96 h LC_{50})	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (35‰)	4970 (48 h LC_{50})	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (35‰)	3525 (72 h LC_{50})	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (35‰)	2316 (96 h LC_{50})	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (15‰)	145 (safe level)	Tsai and Chen (2002)

Table 1 (*continued*)

Species	Developmental stage	Aquatic medium	Toxicological parameter (mg NO_3-N/l)	References
	Juveniles (22–35 mm)	Seawater (25‰)	158 (safe level)	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (35‰)	232 (safe level)	Tsai and Chen (2002)
Marsupenaeus japonicus	Juveniles (8.3–14.9 g)	Seawater (30‰)	105 (24 h LOEC)	Cheng and Chen (2002)

Values of toxicological parameters (LC_{50}, LC_{10}, $LC_{0.01}$, NOAEL, NOEC, LOEC) at different exposure times for several species of freshwater and marine invertebrates. In all cases, animals were exposed to sodium nitrate ($NaNO_3$).

in crayfish exposed to a nitrate concentration of 14 mg NO_3-N/l for seven days, indicating a low branchial permeability to nitrate (Table 1). This minor uptake of nitrate appeared to be passive, the haemolymph nitrate concentration staying far below the ambient nitrate concentration. In addition, nitrate exposure did not induce significant changes in haemolymph chloride, sodium or potassium concentrations, nor in divalent cations and anions, extracellular osmolality and amino acid concentrations (Table 1).

Scott and Crunkilton (2000), examining the acute toxicity of $NaNO_3$ to neonates of the cladocerans *Ceriodaphnia dubia* (<24 h old) and *Daphnia magna* (<48 h old), estimated 48 h LC_{50} values of 374 and 462 mg NO_3-N/l (Table 1). Moreover, Scott and Crunkilton (2000) reported that the no-observed-effect concentration (NOEC) and the lowest-observed-effect concentration (LOEC), for neonate production in *C. dubia* females after 7 days of exposure to nominal nitrate concentrations ranging from 2.2 to 113 mg NO_3-N/l, ranged from 7.1 to 56.5 mg NO_3-N/l (average NOEC value of 21.3 mg NO_3-N/l) and from 14.1 to 113 mg NO_3-N/l (average LOEC value of 42.6 mg NO_3-N/l) (Table 1).

Alonso and Camargo (2003), conducting laboratory experiments to examine the acute toxicity of $NaNO_3$ to the snail *Potamopyrgus antipodarum*, calculated 24, 48, 72 and 96 h LC_{10} and LC_{50} values (Table 1). This aquatic snail appeared to be relatively tolerant to nitrate toxicity, since an exposure of 4 days to a nitrate concentration as high as 585 mg NO_3-N/l (96 h LC_{10} value) could potentially cause 10% mortality in *P. antipodarum*. Alonso and Camargo (2003) also estimated a short-term safe level (96 h $LC_{0.01}$ value) of 195 mg NO_3-N/l (Table 1).

In toxicity tests with *Eulimnogammarus toletanus*, *Echinogammarus echinosetosus* and *Hydropsyche exocellata*, mortality percentages increased with increasing nitrate concentrations and exposure times. Before death, gammarids showed alterations in normal movement, and net-spinning caddisfly larvae tended to migrate from their retreat and capture nets. This sublethal effect of migration in larvae of *H. exocellata* has been previously reported in larvae of other hydropsychid species exposed to high levels of sodium nitrate (Camargo and Ward, 1992, 1995). The 48, 72, 96 and 120 h LC_{10} and LC_{50} values, and their 95% confidence limits, are presented in Table 2. From a simple comparison of LC_{50} values (Tables 1 and 2), we can see that test gammarid species (in particular *E. echinosetosus*) seem to be more sensitive to nitrate toxicity than other freshwater invertebrates, at least during short-term exposures. Furthermore, a nitrate concentration as low as 8.5 mg NO_3-N/l (120 h LC_{10} value) could potentially cause 10% mortality in *E. echinosetosus*. Short-term safe levels (120 h $LC_{0.01}$ values) of nitrate for *E. toletanus*, *E. echinosetosus* and *H. exocellata* are also presented in Table 2. 120 h $LC_{0.01}$ values for gammarid species were lower than those for hydropsychid species (Tables 1 and 2). The lowest

Table 2
LC_{50}, LC_{10} and $LC_{0.01}$ values for *Eulimnogammarus toletanus*, *Echinogammarus echinosetosus* and *Hydropsyche exocellata*

Toxicological parameter (mg NO_3-N/l)	*E. toletanus*	*E. echinosetosus*	*H. exocellata*
48 h LC_{50}	180.3 (135.6–266.4)	106.9 (86.6–140.5)	592.3 (447.5–813.1)
48 h LC_{10}	47.2 (26.5–66.4)	16.2 (11.5–20.9)	62.7 (35.0–92.8)
72 h LC_{50}	109.2 (84.9–148.1)	74.8 (61.4–96.6)	350.4 (289.6–436.6)
72 h LC_{10}	28.5 (14.9–40.9)	11.4 (7.9–14.7)	40.0 (20.9–60.5)
96 h LC_{50}	85.0 (63.6–116.8)	62.5 (50.6–81.9)	269.5 (227.4–327.8)
96 h LC_{10}	22.2 (10.9–33.0)	9.5 (6.5–12.6)	31.8 (15.7–50.2)
120 h LC_{50}	73.1 (52.6–102.8)	56.2 (44.7–74.5)	230.2 (194.3–279.4)
120 h LC_{10}	19.1 (9.0–29.3)	8.5 (5.7–11.4)	27.8 (13.2–45.2)
120 h $LC_{0.01}$	4.4 (1.6–7.9)	2.8 (1.0–5.2)	11.9 (4.6–20.8)

95% confidence limits are presented in parenthesis.

120 h $LC_{0.01}$ value was for *E. echinosetosus* (2.8 mg NO_3-N/l).

Regarding marine invertebrates, Epifano and Srna (1975), studying the acute toxicity of $NaNO_3$ to juveniles of the American oyster *Crassostrea virginica*, estimated a 96 h LC_{50} value of 3794 mg NO_3-N/l (Table 1). Wickins (1976), examining the acute toxicity of $NaNO_3$ to combined species of penaeid shrimps (*Penaeus aztecus*, *P. japonicus*, *P. occidentalis*, *P. orientalis*, *P. schmitti* and *P. setiferus*), estimated a 48 h LC_{50} value as high as 3400 mg NO_3-N/l in 28‰ seawater (Table 1). Basuyaux and Mathieu (1999), studying the effect of elevated nitrate concentrations on growth of the abalone *Haliotis tuberculata* and the sea urchin *Paracentrotus lividus* during 15 days of exposure, reported maximum safe levels of 100 mg NO_3-N/l for *P. lividus* and 250 mg NO_3-N/l for *H. tuberculata* (Table 1).

Cheng and Chen (2002) found that a nitrate concentration of 105 mg NO_3-N/l caused reduction of oxyhemocyanin and protein in individuals (wet weight of 8.28–14.85 g) of the Kuruma shrimp *Marsupenaeus japonicus* (Table 1). Similarly, Cheng et al. (2002) studied nitrate accumulation (from $NaNO_3$) in tissues of the penaeid shrimp *Penaeus monodon*, and found that nitrate accumulated in muscle, hepatopancreas, foregut, heart, gill, hemolymph, midgut and eyestalk by factors of 0.16, 0.20, 0.26, 0.45, 0.60, 0.61, 0.83 and 1.32 over the ambient nitrate concentration. In addition, Tsai and Chen (2002), examining the acute toxicity of $NaNO_3$ on juveniles (average length 28.4 mm) of *P. monodon* at different salinity levels, reported that 48, 72 and 96 h LC_{50} values were: 2876, 1723 and 1449 mg NO_3-N/l in 15‰ seawater (Table 1); 3894, 2506 and 1575 mg NO_3-N/l in 25‰ seawater (Table 1); and 4970, 3525 and 2316 mg NO_3-N/l in 35‰ seawater (Table 1). Safe levels for rearing *P. monodon* juveniles were estimated to be 145, 158 and 232 mg NO_3-N/l at salinity levels of 15‰, 25‰ and 35‰ (Table 1).

In contrast, Muir et al. (1991) reported much lower levels of nitrate toxicity in *P. monodon*. They studied the tolerance of larvae at the Protozoea I stage (55–60 h after hatching) to $NaNO_3$, and found that significant mortality (31–37%) occurred within 40 h at a nitrate concentration as low as 0.226 mg NO_3-N/l (Table 1). Examination of surviving larvae from nitrate treatments indicated sublethal histopatological changes including vacuolation and shrinkage of the ganglionic neuropiles, and minor muscle fragmentation and shrinkage. At higher nitrate concentrations (2.26 and 22.6 mg NO_3-N/l), larval mortality increased (35–43% and 37–58%; Table 1) and additional tissues were affected: vacuolation and splitting of the hypodermis from the cuticle, and cytoplasmatic vacuolation of cells in the midgut and proventriculus. Because *P. monodon* larvae moulted from Protozoea I to Protozoea II stage during the experimental study, and because *P. monodon* larvae occur naturally in offshore, tropical regions which typically contain extremely low levels of dissolved nitrate (<0.05 mg NO_3-N/l; see Spencer, 1975; Kinne, 1984; Motoh, 1985), Muir et al. (1991) concluded that the relatively great sensitivity of *P. monodon* larvae to nitrate toxicity might be related to ontogeny and natural habitat: on the one hand, it is likely that larvae are more susceptible to nitrate during ecdysis; on the other hand, it is possible that larvae are well adapted to natural conditions (very low nitrate concentrations) and, consequently, are intolerant of elevated nitrate concentrations.

4. Toxicity to fishes

Nitrate toxicity to freshwater and marine fishes increases with increasing nitrate concentrations and exposure times (Trama, 1954; Westin, 1974; Colt and Tchobanoglous, 1976; Rubin and Elmaraghy, 1977; Kincheloe et al., 1979; Brownell, 1980; Tomasso and Carmichael, 1986; Pierce et al., 1993; Scott and Crunkilton, 2000). Furthermore, nitrate toxicity can depend greatly upon the cationic composition of the solution (Dowden and Bennett, 1965). As in the case of aquatic invertebrates, freshwater fishes appear to be more sensitive to nitrate toxicity than marine fishes.

Trama (1954) found that the common bluegill *Lepomis macrochirus* was able to tolerate elevated nitrate levels during short-term exposures: a 96 h LC_{50} value of 1975 mg NO_3-N/l was estimated for this fish species (Table 3). Dowden and Bennett (1965) reported that the 24 h LC_{50} values of $NaNO_3$ and KNO_3 for *L. macrochirus* were 2110 and 761 mg NO_3-N/l (Table 3).

Knepp and Arkin (1973) reported that the channel catfish *Ictalurus punctatus* was able to tolerate a nitrate concentration of 90 mg NO_3-N/l without affecting their growth and feeding activity after an exposure of 164 days (Table 3). Colt and Tchobanoglous (1976), evaluating the short-term toxicity of $NaNO_3$ to fingerlings (50–76 mm total length) of *I. punctatus* at 22, 26 and 30 °C, calculated 96 h LC_{50} values of 1355, 1423 and 1400 mg NO_3-N/l (Table 3). They concluded that the acute toxicity of nitrate to *I. punctatus* was independent of water temperature.

Westin (1974) reported that the 96 h LC_{50} values of nitrate for the rainbow trout *Oncorhynchus mykiss* (*Salmo gairdneri*, previously) and the chinook salmon *Oncorhynchus tshawytscha* were 1355 and 1310 mg NO_3-N/l (Table 3). Stormer et al. (1996) exposed fingerlings of *O. mykiss* to a nitrate concentration of 14 mg NO_3-N/l for 8 days. They found that NO_3^- ions were taken up passively, with plasma concentrations remaining below the ambient nitrate concentration. This limited uptake appeared central to the low toxicity of nitrate, and did not measurably influence electrolyte balance or haematology (Table 3).

Table 3
Comparative toxicity of nitrate-nitrogen (NO_3-N) to fishes

Species	Developmental stage	Aquatic medium	Toxicological parameter (mg NO_3-N/l)	References
Lepomis macrochirus	Fingerlings	Freshwater	1975 (96 h LC_{50})[a]	Trama (1954)
	Fingerlings	Freshwater	2110 (24 h LC_{50})[a]	Dowden and Bennett (1965)
	Fingerlings	Freshwater	761 (24 h LC_{50})[b]	Dowden and Bennett (1965)
Ictalurus punctatus	Fingerlings	Freshwater	90 (164 d NOAEL)[a]	Knepp and Arkin (1973)
	Fingerlings (50–76 mm)	Freshwater (22 °C)	1355 (96 h LC_{50})[a]	Colt and Tchobanoglous (1976)
	Fingerlings (50–76 mm)	Freshwater (26 °C)	1423 (96 h LC_{50})[a]	Colt and Tchobanoglous (1976)
	Fingerlings (50–76 mm)	Freshwater (30 °C)	1400 (96 h LC_{50})[a]	Colt and Tchobanoglous (1976)
Oncorhynchus mykiss	Fingerlings	Freshwater	1355 (96 h LC_{50})[a]	Westin (1974)
	Eggs (anadromous)	Freshwater	1.1 (30 d LOEC)[a]	Kincheloe et al. (1979)
	Fry (anadromous)	Freshwater	4.5 (30 d NOEC)[a]	Kincheloe et al. (1979)
	Eggs (nonanadromous)	Freshwater	1.1 (30 d NOEC)[a]	Kincheloe et al. (1979)
	Eggs (nonanadromous)	Freshwater	2.3 (30 d LOEC)[a]	Kincheloe et al. (1979)
	Fry (nonanadromous)	Freshwater	1.1 (30 d NOEC)[a]	Kincheloe et al. (1979)
	Fry (nonanadromous)	Freshwater	2.3 (30 d LOEC)[a]	Kincheloe et al. (1979)
	Fingerlings	Freshwater	14.0 (8 d NOAEL)[a]	Stormer et al. (1996)
Oncorhynchus tshawytscha	Fingerlings	Freshwater	1310 (96 h LC_{50})[a]	Westin (1974)
	Eggs	Freshwater	4.5 (30 d NOEC)[a]	Kincheloe et al. (1979)
	Fry	Freshwater	2.3 (30 d NOEC)[a]	Kincheloe et al. (1979)
	Fry	Freshwater	4.5 (30 d LOEC)[a]	Kincheloe et al. (1979)
Salmo clarki	Eggs	Freshwater	2.3 (30 d NOEC)[a]	Kincheloe et al. (1979)
	Eggs	Freshwater	4.5 (30 d LOEC)[a]	Kincheloe et al. (1979)
	Fry	Freshwater	4.5 (30 d NOEC)[a]	Kincheloe et al. (1979)
	Fry	Freshwater	7.6 (30 d LOEC)[a]	Kincheloe et al. (1979)
Oncorhynchus kisutch	Eggs	Freshwater	4.5 (30 d NOEC)[a]	Kincheloe et al. (1979)
	Fry	Freshwater	4.5 (30 d NOEC)[a]	Kincheloe et al. (1979)
Poecilia reticulatus	Fry	Freshwater	267 (24 h LC_{50})[b]	Rubin and Elmaraghy (1977)
	Fry	Freshwater	219 (48 h LC_{50})[b]	Rubin and Elmaraghy (1977)
	Fry	Freshwater	199 (72 h LC_{50})[b]	Rubin and Elmaraghy (1977)
	Fry	Freshwater	191 (96 h LC_{50})[b]	Rubin and Elmaraghy (1977)
Micropterus treculi	Fingerlings	Freshwater	1261 (96 h LC_{50})[a]	Tomasso and Carmichael (1986)
Pimephales promelas	Larvae (<8 d)	Freshwater	1010–1607 (96 h LC_{50})[a]	Scott and Crunkilton (2000)
	Larvae (<24 h)	Freshwater	358 (7 d NOEC)[a]	Scott and Crunkilton (2000)
	Larvae (<24 h)	Freshwater	717 (7d LOEC)[a]	Scott and Crunkilton (2000)
Catla catla	Fry (static system)	Freshwater	1565 (24 h LC_{50})[a]	Tilak et al. (2002)
	Fry (flow through system)	Freshwater	1484 (24 h LC_{50})[a]	Tilak et al. (2002)
Lithognathus mormyrus	Fingerlings	Seawater (34‰)	3450 (24 h LC_{50})[a]	Brownell (1980)
Diplodus saegus	Fingerlings	Seawater (34‰)	3560 (24 h LC_{50})[a]	Brownell (1980)
Heteromycteris capensis	Fingerlings	Seawater (34‰)	5050 (24 h LC_{50})[a]	Brownell (1980)
Pomacentrus leucostritus	Fingerlings (59–85 mm)	Seawater (32‰)	>3000 (96 h LC_{50})[a]	Pierce et al. (1993)
Centropristis striata	Fingerlings (106–168 mm)	Seawater (32‰)	2400 (96 h LC_{50})[a]	Pierce et al. (1993)
Trachinotus carolinus	Fingerlings (69–115 mm)	Seawater (32‰)	1000 (96 h LC_{50})[a]	Pierce et al. (1993)
Raja eglanteria	Fingerlings (75–125 mm)	Seawater (32‰)	>960 (96 h LC_{50})[a]	Pierce et al. (1993)
Monocanthus hispidus	Fingerlings (39–55 mm)	Seawater (32‰)	573 (96 h LC_{50})[a]	Pierce et al. (1993)

Values of toxicological parameters (LC_{50}, NOAEL, NOEC, LOEC) at different exposure times for several species of freshwater and marine fishes.

[a] Animals were exposed to sodium nitrate ($NaNO_3$).

[b] Animals were exposed to potassium nitrate (KNO_3).

The first indication that relatively low concentrations of nitrate might be harmful to fish came from Grabda et al. (1974). They reported that fry of rainbow trout, exposed to 5–6 mg NO_3-N/l for several days, displayed increased blood levels of ferrihemoglobin, alterations in the peripheral blood and hematopoietic centres, and liver damage. In addition, Kincheloe et al. (1979), examining the tolerance of several salmonid species to nitrate toxicity after an exposure of 30 days, reported that developing eggs and early fry stages of *O. mykiss*, *O. tshawytscha* and the (Lahontan) cutthroat trout *Salmo clarki* exhibited significant increases in mortality at nitrate concentrations from 1.1 to 4.5 mg NO_3-N/l (Table 3). In the case of the coho salmon *Oncorhynchus kisutch*, eggs and fry were not affected at the highest nitrate concentration of 4.5 mg NO_3-N/l (Table 3). Kincheloe et al. (1979) concluded that a nitrate level as low as 2.0 mg NO_3-N/l in surface waters of low total hardness (<40 mg $CaCO_3$/l) would be expected to limit survival of some salmonid fish populations because of impaired reproductive success.

Rubin and Elmaraghy (1977), after examining the acute toxicity of KNO_3 to guppy (*Poecilia reticulatus*) fry, calculated 24, 48, 72 and 96 h LC_{50} values of 267, 219, 199 and 191 mg NO_3-N/l (Table 3). Tomasso and Carmichael (1986) reported that the 96 h LC_{50} value of nitrate for the Guadalupe bass *Micropterus treculi* was 1261 mg NO_3-N/l (Table 3). Tilak et al. (2002), using static and continuous flow through systems, determined 24 h LC_{50} values of 1565 and 1484 mg NO_3-N/l for the Indian major carp *Catla catla* (Table 3).

Scott and Crunkilton (2000), after conducting laboratory experiments to examine the acute toxicity of $NaNO_3$ to larvae (<8 day old) of the fathead minnow *Pimephales promelas*, found that the 96 h LC_{50} value fell within the range of 1010–1607 mg NO_3-N/l (average LC_{50} value of 1341 mg NO_3-N/l; Table 3). Scott and Crunkilton (2000) also reported that the no-observed-effect concentration (NOEC) and the lowest-observed-effect concentration (LOEC), for the growth of newly hatched larvae (<24 h old) of *P. promelas* after an exposure of 7 days, were 358 and 717 mg NO_3-N/l (Table 3). These larvae were lethargic and exhibited bent spines before death at a nitrate concentration of 717 mg NO_3-N/l.

With regard to marine fishes, Brownell (1980) reported 24 h LC_{50} values (mg NO_3-N/l) in 34‰ seawater of 3450 for *Lithognathus mormyrus*, 3560 for *Diplodus saegus*, and 5050 for *Heteromycteris capensis* (Table 3). Pierce et al. (1993) estimated 96 h LC_{50} values (mg NO_3-N/l) in 32‰ seawater of 573 for the planehead filefish *Monocanthus hispidus*, >960 for the clearnose skate *Raja eglanteria*, 1000 for the Florida pompano *Trachinotus carolinus*, 2400 for the black sea bass *Centropristis striata*, and >3000 for the beaugregory *Pomacentrus leucostritus* (Table 3).

5. Toxicity to amphibians

Current field data suggest that nitrogen fertilizers, such as ammonium nitrate (NH_4NO_3), potassium nitrate (KNO_3) and sodium nitrate ($NaNO_3$), may be contributing (with pesticides) to the decline of amphibian populations in agricultural areas (Wederkinch, 1988; Berger, 1989; Hecnar, 1995; Oldham et al., 1997; Birge et al., 2000). Laboratory studies have shown that the toxicity of nitrate compounds to amphibians increases with increasing nitrate concentrations and exposure times (Baker and Waights, 1993, 1994; Hecnar, 1995; Xu and Oldham, 1997; Marco et al., 1999; Schuytema and Nebeker, 1999a,b,c). The tolerance of amphibians to nitrogen fertilizers may however increase with increasing body size (Schuytema and Nebeker, 1999a,b) and environmental adaptation (Johansson et al., 2001).

Baker and Waights (1993), studying the toxicity of $NaNO_3$ to tadpoles of the common toad *Bufo bufo*, found that these animals exhibited reduced feeding activity, weight loss and decreased survival (84.6% mortality) when exposed for 13 days to a nitrate concentration of 9.1 mg NO_3-N/l (Table 4). Similarly, Baker and Waights (1994), examining the toxicity of $NaNO_3$ to tadpoles of the treefrog *Litoria caerulea*, found that these animals exhibited reduced feeding activity, weight loss and decreased survival (58.0% mortality) when exposed for 16 days to a nitrate concentration of 22.7 mg NO_3-N/l (Table 4).

Hecnar (1995), examining the acute toxicity of NH_4NO_3 to tadpoles of the American toad *Bufo americanus*, the chorus frog *Pseudacris triseriata*, the leopard frog *Rana pipiens* and the green frog *Rana clamitans*, reported 96 h LC_{50} values within the range 13.6–39.3 mg NO_3-N/l (Table 4). Hecnar (1995) also examined the chronic (100 days) toxicity of NH_4NO_3 to these amphibian species, and found that tadpoles of chorus frog and leopard frog exhibited lower survivorship at a nitrate concentration of 10.0 mg NO_3-N/l (Table 4). Signs of anormal behavior and development were similar in acute and chronic experiments: tadpoles swam and fed less vigorously, exhibited swelled and transparent bodies, developed head and digestive-system deformities, and suffered edemas and paralysis before death. Although Hecnar (1995) only considered nitrate toxicity when using ammonium nitrate, the toxicity of H_4NO_3 could be due not only to nitrate but also to ammonia (the unionized form of NH_4^+). Because laboratory conditions during toxicity tests were 7.6 for pH and 20 °C for temperature (Hecnar, 1995), it may be estimated that maximum ammonia levels in acute and chronic exposures were 1.0 and 0.20 mg NH_3/l, respectively. These NH_3 levels are higher than the established safe levels of ammonia for aquatic animals (Alabaster and Lloyd, 1982; US Environmental Protection Agency, 1986)

Table 4
Comparative toxicity of nitrate-nitrogen (NO_3-N) to amphibians

Species	Developmental stage	Toxicological parameter (mg NO_3-N/l)	References
Bufo bufo	Tadpoles	9.1 (84.6% mortality 13 d)[a]	Baker and Waights (1993)
	Tadpoles	384.8 (96 h LC_{50})[c]	Xu and Oldham (1997)
	Tadpoles	369.6 (168 h LC_{50})[c]	Xu and Oldham (1997)
	Tadpoles	22.6 (30 d LOEC)[c]	Xu and Oldham (1997)
Litoria caerulea	Tadpoles	22.7 (58% mortality 16 d)[a]	Baker and Waights (1994)
Bufo americanus	Tadpoles (from Ojibway)	13.6 (96 h LC_{50})[c]	Hecnar (1995)
	Tadpoles (from Harrow)	39.3 (96 h LC_{50})[c]	Hecnar (1995)
	Fertilized eggs	9.0 (NOAEL)[a]	Laposata and Dunson (1998)
Pseudacris triseriata	Tadpoles	17 (96 h LC_{50})[c]	Hecnar (1995)
	Tadpoles	10.0 (100 d LOEC)[c]	Hecnar (1995)
Rana pipiens	Tadpoles	22.6 (96 h LC_{50})[c]	Hecnar (1995)
	Tadpoles	10.0 (100 d LOEC)[c]	Hecnar (1995)
	Larvae	30.0 (NOAEL)[a]	Allran and Karasov (2000)
Rana clamitans	Tadpoles	32.4 (96 h LC_{50})[c]	Hecnar (1995)
Rana sylvatica	Fertilized eggs	9.0 (NOAEL)[a]	Laposata and Dunson (1998)
Rana pretiosa	Newly hatched larvae	16.45 (15 d LC_{50})[b]	Marco et al. (1999)
Ambystoma jeffersonianum	Fertilized eggs	9.0 (NOAEL)[a]	Laposata and Dunson (1998)
Ambystoma maculatum	Fertilized eggs	9.0 (NOAEL)[a]	Laposata and Dunson (1998)
Ambystoma gracile	Newly hatched larvae	23.39 (15 d LC_{50})[b]	Marco et al. (1999)
Pseudacris regilla	Embryos	643 (96 h LC_{50})[a]	Schuytema and Nebeker (1999a)
	Embryos	578 (240 h LC_{50})[a]	Schuytema and Nebeker (1999a)
	Embryos	56.7 (10 d NOAEL)[a]	Schuytema and Nebeker (1999a)
	Tadpoles	1749.8 (96 h LC_{50})[a]	Schuytema and Nebeker (1999b)
	Tadpoles	266.2 (240 h LC_{50})[a]	Schuytema and Nebeker (1999b)
	Tadpoles	30.1 (10 d NOAEL)[a]	Schuytema and Nebeker (1999b)
Xenopus laevis	Embryos	438.4 (120 h LC_{50})[a]	Schuytema and Nebeker (1999a)
	Embryos	24.8 (5 d NOAEL)[a]	Schuytema and Nebeker (1999a)
	Tadpoles	1655.8 (96 h LC_{50})[a]	Schuytema and Nebeker (1999b)
	Tadpoles	1236.2 (240 h LC_{50})[a]	Schuytema and Nebeker (1999b)
	Tadpoles	65.6 (10 d NOAEL)[a]	Schuytema and Nebeker (1999b)
	Tadpoles	66.0 (40 d NOAEL)[a]	Sullivan and Spence (2003)
Rana aurora	Embryos	636.3 (16 d LC_{50})[a]	Schuytema and Nebeker (1999c)
	Embryos	29.0 (16 d NOAEL)[a]	Schuytema and Nebeker (1999c)
Rana temporaria	Larvae (northern Scandinavia)	5.0 (8 w LOEC)[a]	Johansson et al. (2001)
	Larvae (southern Scandinavia)	5.0 (10 w NOEC)[a]	Johansson et al. (2001)

Values of toxicological parameters (LC_{50}, NOAEL, NOEC, LOEC) at different exposure times for several species of amphibians.
[a] Animals were exposed to sodium nitrate ($NaNO_3$).
[b] Animals were exposed to potassium nitrate (KNO_3).
[c] Animals were exposed to ammonium nitrate (NH_4NO_3).

and, consequently, we can assume that, in addition to nitrate toxicity, some toxicity might have been caused by NH_3.

Xu and Oldham (1997) examined lethal and sublethal effects of NH_4NO_3 on tadpoles of the common toad *Bufo bufo*. They reported 96 and 168 h LC_{50} values of 384.8 and 369.6 mg NO_3-N/l (Table 4). Tadpoles exhibited certain unusual behavior (either undirected swimming movements or twisting laterally), remaining static unless disturbed. In a subchronic exposure (30 days) at a nitrate concentration of 22.6 mg NO_3-N/l, there was 21% mortality and a further 17% failed to resorb their tails at metamorphosis (Table 4). As in the case of Hecnar (1995), Xu and Oldham (1997) only considered nitrate toxicity when using ammonium nitrate. Because laboratory conditions during toxicity tests were 7.4 for pH and 27.5 °C for temperature (Xu and Oldham, 1997), it may be estimated that maximum ammonia levels in acute and subchronic exposures were 9.2 and 0.51 mg NH_3/l, respectively. In consequence, we can assume that, in addition to nitrate toxicity, some toxicity might have been caused by NH_3.

Laposata and Dunson (1998) exposed fertilized eggs of the wood frog *Rana sylvatica*, the American toad *Bufo americanus*, the Jefferson salamander *Ambystoma jeffersonianum* and the spotted salamander *A. maculatum* to a nitrate concentration of 9.0 mg NO_3-N/l (from $NaNO_3$). They found that there was no significant difference in the hatching success with regard to control eggs in any of the four amphibian species (Table 4).

Marco et al. (1999), studying the effects of KNO_3 on several amphibian species indigenous of the Pacific Northwest (USA), found that newly hatched larvae of *Rana pretiosa* and *Ambystoma gracile*, exposed for 15 days to nitrate concentrations within the range 0.78–25.0 mg NO_3-N/l, reduced feeding activity, swam less vigorously, suffered edemas and paralysis, and eventually died. They calculated 15 d LC_{50} values of 16.45 mg NO_3-N/l for *R. pretiosa* and 23.39 mg NO_3-N/l for *A. gracile* (Table 4).

Schuytema and Nebeker (1999a,b,c) examined the toxic effects of $NaNO_3$ on embryos and tadpoles of the Pacific treefrog *Pseudacris regilla*, the African clawed frog *Xenopus laevis* and the red-legged frog *Rana aurora*. Schuytema and Nebeker (1999a) calculated 96 and 240 h LC_{50} values (mg NO_3-N/l) of 643 and 578 for embryos of *P. regilla*, and a 120 h LC_{50} value of 438.4 mg NO_3-N/l for embryos of *X. laevis* (Table 4). NOAEL (no observed adverse effect level) values, based on reduced growth (wet weight) of embryos, were 56.7 mg NO_3-N/l for *P. regilla* and 24.8 mg NO_3-N/l for *X. laevis* (Table 4). Schuytema and Nebeker (1999b) calculated 96 and 240 h LC_{50} values (mg NO_3-N/l) of 1749.8 and 266.2 for tadpoles of *P. regilla*, and 1655.8 and 1236.2 for tadpoles of *X. laevis* (Table 4). NOAEL values, based on reduced growth (wet weight) of tadpoles, were 30.1 mg NO_3-N/l for *P. regilla* and 65.6 mg NO_3-N/l for *X. laevis* (Table 4). Lastly, Schuytema and Nebeker (1999c) reported, for embryos of *R. aurora*, a 16 d LC_{50} value of 636.3 mg NO_3-N/l and a NOAEL value (based on length) of 29 mg NO_3-N/l (Table 4).

Allran and Karasov (2000), studying $NaNO_3$ toxicity to larvae of the leopard frog *Rana pipiens* exposed from first-feeding stage through metamorphosis, found that a nominal nitrate concentration of 30 mg NO_3-N/l had no significant effect on development rate, percent metamorphosis, time to metamorphosis, percent survival, mass at metamorphosis, or hematocrit. Although the growth of larvae was slowed, this growth inhibition was not biologically important when compared with natural variation in the environment.

Johansson et al. (2001), after conducting a comparison of nitrate tolerance between different populations of the common frog *Rana temporaria*, reported that a nitrate concentration of 5.0 mg NO_3-N/l might reduce the growth rate and metamorphic size in larvae (stage 25) from the northern parts of Scandinavia (less well adapted to cope with high environmental nitrate levels), but not in larvae from the southern parts of Scandinavia (better adapted to cope with high environmental nitrate levels) (Table 4). They concluded that increased anthropogenic nitrate pollution could impact more the northern than the southern Swedish common frog populations.

Sullivan and Spence (2003), examining $NaNO_3$ toxicity to tadpoles of the African clawed frog *Xenopus laevis*, found that a nominal nitrate concentration of 66 mg NO_3-N/l had no significant effect on the survival and metamorphosis of these animals during a exposure of 40 days (Table 4).

6. Concluding remarks

It should be evident, from data presented in this review, that nitrate discharges from anthropogenic sources may result in a serious ecological risk for certain aquatic animals. Indeed, as a consequence of nitrogen pollution, nitrate concentrations in surface waters can actually exceed values of 25 mg NO_3-N/l (Bogardi et al., 1991; Gleick, 1993; Ministry of Agriculture, Fisheries and Food, 1993). Because a nitrate concentration of 10 mg NO_3-N/l (USA federal maximum level for drinking water) can adversely affect, at least during long-term exposures, freshwater invertebrates (*Eulimnogammarus toletanus*, *Echinogammarus echinosetosus*, *Cheumatopsyche pettiti*, *Hydropsyche occidentalis*), fishes (*Oncorhynchus mykiss*, *Oncorhynchus tshawytscha*, *Salmo clarki*), and amphibians (*Pseudacris triseriata*, *Rana pipiens*, *Rana temporaria*, *Bufo bufo*) (Tables 1–4), safe levels below this nitrate concentration are therefore recommended to protect these sensitive freshwater animals from nitrate pollution. Furthermore, following Kincheloe et al.'s (1979) recommendation, we consider that a maximum level of 2.0 mg NO_3-N/l would be appropriate for protecting the most sensitive freshwater species. In the case of marine invertebrates and fishes, we consider that the proposed maximum level of 20 mg NO_3-N/l for culturing seawater animals (Spotte, 1979) may in general be acceptable. However, early developmental stages of some marine invertebrates (Muir et al., 1991), that are well adapted to low nitrate concentrations, may be so susceptible to nitrate as sensitive freshwater invertebrates (Tables 1 and 2).

In spite of this proposal of preliminary safe levels of nitrate for aquatic animals, further studies, especially long-term studies, are required to check and improve the recommended safe levels. Additional studies must also examine the influence of water hardness, salinity, pH, temperature, dissolved oxygen and other chemical compounds on nitrate toxicity to aquatic animals. Lastly, because aquatic organisms are subjected to biotic interactions (e.g., competition, predation, parasitism) and diseases, field and laboratory studies should be carried out to assess the effects of elevated nitrate concentrations on these ecological and evolutionary agents of natural selection.

Acknowledgement

Funds for this research came from Madrid Community (research project 07M/0065/2001) and Ministry of Science and Technology (research project REN2001-1008/HID) in Spain. The University of Alcala provided logistical support. Comments by two anonymous reviewers are gratefully acknowledged. A. Alonso was supported by a pre-doctoral grant from the Council of Castilla-La Mancha. Part of this work was presented at the 5th Iberian and 2nd Iberoamerican Congress on Environmental Contamination and Toxicology, held in Porto (Portugal) from 22 to 24 September of 2003.

References

Alabaster, J.S., Lloyd, R., 1982. Water Quality Criteria for Freshwater Fish, 2nd ed. Butterworths, London.

Allran, J.W., Karasov, W.H., 2000. Effects of atrazine and nitrate on northern leopard frog (*Rana pipiens*) larvae exposed in the laboratory from posthatch through metamorphosis. Environ. Toxicol. Chem. 19, 2850–2855.

Alonso, A., Camargo, J.A., 2003. Short-term toxicity of ammonia, nitrite, and nitrate to the aquatic snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca). Bull. Environ. Contam. Toxicol. 70, 1006–1012.

Amdur, M.O., Dull, J., Klassen, E.D. (Eds.), 1991. Casarett and Doull's Toxicology, 4th ed. Pergamon Press, New York.

Austin, B., 1988. Marine Microbiology. Cambridge University Press, Cambridge.

Baker, J., Waights, V., 1993. The effect of sodium nitrate on the growth and survival of toad tadpoles (*Bufo bufo*) in the laboratory. Herpetol. J. 3, 147–148.

Baker, J., Waights, V., 1994. The effects of nitrate on tadpoles of the tree frog (*Litoria caerulea*). Herpetol. J. 4, 106–107.

Basuyaux, O., Mathieu, M., 1999. Inorganic nitrogen and its effect on growth of the abalone *Haliotis tuberculata* Linnaeus and the sea urchin *Paracentrotus lividus* Lamarck. Aquaculture 174, 95–107.

Berger, L., 1989. Disappearance of amphibian larvae in the agricultural landscape. Ecol. Int. Bull. 17, 65–73.

Birge, W.J., Westerman, A.G., Spromberg, J.A., 2000. Comparative toxicology and risk assessment of amphibians. In: Sparling, D.W., Linder, G., Bishop, C.A. (Eds.), Ecotoxicology of Amphibians and Reptiles. SETAC, Pensacola (USA), pp. 727–792.

Bogardi, I., Kuzelka, R.D., Ennega, W.G., 1991. Nitrate Contamination: Exposure, Consequence, and Control. NATO ASI Series G: Ecological Sciences, vol. 30. Springer-Verlag, New York.

Bouchard, D.C., Williams, M.K., Surampall, R.Y., 1992. Nitrate contamination of groundwater: sources and potential health effects. J. Am. Water Works Assoc. 84, 85–90.

Boumans, L., Fraters, D., van Drecht, G., 2004. Nitrate leaching by atmospheric deposition to upper groundwater in the sandy regions of the Netherlands in 1990. Environ. Monit. Assess. 93, 1–15.

Brownell, C.L., 1980. Water quality requirements for first-feeding in marine fish larvae. I. Ammonia, nitrite and nitrate. J. Exp. Mar. Biol. Ecol. 44, 269–283.

Camargo, J.A., Ward, J.V., 1992. Short-term toxicity of sodium nitrate ($NaNO_3$) to non-target freshwater invertebrates. Chemosphere 24, 23–28.

Camargo, J.A., Ward, J.V., 1995. Nitrate (NO_3-N) toxicity to aquatic life: a proposal of safe concentrations for two species of Nearctic freshwater invertebrates. Chemosphere 31, 3211–3216.

Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W., Sharpley, A.N., Smith, V.H., 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol. Appl. 8, 559–568.

Cheng, S.-Y., Chen, J.-C., 2002. Study on the oxyhemocyanin, deoxyhemocyanin, oxygen affinity and acid–base balance of *Marsupenaeus japonicus* following exposure to combined elevated nitrite and nitrate. Aquatic Toxicol. 61, 181–193.

Cheng, S.-Y., Tsai, S.-J., Chen, J.-C., 2002. Accumulation of nitrate in the tissues of *Penaeus monodon* following elevated ambient nitrate exposure after different time periods. Aquatic Toxicol. 56, 133–146.

Colt, J., Tchobanoglous, G., 1976. Evaluation of the short-term toxicity of nitrogenous compounds to channel catfish, *Ictalurus punctatus*. Aquaculture 8, 209–221.

Conrad, J.J., 1990. Nitrate Pollution and Politics. Aldershot, Avebury.

De Graaf, F., 1964. Maintenance problems in large public aquaria. Arch. Neerland. Zool. 16, 142–143.

Dowden, B.F., Bennett, H.J., 1965. Toxicity of selected chemicals to certain animals. J. Wat. Pollut. Control Fed. 37, 1308–1317.

Epifano, C.E., Srna, R.F., 1975. Toxicity of ammonia, nitrite ion, nitrate ion, and orthophosphate to *Mercenaria mercenaria* and *Crassostrea virginica*. Mar. Biol. 33, 241–246.

Galloway, J.N., Cowling, E.B., 2002. Reactive nitrogen and the world: 200 years of change. Ambio 31, 64–71.

Gleick, P.H. (Ed.), 1993. Water in Crisis: A Guide to the World's Fresh Water Resources. Oxford University Press, New York.

Goodrich, J.A., Lykins Jr., B.W., Clark, R.M., 1991. Drinking water from agriculturally contaminated groundwater. J. Environ. Qual. 20, 707–717.

Grabda, E., Einszporn-Orecka, T., Felinska, C., Zbanysek, R., 1974. Experimental methemoglobinemia in trout. Acta Ichthyol. Piscat. 4, 43–71.

Harte, J., Holdren, C., Schneider, R., Shirley, C., 1991. Toxics A to Z: A Guide to Everyday Pollution Hazards. University of California Press, Berkeley.

Hecnar, S.J., 1995. Acute and chronic toxicity of ammonium nitrate fertilizer to amphibians from southern Ontario. Environ. Toxicol. Chem. 14, 2131–2137.

Jensen, F.B., 1996. Uptake, elimination and effects of nitrite and nitrate in freshwater crayfish (*Astacus astacus*). Aquatic Toxicol. 34, 95–104.

Johansson, M., Räsänen, K., Merilä, J., 2001. Comparison of nitrate tolerance between different populations of the common frog, *Rana temporaria*. Aquatic Toxicol. 54, 1–14.

Kincheloe, J.W., Wedemeyer, G.A., Koch, D.L., 1979. Tolerance of developing salmonid eggs and fry to nitrate exposure. Bull. Environ. Contam. Toxicol. 23, 575–578.

Kinne, O. (Ed.), 1984. Marine Ecology. John Wiley and Sons, London.

Knepp, G.L., Arkin, G.F., 1973. Ammonia toxicity levels and nitrate tolerance of channel catfish (*Ictalurus punctatus*). Prog. Fish-Cult. 35, 221–224.

Laposata, M.M., Dunson, W.A., 1998. Effects of boron and nitrate on hatching success of amphibian eggs. Arch. Environ. Contam. Toxicol. 35, 615–619.

Lee, G., Ellersieck, M.R., Mayer, F.L., Krause, G.F., 1995. Predicting chronic lethality of chemicals to fishes from acute toxicity test data: multifactor probit analysis. Environ. Toxicol. Chem. 14, 345–349.

Marco, A., Quilchano, C., Blaustein, A.R., 1999. Sensitivity to nitrate and nitrite in pond-breeding amphibians from the Pacific Northwest, USA. Environ. Toxicol. Chem. 18, 2836–2839.

Meade, M.E., Watts, S.A., 1995. Toxicity of ammonia, nitrite, and nitrate to juvenile Australian crayfish, *Cherax quadricarinatus*. J. Shellfish Res. 14, 341–346.

Meybeck, M., Chapman, D.V., Helmer, R., 1989. Global Freshwater Quality: A First Assessment, World Health Organization and United Nations Environmental Program. Blackwell, Oxford.

Ministry of Agriculture, Fisheries and Food, 1993. Solving the Nitrate Problem. Ref. PB 1092. MAFF Publications, London.

Moomaw, W.R., 2002. Energy, industry and nitrogen: strategies for decreasing reactive nitrogen emissions. Ambio 31, 184–189.

Motoh, H., 1985. Biology and ecology of *Penaeus monodon*. In: Taki, Y., Primavera, J.H., Jose A.L. (Eds.). Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps. Aquaculture Department, Southeast Asian Fisheries Development Center, Iloilo (Philippines), pp. 27–36.

Muir, P.R., Sutton, D.C., Owens, L., 1991. Nitrate toxicity to *Penaeus monodon* protozoea. Mar. Biol. 108, 67–71.

Nash, L., 1993. Water quality and health. In: Gleik, P.H. (Ed.), Water in Crisis: A Guide to the World's Fresh Water Resources. Oxford University Press, New York, pp. 25–39.

Nixon, S.W., 1995. Coastal marine eutrophication: a definition, social causes, and future concerns. Ophelia 41, 199–219.

Oldham, R.S., Lathan, D.M., Hilton-Brown, D., Towns, M., Cooke, A.S., Burn, A., 1997. The effect of ammonium nitrate fertilizer on frog (*Rana temporaria*) survival. Agric. Ecosys. Environ. 61, 69–74.

Pierce, R.H., Weeks, J.M., Prappas, J.M., 1993. Nitrate toxicity to five species of marine fish. J. World Aquacult. Soc. 24, 105–107.

Rabalais, N.N., 2002. Nitrogen in aquatic ecosystems. Ambio 31, 102–112.

Rubin, A.J., Elmaraghy, G.A., 1977. Studies on the toxicity of ammonia, nitrate and their mixture to guppy fry. Water Res. 11, 927–935.

Russo, R.C., 1985. Ammonia, nitrite and nitrate. In: Rand, G.M., Petrocelli, S.R. (Eds.), Fundamentals of Aquatic Toxicology. Hemisphere Publishing Corporation, Washington DC, pp. 455–471.

Sandstedt, C.A., 1990. Nitrates: Sources and their Effects upon Humans and Livestock. American University, Washington, DC.

Schuytema, G.S., Nebeker, A.V., 1999a. Comparative effects of ammonium and nitrate compounds on Pacific treefrog and African clawed frog embryos. Arch. Environ. Contam. Toxicol. 36, 200–206.

Schuytema, G.S., Nebeker, A.V., 1999b. Comparative toxicity of ammonium and nitrate compounds to Pacific treefrog and African clawed frog tadpoles. Environ. Toxicol. Chem. 18, 2251–2257.

Schuytema, G.S., Nebeker, A.V., 1999c. Effects of ammonium nitrate, sodium nitrate, and urea on red-legged frogs, Pacific treefrogs, and African clawed frogs. Bull. Environ. Contam. Toxicol. 63, 357–364.

Scott, G., Crunkilton, R.L., 2000. Acute and chronic toxicity of nitrate to fathead minnows (*Pimephales promelas*), *Ceriodaphnia dubia* and *Daphnia magna*. Environ. Toxicol. Chem. 19, 2918–2922.

Sharma, B., Ahlert, R.C., 1977. Nitrification and nitrogen removal. Water Res. 11, 897–925.

Smith, V.H., 2003. Eutrophication of freshwater and coastal marine ecosystems: a global problem. Environ. Sci. Pollut. R. 10, 126–139.

Smith, V.H., Tilman, G.D., Nekola, J.C., 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environ. Pollut. 100, 179–196.

Spencer, C., 1975. The micronutrient elements. In: Riley, J.P., Skirrow, G. (Eds.), Chemical Oceanography, vol. II, second ed. Academic Press, London.

Spotte, S., 1979. Fish and Invertebrate Culture: Water Management in Closed System. John Wiley and Sons, New York.

Steinheimer, T.R., Scoggin, K.D., Kramer, L.A., 1998. Agricultural chemical movement through a field-sized watershed in Iowa: subsurface hydrology and distribution of nitrate in groundwater. Environ. Sci. Tech. 32, 1039–1047.

Stormer, J., Jensen, F.B., Rankin, J.C., 1996. Uptake of nitrite, nitrate, and bromide in rainbow trout, *Oncorhynchus mykiss*: effects on ionic balance. Can. J. Fish. Aquat. Sci. 53, 1943–1950.

Stumm, W., Morgan, J.J., 1996. Aquatic Chemistry, third ed. John Wiley and Sons, New York.

Sullivan, K.B., Spence, K.M., 2003. Effects of sublethal concentrations of atrazine and nitrate on metamorphosis of the african clawed frog. Environ. Toxicol. Chem. 22, 627–635.

Tilak, K.S., Lakshmi, S.J., Susan, T.A., 2002. The toxicity of ammonia, nitrite and nitrate to the fish, *Catla catla* (Hamilton). J. Environ. Biol. 23, 147–149.

Tomasso, J.R., Carmichael, G.J., 1986. Acute toxicity of ammonia, nitrite and nitrate to the Guadalupe bass, *Micropterus treculi*. Bull. Environ. Contam. Toxicol. 36, 866–870.

Trama, F.B., 1954. The acute toxicity of some common salts of sodium, potassium and calcium to the common bluegill (*Lepomis macrochirus* Rafinesque). Proc. Acad. Nat. Sci., Philadelphia 106, 185–205.

Tsai, S.-J., Chen, J.-C., 2002. Acute toxicity of nitrate on *Penaeus monodon* juveniles at different salinity levels. Aquaculture 213, 163–170.

US Environmental Protection Agency, 1986. Quality Criteria for Water. EPA 440/5-86-001, Washington, DC.

US Environmental Protection Agency, 1991. Multifactor Probit Analysis. EPA 600/X-91-101, Washington, DC.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G., 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecol. Appl. 7, 737–750.

Wederkinch, E., 1988. Population size, migration barriers, and other features of *Rana dalmatina* populations near Køge, Zealand, Denmark. Memo. Soc. Fauna Flora Fenn. 64, 101–103.

Welch, E.B., Lindell, T., 1992. Ecological Effects of Wastewater, 2nd ed. Chapman and Hall, London.

Westin, D.T., 1974. Nitrate and nitrite toxicity to salmonid fishes. Prog. Fish-Cult. 36, 86–89.

Wetzel, R.G., 2001. Limnology, 3rd ed. Academic Press, New York.

Wickins, J.F., 1976. The tolerance of warm-water shrimps to recirculated water. Aquaculture 9, 19–37.

Wolfe, A.H., Patz, J.A., 2002. Reactive nitrogen and human health: acute and long-term implications. Ambio 31, 120–125.

Xu, Q., Oldham, R.S., 1997. Lethal and sublethal effects of nitrogen fertilizer ammonium nitrate in common toad (*Bufo bufo*) tadpoles. Arch. Environ. Contam. Toxicol. 32, 298–303.

Dr. Julio A. Camargo teaches ecotoxicology, limnology, and evolutionary ecology at the University of Alcala. His research interest concerns aquatic ecology and ecotoxicology, with the primary goal of understanding and conserving aquatic ecosystems.

Alvaro Alonso is a PhD student at the University of Alcala. His research interest concerns aquatic ecology and ecotoxicology, particularly the effects of nitrogen compounds on riverine communities.

Annabella Salamanca is a PhD student at the University of Alcala. Her research interest concerns aquatic ecology and ecotoxicology, particularly the effects of inorganic nutrients on lacustrine communities.

Environmental Toxicology and Chemistry, Vol. 32, No. 2, pp. 288–295, 2013

Printed in the USA
DOI: 10.1002/etc.2054

Water Quality

A METHOD FOR ASSESSING THE POTENTIAL FOR CONFOUNDING APPLIED TO IONIC STRENGTH IN CENTRAL APPALACHIAN STREAMS

Glenn W. Suter II* and Susan M. Cormier
National Center for Environmental Assessment, U.S. Environmental Protection Agency, Cincinnati, Ohio

(*Submitted* 11 *November* 2011; *Returned for Revision* 9 *January* 2012; *Accepted* 12 *April* 2012)

Abstract—Causal relationships derived from field data are potentially confounded by variables that are correlated with both the cause and its effect. The present study presents a method for assessing the potential for confounding and applies it to the relationship between ionic strength and impairment of benthic invertebrate assemblages in central Appalachian streams. The method weighs all available evidence for and against confounding by each potential confounder. It identifies 10 types of evidence for confounding, presents a qualitative scoring system, and provides rules for applying the scores. Twelve potential confounders were evaluated: habitat, organic enrichment, nutrients, deposited sediments, pH, selenium, temperature, lack of headwaters, catchment area, settling ponds, dissolved oxygen, and metals. One potential confounder, low pH, was found to be biologically significant and eliminated by removing sites with pH < 6. Other potential confounders were eliminated based on the weight of evidence. This method was found to be useful and defensible. It could be applied to other environmental assessments that use field data to develop causal relationships, including contaminated site remediation or management of natural resources. Environ. Toxicol. Chem. 2013;32:288–295. © 2012 SETAC

Keywords—Aquatic invertebrates Conductivity Weight of evidence Salinity Causation

INTRODUCTION

The use of field data to understand and manipulate causal relationships is limited by the possibility that the apparent relationship is confounded. *Confounding* is a bias in the analysis of causal relationships due to the influence of extraneous factors (confounders). Confounding can occur when a variable is correlated with both the potential cause and its effect. The correlations are usually due to a common source of multiple potentially causal agents. However, they may be observed for other reasons (e.g., when one variable is a by-product of another) or due to chance associations.

Confounding is not, in general, well treated in ecological studies. Investigators often assume that confounding is not a problem if the association of interest is strong (e.g., has a high correlation coefficient). Alternatively, they assume that multiple regression, path analysis, or other multivariate statistics adequately deal with confounding, even though assumptions are violated and important potential confounders are often excluded for lack of adequate data. Correlation and regression do not even tell us whether *C* causes *E* or *E* causes *C*. In the present study, we present an alternative approach based on weighing all available evidence for and against plausible confounders. The approach includes a list of types of evidence that could indicate that confounding interferes with our ability to characterize the causal relationship and uses explicit criteria and scoring to transparently evaluate the evidence.

The method is applied to potential confounders of the relationship between stream invertebrate presence and the salts that leach from crushed rock in central Appalachia [1; this issue]. The goal of the present analysis was to determine which environmental variables must be treated as confounders in the development of the benchmark value. It was not to eliminate confounding variables. Most of them are natural variables, such as temperature and habitat structure, that cannot be literally eliminated, like eliminating women or smokers in an epidemiological study. Nor was the goal to equate the levels of confounders to an ideal or pristine level. Furthermore, the goal was not to demonstrate that these variables never cause effects. It is known that these factors all cause some effects in some circumstances. The goal was to support estimation of the ionic strength, measured as specific conductance, that protects against unacceptable effects on the invertebrate communities in those streams without significant influence by confounding variables [1,2].

METHODS

General approach

We developed a weight-of-evidence approach for evaluating potential confounders. Both logical arguments and statistical analyses are used to indicate whether an environmental factor affects or does not affect our ability to model the causal relationship. If the body of evidence indicates that the factor was not a potential confounder, no action was taken. If the body of evidence indicates that an environmental factor was a likely confounder, then the data set was truncated to reduce the effect of the confounder. Truncation removes the observations for which the confounder was beyond its threshold for effects. Although it was not necessary in this case, other methods might be used to adjust for any discovered confounding of the causal relationship.

Evidence of confounding

Most confounders are causal agents that are correlates of the cause of interest. Causation is commonly addressed by applying Hill's [3] considerations or some equivalent set of criteria for causation [4]. This is done because statistics alone cannot determine the causal nature of relationships [5–7]. Confound-

All Supplemental Data can be found in the online version of this article.
* To whom correspondence may be addressed
(suter.glenn@epamail.epa.gov).
Published online 12 November 2012 in Wiley Online Library
(wileyonlinelibrary.com).

ing, whether due to causation or chance correlations, can bias a causal model resulting in uncertainty concerning the actual magnitude of the effects. A variety of types of evidence may be used to determine whether confounders significantly affect a field-derived benchmark. We have identified 10 types, but there may be others. They are related to three of the characteristics of causation used to determine that elevated ionic strength is a cause of impairment of stream communities (co–occurrence, sufficiency, and alteration) [1]. In some cases, one piece of evidence may rule out a potential confounder but more than one piece of evidence provides more confidence. Also, exclusion of relevant evidence can lead to false conclusions or accusations of bias. Only a weight-of-evidence approach allows assessors to consider all relevant statistical and logical evidence [8].

We used 10 types of evidence to assess confounding. They are listed below, beginning with a short description and followed by an explanation. In the descriptions, "the cause" refers to the cause of concern (ionic strength in this case) and "the confounder" refers to any potential confounder of the causal relationship. Type 1, correlation of confounder and cause: Confounders are correlated with the cause of interest. A low correlation coefficient is evidence against the potential confounder. Type 2, correlation of confounder and effect: Confounders are correlated with the effect of interest. A low correlation coefficient is evidence against the potential confounder. Type 3, influence of the confounder at extreme levels: Even when the confounder is not correlated with the cause of interest, it may be influential at extreme levels. A lack of influence at extreme levels of the potential confounder is evidence against the potential confounder. Type 4, influence of the presence of the confounder: If the frequency of the effect does not diminish when the potential confounder is never present or is present in all cases, it can be discounted in that subset. Type 5, occurrence of confounder at sufficient levels: The magnitude of the potential confounder (e.g., concentration of a cocontaminant) may be compared to exposure–response relationships from elsewhere (e.g., laboratory toxicity tests) to determine if the exposure to the potential confounder is sufficient to influence the effect. If it is not sufficient, that is evidence that it is not acting as a confounder. Type 6, influence of removing a confounder where it is at sufficient levels: If the confounder is estimated to be sufficient in a subset of cases, those cases may be removed from the data set and the remaining set reanalyzed to determine the influence of their removal on the results. If the cause–effect relationship is unchanged, the confounder was not causal or influential. Note that this evidence of confounding may also identify a treatment for confounding. Type 7, influence of the confounder in multivariate correlations: Multiple regression and other multivariate statistical techniques may be used to estimate the relative degree of association of the cause and potential confounders with the effect. Type 8, frequency of occurrence of the confounder: If the potential confounder occurs in a sufficiently small proportion of cases, it can be ignored. That is because if it occurs rarely, it cannot significantly influence the causal relationship. Type 9, occurrence of characteristic effects of the confounder: If a potential confounder has characteristic effects that are distinct from those of the cause of concern, then the absence of those effects can eliminate the potential confounder as a concern in either individual cases or the entire data set. Type 10, occurrence of characteristic effects of the cause: If the effects are characteristic of the cause of concern and not of the potential confounder, then the potential confounder can be eliminated as a concern in either individual cases or the entire data set.

Scoring evidence

Weighing evidence for confounding differs from weighing evidence for causation. A causal assessment determines whether the contaminant of concern (e.g., dissolved ions) is an important cause of biological impairment in the region [1]. This assessment of confounding accepts the result of the causal assessment and attempts to determine whether any of the known potential confounders substantively interferes with estimating the effects of ionic strength in the causal model. If there is significant interference, the confidence in the model predictions would be weakened unless the model is modified. Although the general approach of weighing evidence by assigning scores using explicit criteria is the same as that used in the assessment of general causation (e.g., have dissolved ions caused impairment? [1]) or specific causation (e.g., what caused impairment of this community? [9]), the inference is different.

Two biological effect end points are used to develop evidence: (1) the species sensitivity distribution of invertebrate genera and the resulting 5th percentile hazard concentration (HC05), and (2) the number of ephemeropteran genera. The species sensitivity distribution and HC05 are used because they are the model and resulting output used to develop the benchmark. If a potential confounder does not influence that end point, it is not a confounder. However, the HC05 does not lend itself to correlation, contingency tables, or regression because it has only one value for the region and values are needed for individual sites. For those statistical analyses, the number of ephemeropteran genera is used as a surrogate end point. That metric was chosen because these genera are consistently among the most sensitive to salts. However, because of a resistant mayfly genus, it is not expected that all Ephemeroptera will be missing at high specific conductance, hereafter referred to as "conductivity."

The primary data source for evidence of confounding is West Virginia's watershed analysis database, which was used to derive the benchmark [1]. Except where indicated, reported results are derived from those data, which are referred to as the West Virginia data. However, where possible and appropriate, the U.S. Environmental Protection Agency (U.S. EPA) Region 3 data set from West Virginia samples (referred to as the U.S. EPA data) is used for independent corroboration. The U.S. EPA data set is much smaller and often does not have enough extreme values of the potential confounder to calculate reliable contingency tables or regressions of censored data.

The evidence is weighted using a system of plus (+) for supporting the potential confounder (i.e., the evidence suggests that the potential confounder is actually causing the effect to a significant degree), minus (−) for weakening the potential confounder (i.e., the evidence suggests that the potential confounder does not contribute to the effect to a significant degree), and zero (0) for no effect, usually due to ambiguity. One to three (+) or (−) symbols are used to indicate the weight of a piece of evidence: (+ + +) or (− − −) indicates convincing support or weakening, (+ +) or (− −) indicates strong support or weakening, (+) or (−) indicates some support or weakening, and 0 indicates no effect on the hypothesis of confounding.

Any relevant evidence receives a single plus, minus, or zero to register the relevance of the evidence and to indicate its logical implication (i.e., does it decrease or increase the potential for confounding) (Table 1). The strength of evidence is considered next. Criteria for scoring the strength of evidence are presented below for the common types. The criteria were developed for transparency and consistency and are based on

Table 1. Relationships between qualities of evidence and scores for weighing evidence

Qualities of evidence	Score, not to exceed three minus or three plus
Logical implications and relevance	+, 0, −
Strength	Increase score
Other qualities	Increase score

the authors' judgments. After strength, the other possible unit of weight is assigned depending on the type of evidence.

For evidence based on co–occurrence (types $1-4$), the strength and consistency of the association are the primary considerations. The primary measure of association is Spearman's correlation coefficients. For comparison to the potential confounders, the correlation coefficients for conductivity and number of ephemeropteran genera are −0.61 for the West Virginia data set and −0.72 for the U.S. EPA Region 3 data set; these values fall in the upper end of the moderate range. Correlations, as measures of co–occurrence, can be scored as in Table 2.

These scores are based on conventional expectations for a confounder that is itself a cause. That is, a potential confounder such as deposited sediment by itself can cause extirpation of invertebrate genera (independent combined action) or can act in combination with conductivity to extirpate invertebrate genera (additive or more than additive combined action). However, sometimes correlations are anomalous. For example, a potential confounder may actually decrease effects. Such anomalous results require case–specific interpretation, based on knowledge of mechanisms and characteristics of the ecosystems being analyzed.

Anomalous results may also result from violation of the expectation that a confounder should be correlated with both conductivity and the effect. If only one of the correlations is observed, that result requires additional interpretation. If the potential confounder is correlated with the effect but not with conductivity, the result may be due to chance or to a partitioning of causation in space. That is, the cause and potential confounder are independent because they impair communities at different locations. This could occur if the potential confounder and conductivity have different sources. In any case, it is not a confounder of conductivity.

In the contingency tables (evidence type 3), the frequency of occurrence of any Ephemeroptera (i.e., of the failure to extirpate all ephemeropteran genera) is presented for combinations of high and low levels of conductivity and of the potential confounder. If the frequency of occurrence is much lower when the confounder is present at high levels, this is supporting evidence for confounding. Note that the goal here is not to determine the effects of exceeding a criterion or other benchmark. Rather, the goal is to clarify the co–occurrence of conductivity, confounders, and effects by determining the frequency of effects at each possible combination of extremely high and low levels of conductivity and the potential confounder. It is expected that if a variable is indeed a confounder, its influence on the occurrence of effects would be seen at an extreme level. This use of contingency tables could reveal influences of confounders that are obscured when the entire ranges of data are correlated. Therefore, clearly high and low levels of conductivity and the potential confounder are used in contingency tables.

A potential confounder gets a plus score if its presence at a high level reduces the probability of occurrence by more than 25% and a minus score if it does not (Table 3). It gets a double plus score if its presence at a high level reduces the probability of occurrence by more than 75% and a double minus score if it raises it by less than 10%. These cutoff levels delimit the indicated strength categories, based on the experience and judgment of the authors and reviewers. Any decrease in effects at high levels of a potential confounder is anomalous and treated as strong negative evidence.

The evidence concerning sufficiency of the confounder (evidence types $5-8$) is diverse. Only evidence type 6 was sufficiently common and consistent to develop scoring criteria. For evidence type 6, the primary consideration is the degree of departure of the correlation in the truncated data set from the correlation of conductivity and Ephemeroptera in the full data set (Table 4). However, no more than one negative score was given if less than 10% of the data were removed.

For alteration, the primary consideration is the degree of specificity of the effects of the confounder relative to those of the dissolved ions. This type of evidence is rare and scored ad hoc when it occurs.

Additional considerations that may result in a higher score are presented in Table 5.

Weighing the body of evidence

After the individual pieces of evidence had been weighted, the body of evidence for a potential confounder was weighed based on the credibility, diversity, strength, and coherence of the body of evidence (Table 6). The body of evidence, rather than a single piece of evidence, was considered to determine how strongly these potential confounders might affect the model. Seven potential confounders (habitat quality, deposited sediment, high and low pH, selenium [Se], catchment area, settling ponds, and metals) are presented here. Five other potential confounders (organic enrichment, nutrients, temperature, loss of headwaters, and dissolved oxygen) are assessed in the U.S. EPA report [2].

Table 2. Weighting co–occurrence using correlations for types 1 and 2

Assessment	Strength	Score
Absent	$r \leq 0.1$	− −
Weak	$0.1 < r < 0.25$	−
Moderate	$0.75 \geq r \geq 0.25$	+
High	$r > 0.75$	+ +

Table 3. Weighting co–occurrence for evidence type 3 using contingency tables

Assessment	Strength	Score
High levels of a confounder should increase	Increased effect >25%	+ for co-occurrence
the probability that a site lacks Ephemeroptera	Increased effect >75%	+ + for co-occurrence and strength
at low conductivity, and low levels of the confounder	Increased effect <25%	− for co-occurrence
should decrease the effect at high conductivities	Increased effect <10% or decreased effect	− − for co-occurrence and strength

Table 4. Weighting sufficiency for evidence type 6: Alteration of the correlation of conductivity with the number of ephemeropteran genera after removal of elevated levels of a confounder

Assessment	Strength	Score
Removal of elevated levels of a confounder should change the correlation coefficient	Coefficients deviating by <10%	– – for a lack of change in effect with removal of confounder
	Coefficients deviating by <20%	– for a small change in effect with removal of confounder
	Coefficients deviating by >20%	+ for a strong increase or decrease in effect with removal of confounder

Table 5. Considerations used to weight the evidence concerning the influence of potentially confounding variables

Quality of evidence	Descriptor
Logical implication	Negative or positive
Directness of cause	Proximate cause, sources, or intermediate causal connections
Specificity	Effect attributable to only one cause or to multiple causes
Relevance to effect	From the case or from other similar situations
Nature of association	Quantitative or qualitative
Strength of association	Strong relationships and large range or weak relationships and small range
Consistency of information	All consistent or some inconsistencies
Quantity of information	Many data or few data
Quality of information	Good study or poor study

RESULTS FOR POTENTIAL CONFOUNDERS

Habitat quality

Stream habitat may be modified by physical disturbance, changes in flow, or increased sediment loads in reaches that receive high conductivity effluents. Habitat quality was represented by an index, the rapid bioassessment protocol derived by the West Virginia Department of Environmental Protection, which increases as habitat quality increases. Component metrics were not used because they were less correlated with Ephemeroptera than the index.

Habitat quality was analyzed as part of groups of variables judged a priori to be more likely than others to have combined effects. Therefore, sites at which the rapid bioassessment protocol and pH were low and fecal coliform count was high were removed to determine whether the HC05 was affected (Fig. 1). Similarly, the rapid bioassessment protocol score was used with fecal coliform count and temperature in a multiple linear regression with conductivity (Supplemental Data, Table S1).

The body of evidence was mixed. Habitat scores were moderately correlated with both conductivity and biological response, indicating a potential for confounding. However, removal of poor habitat had little effect on the correlation of conductivity with Ephemeroptera or on the derivation of the HC05 for conductivity (Fig. 1 and Supplemental Data, Table S2). Habitat score had a very slight effect on the intercept and the slope for conductivity in a multiple regression (Supplemental Data, Table S1). In addition, Ephemeroptera occur even when habitat is poor (Supplemental Data, Table S2). The weight of the scored body of evidence indicated habitat was not a substantial confounder (Supplemental Data, Table S3).

Deposited sediment

Mining and other activities that result in crushing and exposing rocks are sources of salts and potentially of silt that may affect stream organisms. A qualitative measure of embeddedness (WABase embeddedness score) was evaluated by contingency table and by correlation [10] (Supplemental Data, Tables S1 and S4). No evidence supported embeddedness as a confounder (Supplemental Data, Table S5).

High pH

The dissolution of limestone, dolomite, and sandstone increases as unweathered surface area of rock increases. Waters draining crushed limestone, dolomite, or lime–cemented sandstone contain HCO_3^-, which contributes to higher pH and alkalinity. The HCO_3^- that raises the pH is also a major anion moiety that contributes to conductivity. Hence, pH directly reflects a major constituent of conductivity (HCO_3^-), so it could not be a conventional confounder. In addition, salts influence hydrogen ion activity, which is measured as pH. In any case, the available evidence indicates that the variance in pH has little effect on the derivation of the HC05 for conductivity in waters above pH 7 (Supplemental Data, Tables S6 and S7).

Low pH

Because low pH from acid mine drainage is known to be an important cause of impairment where coal is mined, it was judged a priori to be a potentially important environmental variable. That preconception was supported by the evidence summarized in the Supplemental Data (Table S8). Therefore, sites with pH < 6 were not used to calculate the benchmark values. However, 84% of sites with low pH still had at least one genus of Ephemeroptera, whereas none occurred at either the low- or high-pH sites with high conductivity (Supplemental Data, Table S6). This suggests that even below pH 4.5, ionic

Table 6. Weighing confidence in the body of evidence for a potential confounder

Assessment	Score	Body of evidence	Action
Very confident	– – –	All minus, some strongly negative evidence	No treatment for confounding
Moderately confident	– –	All minus, no strongly negative evidence	No treatment for confounding
Reasonably confident	–	Majority minus	No treatment for confounding
Undetermined	0	Approximately equal positive and negative, ambiguous evidence, or low-quality evidence	Additional study advised
Potential confounding	+	Majority plus	Correction for confounding may be advised

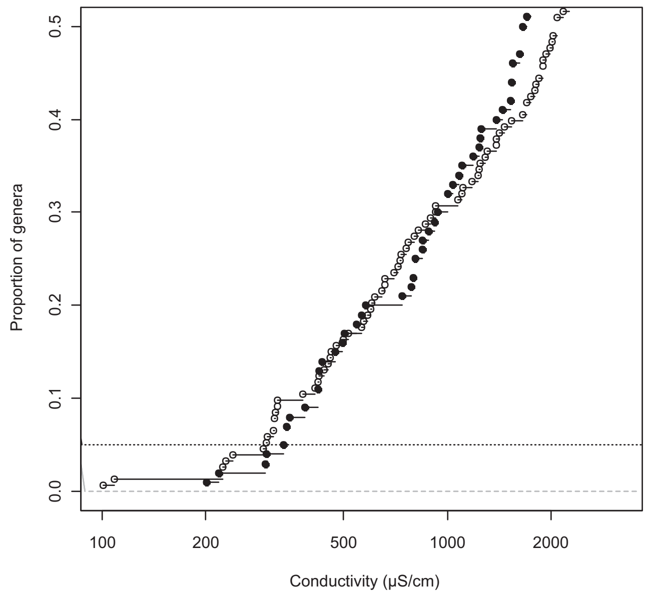


Fig. 1. Species sensitivity distribution used to derive the conductivity benchmark (open circles) and one for sites with good habitat (rapid bioassessment protocol ≥135) and low organic enrichment (fecal coliform fewer than 400 colonies/100) (closed circles). The similarity of the relationships shows that even when both common potential confounders are removed, the results do not significantly change. The lower and upper confidence bounds on 300 µS/cm (5th percentile of distribution of open circles) are 225 and 350 µS/cm, respectively.

strength is more important than acidity to the occurrence of Ephemeroptera. In sum, although the benchmark applies to waters with neutral or basic pH, high ionic strength appears to also cause effects at low pH.

Selenium

Selenium is a potential confounder because it is commonly associated with coal and elevated levels have been reported in the region, but the evidence does not support confounding (Supplemental Data, Table S9). No correlations were found between Se and Ephemeroptera or between Se and conductivity in the West Virginia data set or in the U.S. EPA data set. This result is unreliable because most of the Se values were detection limits, and many of the detection limits were relatively high, even equaling the water-quality criterion of 5.0 µg/L. In addition, there were too few high Se concentrations in the West Virginia data to perform a contingency table analysis. For these reasons, correlational evidence of confounding was ambiguous.

Evidence of the sufficiency of observed Se levels to cause extirpation of stream macroinvertebrates is weakly negative. The national ambient water quality criterion (5 µg/L) is irrelevant because it is based on more sensitive vertebrates [11]. Field and laboratory studies have found invertebrates to be relatively insensitive and unaffected at levels observed in West Virginia streams [12,13]. In outdoor artificial streams dosed with Se, insects were less sensitive than fish, crustaceans, and oligochaetes; baetid mayfly nymphs (*Baetis*, *Callibaetis*), damselfly nymphs (*Enallagma*), and chironomid larvae were not statistically significantly reduced, even at 30 µg/L [14]. Relatively few invertebrate species have been tested, and highly sensitive species may be identified in the future [15]; but the available toxicological evidence does not indicate that Se confounds the relationship between conductivity and invertebrate extirpation.

The effects of removing high Se on the conductivity relationship (evidence type 6) were addressed using the West Virginia data set. When data from streams with Se concentrations above the water-quality criterion (5 µg/L) were removed, the linear correlation coefficient for number of ephemeropteran genera and log conductivity was barely changed ($r = -0.56$, $n = 339$) relative to the full data set. When the same analysis was performed with the U.S. EPA data set, the correlation was actually greater than that for the full data set ($r = -0.84$, $n = 32$) (Fig. 2), which is contrary to expectations for a confounder. This result indicates that the conductivity relationship is not confounded by the toxic effects of Se.

Consideration of the specific effects of Se (evidence type 9) suggests that it is not an important contributor to the impairment. First, the most sensitive organisms to aqueous Se are fish and other oviparous vertebrates [12]; however, in this case, relatively Se–insensitive insects are most affected. Second, Se causes characteristic deformities in fish, which have not been reported in West Virginia streams. Third, the effects of Se at low concentrations are seen in lentic ecosystems (lakes, reservoirs, ponds, wetlands), not in streams like those from which the conductivity relationship and benchmark were derived [12]. Finally, because Se is biomagnified, it primarily affects top predators, not the herbivores and detritivores that are affected in this case. This specificity is supported by the fact that, in the region, the reported effects of Se are greatly elevated body burdens and associated deformities in a top predator fish (largemouth bass) in a lentic system (Upper Mud River Reservoir) [16,17].

The weight of evidence does not support confounding by Se, so no action was taken to adjust the data set or analysis. However, because existing Se data are poor, the occurrence of Se in central Appalachian streams should be investigated further.

Catchment area

Larger streams tend to have more moderate chemical properties than small streams because they receive waters from more sources. Consequently, extreme values—in this case, both low and high conductivity—tend to occur less frequently in large streams. One of the initial data filters for this analysis was to exclude streams larger than 155 km^2 (or 60 mi^2). Small streams are numerically more abundant than large streams, and the

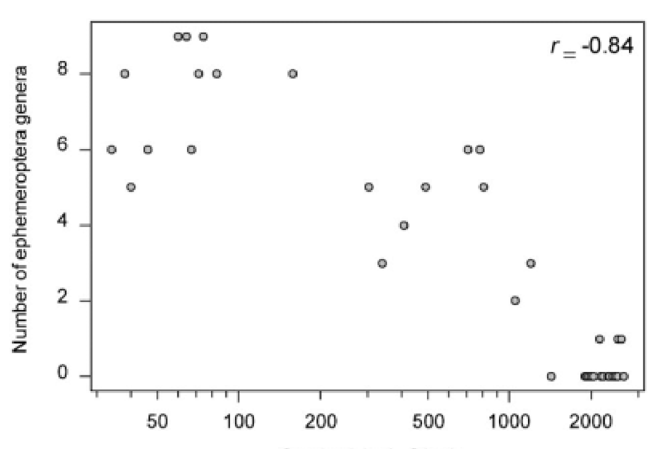


Fig. 2. Spearman's correlation coefficient and scatterplot between the number of ephemeropteran genera and conductivity for 32 sites with low selenium concentrations (<5 µg/L).

inclusion of large streams might introduce extraneous variance. This raises the issue of whether stream size is a potential confounder and whether the results from small streams might be extrapolated to larger streams. That is, do the same effects of conductivity occur in larger streams as were found in the detailed analysis of smaller streams? We examined these issues by analyzing the influence of stream size (as catchment area) on the effects of conductivity and on the occurrence of Ephemeroptera.

We categorized streams by catchment area into three groups: small catchments less than 6 mi^2 (15.5 km^2), medium catchments of 6 to 60 mi^2 (15.5 km^2 to 155 km^2), and large catchments greater than 60 mi^2 (155 km^2). These categories were distinguished because small catchments correspond to headwater streams, which have few pollutant sources and large terrestrial influence, and large catchments may have different sampling methods. In all three stream size categories, if conductivity was <200 μS/cm, 99% or more of streams had Ephemeroptera, but if conductivity was above 1,500 μS/cm, fewer streams had Ephemeroptera (Supplemental Data, Table S10). The number of Ephemeroptera taxa declines with increasing conductivity in all streams with measured catchment areas, independent of classification of catchment area ($r = -0.59$). Correlation of log conductivity with log catchment area is weak (Supplemental Data, Table S11).

The weight of evidence for confounding by catchment area (Supplemental Data, Table S11) is uniformly negative, so we conclude that catchment area has little or no effect on invertebrate response to conductivity.

Settling ponds

The effluents from most valley fills flow into settling ponds, and it has been suggested that those ponds are the actual cause of downstream community impairments. This issue was addressed using the U.S. EPA Region 3 data set because it identifies the presence of ponds. When data from only streams with ponds are used (i.e., the occurrence of ponds is removed as a variable, evidence type 4), the correlation coefficient for number of ephemeropteran genera and log conductivity is $r = -0.84$ (Fig. 3). This result is somewhat higher than the result for the uncensored U.S. EPA Region 3 data set ($r = -0.73$), which is contrary to the expectation if ponds were the cause or contributed to the effects of ionic strength. This

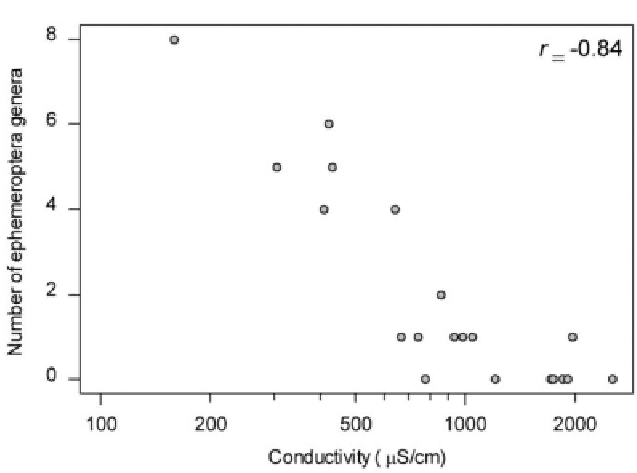


Fig. 3. Spearman's correlation coefficient and scatterplot between the number of ephemeropteran genera and conductivity for 20 sites below settling ponds for valley fills. Data from the U.S. Environmental Protection Agency Region 3 data set.

result clearly shows that the conductivity relationship is not a result of co–occurrence with ponds. In addition, when ponds are removed and the streams are reclaimed, conductivity remains high and the effects continue. For example, Venter's Branch and Jones Branch in Martin County, Kentucky, USA, were mined in the mid-1990s and the ponds removed. When the streams were sampled in 2009, conductivity was >2,000 μS/cm and no Ephemeroptera were found in either stream (Greg Pond, U.S. EPA, personal communication).

The weight of evidence for confounding from ponds is uniformly negative, so we conclude that the presence of ponds has little or no effect on invertebrate response to conductivity.

Metals

Iron (Fe), aluminum (Al), and manganese (Mn) are the metals most associated with acid mine drainage; and commenters have suggested that they may cause the impairment associated with ionic strength. However, for the following reasons, streams that are circumneutral to moderately alkaline are unlikely to experience toxicity from these metals [18].

The most toxic form of Fe (free Fe^{2+}) does not occur in oxygenated waters above pH 4. Under those conditions, Fe occurs as hydroxide particles or, if significant dissolved organic matter is present, as Fe colloids. In these forms, Fe is thought to reduce the toxicity of co–occurring metals by adsorption and coprecipitation. Toxic divalent Al precipitates similarly above pH 5 as hydroxide flocs or polymeric Al. Divalent Mn is converted to insoluble Mn^{4+} in mildly alkaline waters. The precipitates of these metals may adversely modify habitats and directly affect organisms; however, the valley fill effluents that are primarily responsible for the relationship between conductivity and extirpation of invertebrates are not equivalent to the acid drainage into neutralizing streams that results in heavy accumulations of precipitates. Finally, the toxicity of these divalent anions is mitigated by divalent calcium, which is the dominant cation in the ionic mixtures. Hence, because the calcium increase is much greater than the increase in these metals, it is expected that as conductivity increases, the toxicity of these metals will decrease per unit concentration.

Because of concern for combined effects of metals, multiple linear regression of conductivity, Fe, Al, and Mn was performed. The metals reduced the coefficient for conductivity by only 8.6% (Supplemental Data, Table S12).

Based on contingency table analyses, weak correlations, and other evidence [2], Fe and Al are clearly not confounders. However, Mn is more ambiguous since it is moderately correlated with both conductivity and ephemeropteran genera (Supplemental Data, Tables S13 and S14). Manganese has been relatively poorly studied because it has seldom been found at toxic levels. Like other divalent cationic metals, Mn^{2+} is less toxic in hard (i.e., high Ca) waters; and the high conductivity waters in this region are inherently hard. Based on a linear relationship of hardness to conductivity in the West Virginia data, 300 μS/cm conductivity is equivalent to a hardness of approximately 200 mg/L $CaCO_3$. The equivalent hardness–adjusted British Columbia Chronic Water Quality Guideline for Mn is 1.5 mg/L [19]. Dittman and Buchwalter [20] provide the laboratory study with the most directly relevant taxa: aquatic insects from Appalachia. They quantified bioaccumulation and performed biomarker studies that found reduced levels of cysteine and glutathione at 0.10 and 0.50 mg/L, but they saw no overt toxic effects. The most relevant conventional toxicity tests of aquatic invertebrates were 21-d reproduction tests of *Daphnia magna*, which yielded inhibiting concentration 25%

(IC25) values of 5.4 and 9.4 mg/L for hardness levels of 100 and 250 mg/L, respectively [21]. A recent assessment of the Clear Fork watershed, West Virginia, concluded that total Mn at 0.002 −0.50 mg/L was a minor contributor to biotic impairment because Mn was weakly correlated ($r = -0.16$) with the West Virginia Stream Condition Index when corrected for stronger causes [22].

In summary, Fe and Al are clearly not confounders. Equivocal evidence suggests that Mn is potentially a weak confounder.

Summary of actions taken to address potential confounding

The primary means of dealing with confounding is categorization [23]. For example, in epidemiology, it is common to categorize people by gender and age (e.g., child, adult, elderly). In this case, pH is an apparent confounder and, because the problem of concern was associated with circumneutral to alkaline pHs, acidic sites (pH <6) were censored from the data set.

An alternative is to retain the apparent confounders and use multivariate statistics. This offers the opportunity to treat the agent of concern and the confounder as a combined cause if they interact or to statistically partition out the contribution of the confounder if they are independent. However, this requires a large data set that meets the assumptions of the statistical method (e.g., independence, additivity, and normality). It also requires that the contributions of the confounders be sufficiently large relative to the agent of concern and relative to the background variability among sites, sampling events, and analyses. Although habitat and Mn showed signs of being weak confounders, their contributions to multivariate models were too small and the violations of the assumptions of multiple regression were too great for them to be used with any confidence to adjust the conductivity model (Supplemental Data, Tables S13 and S14).

Other potential confounders were eliminated from consideration with confidence. We do not argue that these variables do not cause impairment at some locations in the region. Neither do we argue that they have no influence at all on ionically impaired sites. Rather, given the inevitable variability in sites to which the benchmark would be applied and the relatively strong relationship of conductivity and loss of sensitive genera, the evaluated confounders do not substantially affect the model that is used to develop and apply the conductivity benchmark.

CONCLUSIONS

A weight-of-evidence analysis proved to be effective in analyzing diverse information to determine whether the relationship of ionic strength to an effect on stream invertebrates (loss of ephemeropteran genera) was confounded by specific environmental variables. Quantitative methods are used to generate the individual pieces of evidence, but the combining of heterogeneous evidence is inevitably qualitative. However, the weighing method used in the present study is defensible because it is transparent and, as far as possible, uses a consistent logic, criteria, and scoring rules.

Too often, analyses of causal relationships in nature are conducted without a serious analysis of the potential for confounding. We believe that the weight-of-evidence method used in the present study provides a flexible yet rigorous means to use available evidence to evaluate confounding. Its utility potentially extends to other applications of causal models derived from field data such as resource management or the development of remedial goals for contaminated sites.

SUPPLEMENTAL DATA

Tables S1–S14. (33 KB DOC).

Acknowledgement—We thank the West Virginia Department of Environmental Protection and G. Pond, U.S. EPA, for providing sampling data. L. Zheng, Tetratech, performed all analyses. Many anonymous and named reviewers improved the quality of the manuscript, including the following individuals: M. Griffith, C. Delos, M. Passmore, J. VanSickle, C. Schmitt, C. Menzie, C. Hawkins, and members of the U.S. EPA Biological Advisory Committee. The following members of the U.S. EPA Science Advisory Board provided careful review, interdisciplinary insights, and encouragement: D. Patten, E. Boyer, W. Clements, J. Dinger, G. Geidel, K. Hartman, R. Hilderbrand, A. Huryn, L. Johnson, T.W. La Point, S.N. Luoma, D. McLaughlin, M.C. Newman, T. Petty, E. Rankin, D. Soucek, B. Sweeney, and R. Warner. We thank U.S. EPA management for its support throughout this project. The present study is based on work supported by the U.S. EPA. The views expressed in this article are those of the authors and do not necessarily represent the views or policies of the U.S. EPA.

REFERENCES

1. Cormier SM, Suter GW II, Zheng L. 2013. Derivation of a benchmark for freshwater ionic strength. *Environ Toxicol Chem* 32:263–271 (this issue).
2. U.S. Environmental Protection Agency. 2011. A field-based aquatic life benchmark for conductivity in central Appalachian streams. EPA 600/R-10/023F. Office of Research and Developemnt, National Center for Environmental Assessment, Washington, DC.
3. Hill AB. 1965. The environment and disease: Association or causation. *Proc R Soc Med* 58:295–300.
4. Cormier SM, Suter GW II, Norton SB. 2010. Causal characteristics for ecoepidemiology. *Hum Ecol Risk Assess* 16:53–73.
5. Jewell NP. 2004. *Statistics in Epidemiology*. Chapman & Hall/CRC, Boca Raton, FL, USA.
6. Pearl J. 2009. *Causality: Models, Reasoning, and Inference*, 2nd ed. Cambridge University Press, Cambridge, UK.
7. Stewart-Oaten A. 1996. Problems in the analysis of environmental monitoring data. In Schmitt RJ, Osenberg CW, eds, *Detecting Environmental Impacts*. Academic Press, New York, NY, USA, pp 109–131.
8. Suter GW II, Cormier SM. 2011. Why and how to combine evidence in environmental assessments: Weighing evidence and building cases. *Sci Total Environ* 409:1406–1417.
9. U.S. Environmental Protection Agency. 2010. The Causal Analysis/Diagnosis Decision Information System (CADDIS). Washington, DC.
10. West Virginia Department of Environmental Protection. 2008. 2008 Standard operating procedures. Watershed Assessment Branch, Charleston, WV, USA.
11. U.S. Environmental Protection Agency. 2004. Draft aquatic life water quality criteria for selenium. EPA 822-D-04-001. Technical Report. Washington, DC.
12. Chapman PM, Adams WJ, Brooks ML, Delos CG, Luoma SN, Maher WA, Ohlendorf HM, Presser TS, Shaw DP, eds. 2010. *Ecological Assessment of Selenium in the Aquatic Environment*. SETA/CRC Press, Boca Raton, FL, USA.
13. Lemly AD. 1993. Guidelines for evaluating selenium data from aquatic monitoring and assessment studies. *Environ Monit Assess* 28:83–100.
14. Swift MC. 2002. Stream ecosystem response to, and recovery from, experimental exposure to selenium. *J Aquat Ecosyst Stress Recovery* 9:159–184.
15. deBruyn AMH, Chapman PM. 2007. Selenium toxicity to invertebrates: Will proposed thresholds for toxicity to fish and birds also protect their prey? *Environ Sci Technol* 41:1766–1770.
16. West Virginia Department of Environmental Protection. 2009. Selenium bioaccumulation among select stream and lake fisheries. Charleston, WV, USA.
17. West Virginia Department of Environmental Protection. 2010. Selenium-induced developmental effects among fishes in select West Virginia waters. Charleston, WV, USA.
18. Luoma SN, Rainbow PS. 2008. *Metal Contamination in Aquatic Environments: Science and Lateral Management*. Cambridge University Press, Cambridge, UK.
19. Ministry of Environment. 2001. Ambient water quality guidelines for manganese. Province of British Columbia, Canada.

20. Dittman EK, Buchwalter DB. 2010. Manganese bioaccumulation in aquatic insects: Mn oxide coatings, molting loss, and Mn(II) thiol scavenging. *Environ Sci Technol* 44:9182–9188.
21. Reimer PS. 1999. *Environmental Effects of Manganese and Proposed Freshwater Guidelines to Protect Aquatic Life in British Columbia*. University of British Columbia, Vancouver, Canada.
22. Gerritsen J, Zheng L, Burton J, Boschen C, Wilkes S, Ludwig J, Cormier SM. 2010. Inferring causes of biological impairment in the Clear Fork Watershed, West Virginia. EPA 600/R-08/146. U.S. Environmental Protection Agency, Cincinnati, OH.
23. Rothman KJ, Greenland S. 1998. *Modern Epidemiology*, 2nd ed. Lippincott, Williams & Wilkins, Philadelphia, PA, USA.

Environmental Toxicology and Chemistry, Vol. 32, No. 2, pp. 296–303, 2013

Printed in the USA
DOI: 10.1002/etc.2055

Water Quality

RELATIONSHIP OF LAND USE AND ELEVATED IONIC STRENGTH IN APPALACHIAN WATERSHEDS

SUSAN M. CORMIER,*† SAMUEL P. WILKES,‡ and LEI ZHENG§
†National Center for Environmental Assessment, U.S. Environmental Protection Agency, Cincinnati, Ohio
‡Tetra Tech, Inc., Charleston, West Virginia, USA
§Tetra Tech, Inc., Owings Mills, Maryland, USA

(*Submitted* 29 *March* 2012; *Returned for Revision* 14 *August* 2012; *Accepted* 20 *September* 2012)

Abstract—Coal mining activities have been implicated as sources that increase stream specific conductance in Central Appalachia. The present study characterized potential sources of elevated ionic strength for small subwatersheds within the Coal, Upper Kanawha, Gauley, and New Rivers in West Virginia. From a large monitoring data set developed by the West Virginia Department of Environmental Protection, 162 < 20–km^2-watersheds were identified that had detailed land cover information in southwestern West Virginia with at least one water chemistry sample. Scatter plots of specific conductance were generated for nine land cover classifications: open water, agriculture, forest, residential, barren, total mining, valley fill, abandoned mine lands, and mining excluding valley fill and abandoned mine lands. Conductivity was negatively correlated with the percentage of forest area and positively associated with other land uses. In a multiple regression, the percentage of area in valley fill was the strongest contributor to increased ionic strength, followed by percentage of area in urban (residential/buildings) land use and other mining land use. Based on the 10th quantile regression, 300 μS/cm was exceeded at 3.3% of area in valley fill. In most catchments, HCO_3^- and SO_4^{2-} concentrations were greater than Cl^- concentration. These findings confirm coal mining activities as the primary source of high conductivity waters. Such activities might be redressed with the goal of protecting sources of dilute freshwater in the region. Environ. Toxicol. Chem. 2013;32:296–303. © 2012 SETAC

Keywords—Conductivity Coal mining Dissolved ions Valley fill Sulfate

INTRODUCTION

Coal mining activities have been implicated as causes of adverse biological effects in Central Appalachia, which are potentially associated with bicarbonate and sulfate salts that increase stream-specific conductance (hereafter referred to as conductivity) [1–6]. The concentration of dissolved ions has been recognized as a key physiological determinant of the distribution of aquatic organisms, but the ionic constituents are also important [7–9]. In freshwater Appalachian streams, 5% of genera are extirpated when ion concentration exceeds 295 μS/cm [10]. One mechanism appears to be the disruption of bicarbonate gradients that maintain pH and ionic homeostasis [8,9]. The predominant ionic mixture that raises conductivity levels above background in Central Appalachia contains much greater concentrations of HCO_3^-/CO_3^{2-}, as well as SO_4^{2-}, Cl^-, Ca^{2+}, and Mg^{2+} [5,10]. In the present study, a regional analysis was undertaken to determine the contribution of different types of land cover to high ionic strength from mixtures of predominately HCO_3^-/CO_3^{2-} and SO_4^{2-} anions.

Mineral extraction in the region has been shown to be a source of predominately HCO_3^-/CO_3^{2-}, Cl^-, and SO_4^{2-} anionic mixture [1,5,6]. Some of these sources include surface and underground coal mining, effluent from coal preparation plants and associated slurry impoundments, and effluent from coal fly ash impoundments [3,11,12]. Besides coal mining, other sources that could increase ionic strength in the study region include road construction, winter road maintenance, treatment of waste water, human and animal waste, scrubbers at coal-fired electric plants, soil amendments and fertilizers, natural gas and coal-bed methane extraction, and construction run–off. The dominant anion associated with these sources, however, is Cl^- (Table 1) [12–14].

The present paper is one of a series related to a new method that uses field data to develop water quality benchmark values. The series begins by describing the method for deriving the benchmark [15] and a separate paper applies that method to determine the relationship between dissolved ions and the extirpation of benthic invertebrates [10]. Another paper in this special section explains how to determine whether a field-derived exposure–response relationship is confounded and assesses potential confounders of the conductivity–extirpation relationship [16]. Two papers in this special section first describe a method to determine whether an association observed in the field is causal [17] and then the method is used to show that increased concentrations of ions have caused the extirpation of benthic invertebrates in Appalachian streams [9]. One type of evidence for assessing causes of extirpation of stream invertebrates is to demonstrate that there are sources that can and do increase dissolved ions in Appalachian watersheds. Developing that evidence is the focus of the present paper.

The extent of land use alteration associated with surface mining is greater in Appalachia than anywhere else in the United States [3]. Our analysis of land use/land cover characterizes ionic strength associated with land uses and estimates the relative contribution of surface coal mining as a source of ions in streams. The present study also provides information to estimate expected conductivity levels associated with the percentage of watershed area filled with crushed rock; that is, stream valleys filled with the overburden from blasting and removing mountaintops to access underlying coal. This form of coal mining is called mountain top mining with valley fill construction.

All Supplemental Data may be found in the online version of this article.

* To whom correspondence may be addressed
(Cormier.Susan@epa.gov).

Published online 12 November 2012 in Wiley Online Library
(wileyonlinelibrary.com).

Table 1. Dominant ions associated with different sources

Source	Dominant ions	Reference
Crushed rock (e.g., surface coal mining)[a]	Ca^{2+}, Mg^{2+}, HCO_3^-, Cl^-, SO_4^{2-}	[5,30]
Wastewater treatment plants	Na^+, Cl^-, NH_4^+, NO_3^-, PO_4^{3-}	[31]
Road salt	Na^+, Cl^-, Ca^{2+}, Mg^+	[32]
Salt water intrusion	Na^+, Cl^-	[33]
Produced water from natural gas and coal bed methane production	Na^+, Ca^{2+}, Mg^{+-}, Cl^-, HCO_3^-	[34]
Agricultural runoff	Na^+, NH_4^+, NO_3^-, PO_4^{3-} (irrigation water related ions may vary)	[29]

[a] Deep coal mine discharges can have higher chloride levels compared to those from surface coal mines [30].

METHODS

General approach

Small watersheds were delineated from the upland to the pore point, and the proportions of land cover types were regressed against water quality chemical parameters. Watershed size was limited to <20 km^2 and from headwater to pore point ranged between 1.85 to 19.53 km for the smallest to largest catchment based on stream length. Catchments represent true watersheds [18]. Watershed size was kept small to minimize the variety of land use and cover types within a single watershed, thereby providing a clearer signal for each potential source of ionic strength. Because the region has a long history of mining, however, and land cover information may not include legacy mining, persistent effects of legacy mining are potentially present even when no current record of past or present mining activity exists in publically available land cover databases. Also, buildings and roads are present in areas where

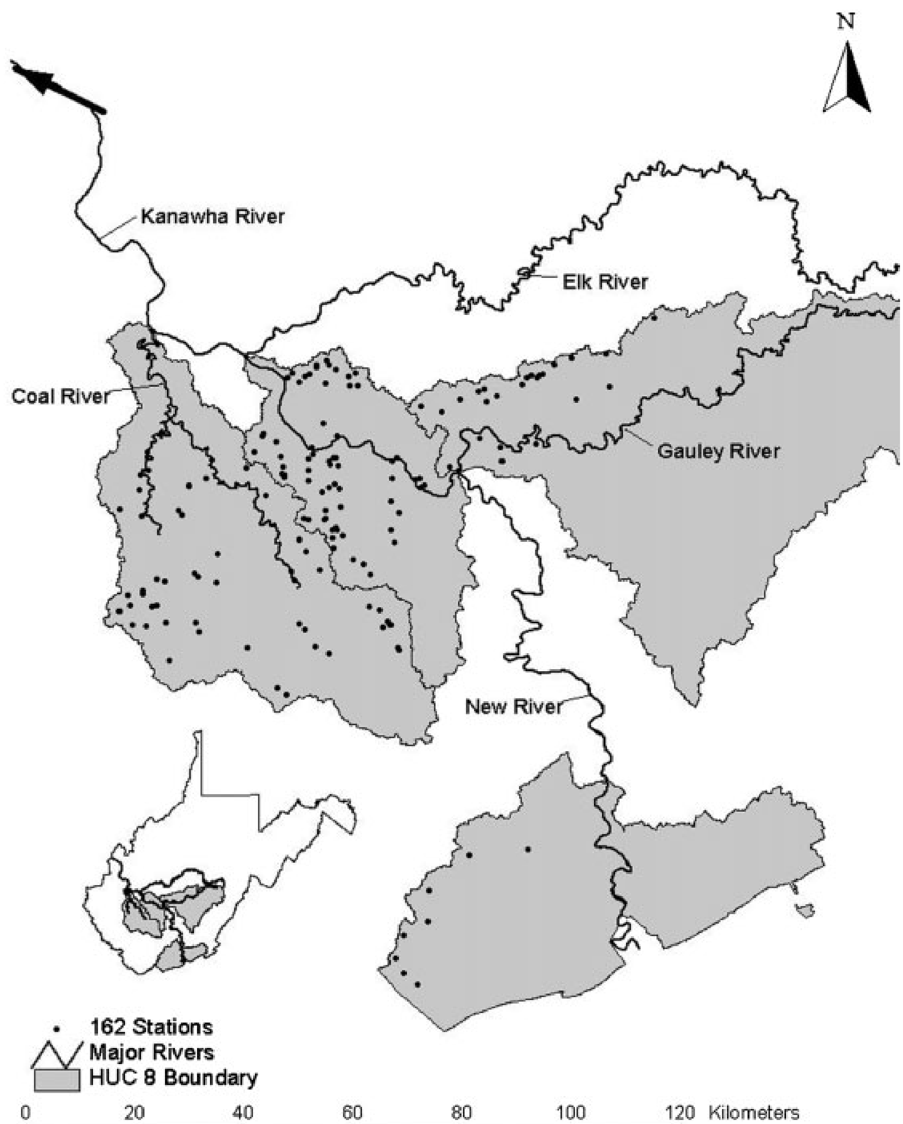


Fig. 1. Sampling locations used to develop evidence of sources of high conductivity inputs. The 162 stations (black dots) at the terminus of each <20–km^2 catchment are shown within the larger 8–digit hydrologic unit catchments (HUC) in southwestern West Virginia. Major rivers are depicted as solid lines draining northward.

mining occurs. Therefore, there are potential influences from multiple sources in most of the 162 watersheds, but these are minimized by using smaller subwatersheds.

These smaller subwatersheds were delineated from the larger basins of the Coal, Upper Kanawha, Gauley, and New Rivers of Ecoregion 69D (Dissected Appalachian Plateau) in West Virginia (Fig. 1) [19]. Selecting the subwatersheds was based on the availability of at least one water chemistry sample and detailed land cover information. Water quality parameters were obtained from the West Virginia Department of Environmental Protection's (WVDEP) Watershed Assessment Branch database. The number of water samples from each station varied from 1 to 18 samples (median number of samples was 12 with 161 out of 162 stations sampled at least twice). In a prior analysis [10], we examined the seasonal patterns of conductivity with at least one water quality sample taken in the first and last halves of the year. On average, the mean conductivity between January and June was less than the mean conductivity between July and December. Although we recognized that this would increase variability in our measurement of water chemistries, we chose to maximize the overall sample size and calculated the average of the sample values for each station and compared the average with land uses. Land use information came from many sources (Table 2). No new data were collected for the present study.

Geographical Information Systems (GIS) data descriptions

Numerous GIS data sets are available for the state of West Virginia. One such repository for data, the West Virginia GIS Technical Center (WVGISTC) [20], maintains publicly available shapefiles. The West Virginia Department of Environmental Protection [21] also maintains a publicly available repository of statewide GIS data sets (http://gis.dep.wv.gov/). All relevant GIS metadata are available for the data housed at each repository site. All GIS coverages are in or were converted to universal Transverse Mercator 1983 Zone 17, with the units in meters. Table 2 describes some of the publicly available GIS shapefiles that were originally used to develop base files for WVDEP's program to remediate waters listed as impaired. We used the WVDEP's land cover data from this program as the starting point to select stations for the analyses (Table 3). The area in valley fill is from a 2003 coverage that the WVDEP developed with ground-truthing performed; note that some water samples were taken in 2004. Both the analytical sample and land use data are from a discrete time span (2001 through 2004) and accurately reflect the existing chemistry and land use at that time.

Catchments with available data that met the needs of the analysis involved a multistep selection process that resulted in 162 small watershed catchments. The steps were performed in sequence. From the WVDEP Watershed Assessment Branch database, we selected all small catchments ($\leq 20\,km^2$) located within Ecoregion 69D that were sampled between 2001 and 2004. We then removed sites with an average pH <6, thereby focusing the present study on sources of increased ionic concentrations in the neutral to alkaline range. We selected chemistry stations that coincided with an appropriate scale of land use that were already determined by previous WVDEP efforts. Catchments were located in the Coal, Upper Kanawha, Gauley, and New River watersheds. The total number of water chemistry stations and catchments within Ecoregion 69D is 162 (Fig. 1).

Table 2. Publicly available GIS data used to generate land cover estimates

Source Type	Data description
General sources of land use/land cover information	General West Virginia Universities GIS data repository location [20] was accessed at http://wvgis.wvu.edu/data/data.php General WVDEP's GIS data repository location [21] was accessed available at http://gis.dep.wv.gov/. Coal River TMDL Land use spreadsheet appendix [22] was accessed at http://www.epa.gov/waters/tmdldocs/WV/Coal_AL_DR.pdf. Upper Kanawha River TMDL Land use spreadsheet appendix [23] is was accessed http://www.dep.wv.gov/WWE/watershed/TMDL/grpa/Documents/Upper%20Kanawha/7950_Final_Upper_Kanawha_TMDL_January_2005.pdf. Gauley River TMDL Land use spreadsheet appendix [24] was accessed http://www.dep.wv.gov/wwe/watershed/tmdl/grpc/documents/gauley%202008/_gauley_final_tmdl_report_03_27_08.pdf New River TMDL Land use spreadsheet appendix [25] was accessed at http://www.prp.cses.vt.edu/Reports_11/Timpano-TDSinStreams-2011.pdf.
Base land use/land	GAP land use [35] was accessed from at http://wvgis.wvu.edu/data/dataset.php?ID=62. NLCD 2001 land use [36] was accessed from http://wvgis.wvu.edu/data/dataset.php?ID=269.
Watershed boundary data sets	USGS 8–digit hydrologic unit code boundaries [37] was accessed from http://wvgis.wvu.edu/data/dataset.php?ID=123.
NHD streams	National Hydrography Data Set streams [38] was accessed from http://wvgis.wvu.edu/data/dataset.php?ID=235.
Abandoned mine lines (AML–highwalls) and polygons (AML areas)	West Virginia abandoned mine lands coverages. Highwall mine coverage and AML area [39] was accessed http://wvgis.wvu.edu/data/dataset.php?ID=150.
DMR mining NPDES permits and outlets	WVDEP Office of Mining and Reclamation NPDES permit and outlet coverages [40] was accessed from http://gis.dep.wv.gov/data/omr.html.
Mining related fills, southern West Virginia	WVDEP valley fills coverage from 2003 [41] was accessed from http://gis.dep.wv.gov/data/omr.html.
Mining permit boundaries	WVDEP mining permit boundaries [21] was accessed from http://gis.dep.wv.gov/.
Roads paved	2000 TIGER/Line GIS and WV_roads shapefiles [20,42] was accessed from Available at http://wvgis.wvu.edu/data/data.php and from http://www.census.gov/geo/www/tiger/tiger2k/tgr2000.html.
Roads unpaved	2000 TIGER/Line GIS shapefile and digitized from aerial photographs and topographic maps [20,43] was accessed from http://wvgis.wvu.edu/data/data.php and http://www.census.gov/geo/www/tiger/tiger2k/tgr2000.html.

AML = abandoned mine lines; DMR = Division of Mining Reclamation; GAP = Gap Analysis Program; GIS = geographic information system; NHD = National Hydrography Data Set; NLCD = National Land Cover Database; NPDES = National Pollutant Discharge Elimination System; TMDL = total maximum daily load; USGS = U.S. Geological Survey; WVDEP = West Virginia Department of Environmental Protection

Table 3. Detailed land use category derivation and land use derivation

Detailed WV TMDL land use category	Data source	Base land use from which new source area was subtracted	Land use categories used in scatter plots in Fig. 2
Water	Water—base LU coverage	NA	Water
Wetland	Wetland—base LU coverage	NA	Water
Forest	Forest—consolidated all forested types from base LU coverage	NA	Forest
Grassland	Grassland—base LU coverage	NA	Agriculture
Cropland	Cropland—consolidated all cropland types from base LU coverage	NA	Agriculture
Urban pervious	Urban—consolidated urbanized types from base LU coverage	NA	Urban/residential
Urban impervious	Urban—consolidated urbanized types from base LU coverage	NA	Urban/residential
Barren	Barren—base LU coverage	NA	Barren
Pasture	WVDEP source tracking	New area subtracted from grassland	Agriculture
Paved roads	Roads shapefiles	New area subtracted from urban impervious	Urban/residential
Unpaved roads	Roads shapefiles	New area subtracted from urban pervious	Urban/residential
Revoked mining permits	AML information	New area subtracted from Barren	AML
Abandoned mine land	AML shapefile	New area subtracted from barren	AML
Quarry	Mining shapefile	New area subtracted from barren	Mining
Highwall	AML shapefile	New area subtracted from barren	Mining
Oil and gas	Oil and gas shapefile	New area subtracted from barren	Mining
Surface mine water quality permits	Mining shapefile	New area subtracted from barren	Mining
Surface mine technology permits	Mining shapefile	New area subtracted from barren	Mining
Comingled mine deep ground gravity discharge	Mining shapefile	New area subtracted from barren	Mining
Comingled mine deep ground pump discharge	Mining shapefile	New area subtracted from barren	Mining
Undeveloped surface mine WQ permits	Mining shapefile	New area subtracted from forest	Mining
Undeveloped surface mine technology permits	Mining shapefile	New area subtracted from forest	Mining
Undeveloped comingled mine gravity discharge	Mining shapefile	New area subtracted from forest	Mining
Undeveloped comingled mine pump discharge	Mining shapefile	New area subtracted from forest	Mining
Burned forest	Forestry Dept. information	New area subtracted from forest	Barren
Harvested forest	Forestry Dept. information	New area subtracted from forest	Barren
Skid roads	Forestry Dept. information	New area subtracted from forest	Barren
TMDL land use considers valley fill[a] area as part of the surface mine water quality and technology permit information	WVDEP valley fills coverage from 2003	New area subtracted from mining, barren, and forest, as appropriate	Valley fill

[a] Valley fill land use was not part of the base TMDL land use and was specifically incorporated into the detailed land use analysis.
See Table 2 for the source file.
AML = abandoned mine line; LU = land use; TMDL = total maximum daily load; WQ = water quality; WV = West Virginia; WVDEP = West Virginia Department of Environmental Protection; NA = not available.

Land use analysis

Our land use analysis began by first obtaining the WVDEP's existing electronic land use information in spreadsheet format for the following WVDEP studies: Coal [22], Upper Kanawha [23], Gauley [24], and New River [25]. These land uses were used as the starting point for further analysis. The WVDEP's land uses were created originally by consolidating the available base land use GIS raster files (GAP Analysis Program 2000 or National Land Cover Data 2001) into more general categories and then adding more detailed source land use categories (e.g., mining, oil and gas, and roads) from detailed source information such as permits or field verification. Table 3 contains the land use categories, the data source from which the extent of the area and its location were determined, and the base land use from which any newly created land use categories were subtracted. In brief, nine land use categories were generated: (1) total percentage area in mining (% total mining), which is the sum of % abandoned mine, % valley fill, and % mining; (2) percentage in mountaintop mining valley fill (% valley fill); (3) percentage of abandoned mine lands (% abandoned mine); (4) percentage of mining (% mining), which is % total mining minus % valley fill and % abandoned mine; (5) percentage barren land use (% barren); (6) percentage of residences, buildings, and roads (% urban); (7) percentage in agriculture, pasture and grassland (% agriculture); (8) percentage in forest (% forest); and (9) percentage in open water (% water).

Because the WVDEP land use characterization process has been revised and enhanced over the years, the land use data sets for the Upper Kanawha, Coal, Gauley, and New Rivers were normalized to have equivalent land use classifications. This yielded seven basic land use categories for the 162 sampling stations. The valley fill GIS coverage was then incorporated into the land use characterization by subtracting the valley fill acreage [26] from the mining land use category. If more area was present in the valley fill coverage than was present in the

Table 4. Summary statistics of water quality parameters in the 162 catchments

Parameter	Units	Min	25th percentile	Median	75th percentile	Max	Mean	Valid *n*
Conductivity	μS/cm	5.68	254	473	896.5	3,964	451.8	1,479
Fecal	counts/mL	1	6	50	355	60,000	52.7	1,047
Alkalinity	mg/L	0.02	21.075	52.7	108	710	44.5	1,168
Hardness	mg/L	11	62	159	249	558	111.4	25
Sulfate	mg/L	5	85	187	367	2,915	172.3	1,169
Chloride	mg/L	1.8	2.46	7.2	16	90.2	8.3	19
TSS	mg/L	0.3	3	5	8	1,217	5.9	1,170
Al, total	mg/L	0.02	0.05	0.12	0.41	14.8	0.162	1,170
Al, dissolved	mg/L	0.02	0.02	0.05	0.05	11.3	0.047	1,169
Ca, total	mg/L	1.93	14.1	23.4	58.3	103	23.4	25
Cu, total	mg/L	0.001	0.003	0.003	0.004	0.01	0.003	16
Cu, dissolved	mg/L	0.001	0.003	0.003	0.004	1.91	0.005	19
Fe, total	mg/L	0.02	0.09	0.2	0.48	32.8	0.228	1,170
Fe, dissolved	mg/L	0.02	0.02	0.03	0.06	5.26	0.039	1,165
Mg, total	mg/L	1.28	5.6	18.4	28.9	88.9	12.2	25
Mn, total	mg/L	0.003	0.021	0.09	0.299	27.3	0.096	1,169
Se, total	mg/L	0.001	0.005	0.005	0.005	0.045	0.005	395
Zn, total	mg/L	0.005	0.005	0.01	0.01	0.04	0.01	16
Zn, dissolved	mg/L	0.005	0.008	0.01	0.012	0.726	0.013	19
Flow	ft^3/s	0.004	0.435	1.48	4.58	63.01	1.27	731
Temperature	°C	0.1	8.7	12.7	17.8	30.7	13.2	1,480
pH	standard units	4.4	7.2	7.7	8.0	10.5	7.6	1,479
DO	mg/L	1.22	9.3	10.46	11.8	16.5	10.6	1,475

TSS = total suspended solids; Mean = geometric mean except for temperature, pH, and dissolved oxygen (DO).

original mining area for each subwatershed, the remainder was subtracted from forest. The nine land use categories calculated for each of the 162 Watershed Assessment Branch database sampling stations used seven categories consolidated from the land use (Table 3) and then included the addition of the valley fill area. The % total mining category is the sum of the % mining, % valley fill, and % abandoned mine land categories. The % mining land use represents all known types of mining activities minus % abandoned mine and % valley fill areas.

Water quality data correspond to land use percentages at the time of sampling from 2001 through 2004. Mining areas present at the time of land use characterization are based on active mining permits obtained from the WVDEP. Subsequent permits that became active after the land use characterizations were developed are not used in our analysis because using them would over estimate mining land use and would not accurately reflect the condition of the water quality at the time of sampling.

Statistical analysis

Summary statistics were computed for physical–chemical variables. Environmental variables were logarithm-transformed as appropriate to obtain normal distributions.

The percentage of catchment area for nine land cover classes were determined including open water (% water), agriculture (% agriculture), forest (% forest), urban/residential/buildings (% urban), barren (% barren), total mining (% total mining), valley fill (% valley fill), abandoned mine lands (% abandoned mine), and mining (% mining) (excluding valley fill and abandoned mine lands). All but one land use class (% forest) were transformed ($\log_{10} + 1$) to normalize the data distribution.

Scatter plots between conductivity and the nine land cover classes were generated and their correlations were estimated using Spearman correlation. The trends of relationships between conductivity and each land use class were visualized using locally weighted scatterplot smoothing (span = 0.75). We also used ordinary least square regression and quantile regression to model the relationship between % valley fill and conductivity levels. Quantile regression and ordinary least square models were used to predict the % valley fill associated with conductivity. A multiple regression model was also evaluated, because multiple predictors, that is, land uses, could contribute to increased ionic strength in streams.

RESULTS

Characterization of catchments and ionic matrix

The 162 small watersheds used in the present analysis are located near the borders of the 8–digit hydrologic unit codes where elevations are greater and headwaters of these small perennial streams are located (Fig. 1). The ionic composition of these waters is not uniform, but bicarbonate and sulfate are usually greater than chloride (Table 4) [5,10]. Because we were interested in all ions as well as the mixture, we did not exclude high Cl^- sites.

Correlations with in–stream water quality parameters

Pairs of land use and anionic water quality parameters from >25 stations and at least one Spearman's correlation coefficient with an $r > |0.50|$ are listed in Table 5.

Table 5. Spearman correlation coefficients between pairs of land use and anionic water quality parameters in the land use data set[a]

Water quality parameter	% Valley fill	% Total mining	% Mining	% Forest	*n*
Conductivity	0.66	0.57	0.42	−0.55	162
Alkalinity	0.47	0.46	0.33	−0.48	123
Sulfate	0.70	0.56	0.41	−0.55	123

[a] Parameters yielding $r < |0.50|$ and sample sizes of less than 30 are not shown.

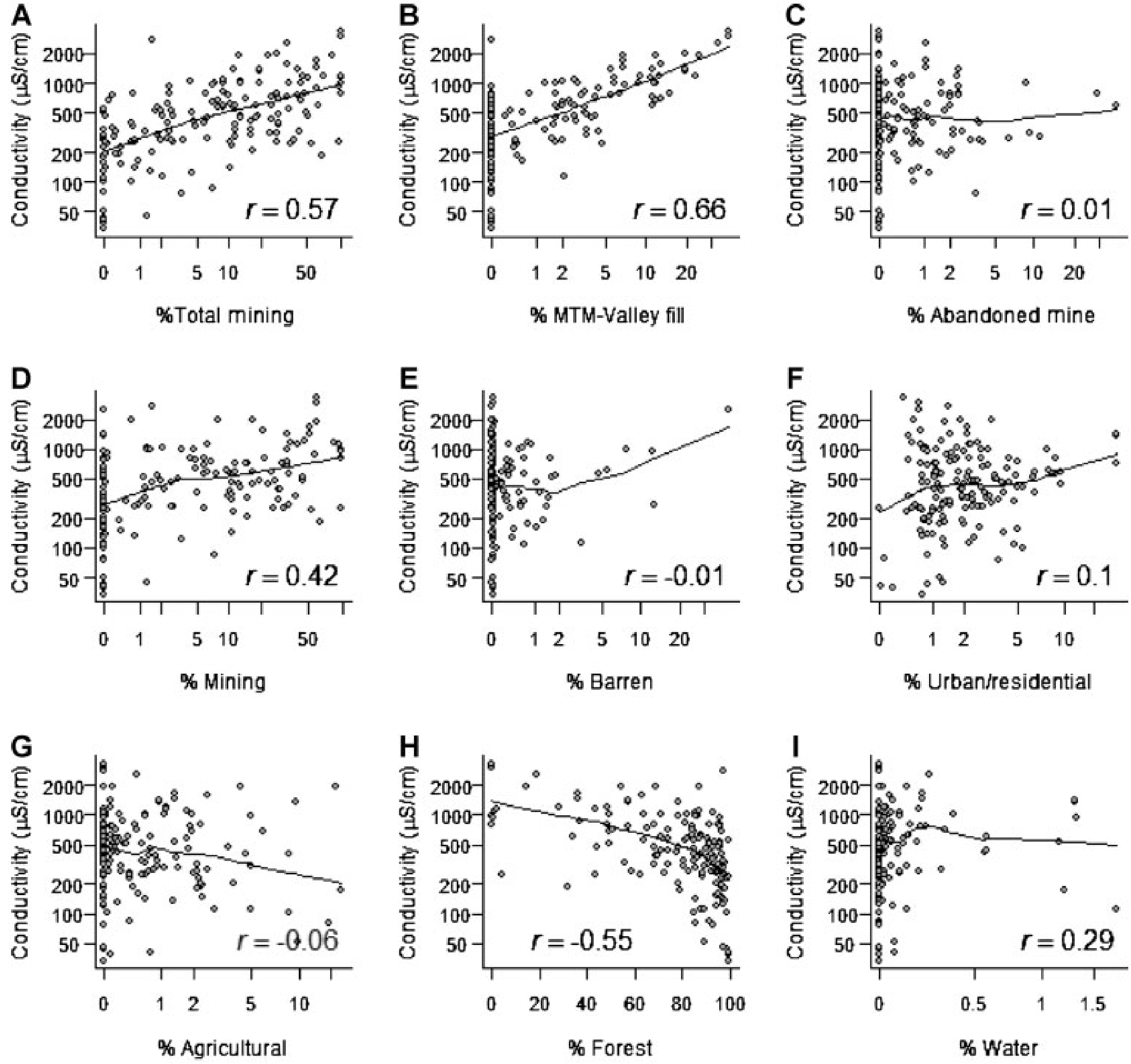


Fig. 2. Geometric mean conductivity associated with different land uses in 162 watersheds in Ecoregion 69D and Spearman's correlation coefficient. Conductivity increases with increasing % valley fill and % total mining, and decreases with increasing % forest, but other land uses are either less clear or show no pattern. From left to right, they are (**A**) % total mining (percentage of deep, surface, quarry mining, valley fill, and abandoned mine land); (**B**) % valley fill (from mountaintop mining [MTM] overburden); (**C**) % abandoned mine; (**D**) % mining (inclusive of all types of mining except valley fill and abandoned mine), (**E**) % barren, (**F**) % urban, (**G**) % agricultural, (**H**) % forest, and (**I**) % water. All but one land use class (% forest) were transformed (log10 + 1) to normalize the data distribution, and were then were transformed back to the original scale to inspect trends conveniently. The fitted lines are the locally weighted scatter plot smoothing lines with span set at 0.75.

Mining-related land uses are strongly correlated with each other and negatively correlated with % forest but not with other land uses. The % total mining is strongly correlated with % mining ($r = 0.89$) and the % valley fill ($r = 0.69$), and negatively correlated with % forest ($r = -0.88$).

At relatively low residential land use, the range of conductivity is highly variable. In contrast, there is a clear pattern of increasing conductivity as percentage of area in valley fill increases and of decreasing conductivity with increasing forest cover (Fig. 2). The scatter plots illustrate that there are clear sources of increased conductivity, but that the percentage of area in valley fill has the strongest correlation with conductivity ($r = 0.66$), and percentage of mining without a valley fill has a moderate correlation ($r = 0.42$). Multiple regression analysis

Table 6. Regression coefficients between % land uses ($\log_{10} + 1$ transformed) and conductivity ($\log_{10}$ transformed) in the land use data set. All % land uses included in the equations are significant at $p = 0.01$ level.

Water quality parameter	Equation	r^2
All data (OLS, $n = 162$)	Cond = 2.42 + 0.58VF	0.45
Samples removed with 0% valley fill (OLS, $n = 78$)	Cond = 2.41 + 0.59VF	0.56
25th quantile regression line	Cond = 2.25 + 0.67VF	
10th quantile regression line	Cond = 2+ 0.75VF	
Multiple regression	Cond = 2.28 + 0.50VF + 0.21Urban + 0.1Mining	0.49

OLS = ordinary least square; VF = valley fill

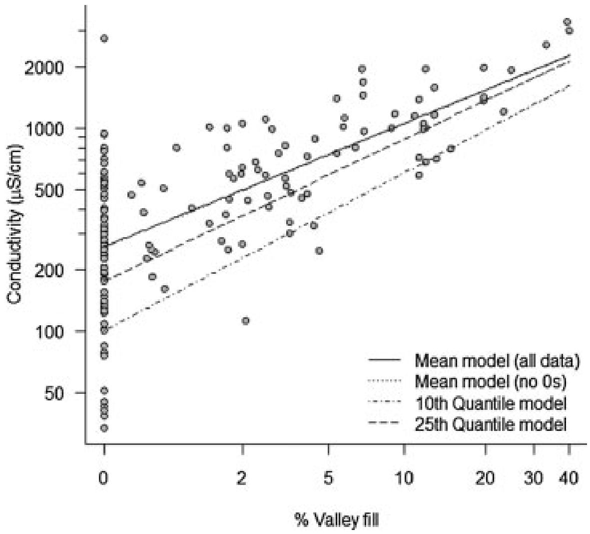


Fig. 3. Ordinary least square regression and quantile regression of percentage of area in valley fill and conductivity in 162 small watersheds in Ecoregion 69D. The percentage of area in valley fill was first log10 + 1 transformed to perform regression analysis and then was transformed back to show the pattern in the original scale. Assuming the lowest conductivity points represent some of the best fill construction practices, the 10th and 25th quantile regression lines are shown. The intercepts for 300 μS/cm are 3.3 and 1.2% valley fill and for 500 μS/cm are approximately 7.6 and 3.7% valley fill for the 10th and 25th quantiles, respectively. The mean model based on samples minus those with 0% valley fill shows that the relationship is unaffected by the removal of sites without valley fills. See Table 6 for formulae for regression lines.

using the three land use categories that could potentially contribute to rising conductivity in streams (% valley fill, % mining, and % urban), showed that all three variables are strong predictors ($p < 0.01$). Notably, the slope of % valley fill is the steepest (0.5), followed by % urban (0.21) and % mining (0.1), indicating that % valley fill has the strongest contribution to ionic strength in these streams (Table 6).

Assuming that the lower conductivity values represent the best achieved with current practice, we modeled the lower 10th and 25th quantile of the percentage of area in valley fill against conductivity (Fig. 3, Table 6). For comparison, the intercept for the 10th and 25th quantile regressions at 300 μS/cm are 3.3% and 1.2% valley fill, respectively. The mean model based on samples minus those with 0% valley fill shows that the relationship is unaffected by removing the sites without valley fills. Because these estimates assesses the percentage of area and do not consider the size of the mined area, volume of the fill, construction practices, distance from the fill, or dilution from tributaries, the estimate of conductivity associated with percentage of valley fill is useful as a general characterization but will vary for specific cases.

CONCLUSIONS

Conductivity typically increases with increasing land use, [27], but in Ecoregion 69D, the densities of agricultural and urban land cover are relatively low and associations of these land uses and conductivity are not strong. In contrast, there are clear patterns of increasing conductivity as the percentage of valley fill area increases and of decreasing conductivity with increasing percentage of forest cover area (Fig. 2). Of the land uses in the small watersheds analyzed in the Upper Kanawha, Coal, Gauley, and New Rivers, mining associated with valley fills is strongly associated with the dissolved ions that are measured as conductivity. Although the presence of buildings (% urban) was also a strong predictor in a multiple linear regression, it is not necessarily the source of increasing ion concentrations in streams. Where there is active mining, there are more buildings. However, the type of ions associated with urban land uses differs (i.e., Cl^- dominated), from that of coal mining land use (i.e., HCO_3^-, Cl^-, and SO_4^{2-} dominated). The present study provides evidence of at least one strong source of high conductivity in the region and is consistent with similar reports [1–6,9,11,28].

The recommended benchmark for ionic strength measured as conductivity is 300 μS/cm [10]. At 300 μS/cm, 5% of genera are extirpated. For comparison, at 500 μS/cm, 17% of genera are extirpated [10]. The 300 μS/cm benchmark has been met in some catchments with valley fills, albeit with a small percentage area in fill (Fig. 3; 0.3% on average and 3.3% at the 10th quantile). Additional analyses is needed to determine if the low conductivity levels at these specific locations are associated with construction design, with the size of the mined area, with dilution from tributaries, or high flow conditions, or other factors.

SUPPLEMENTAL DATA

Table S1. (22 KB DOC)

Acknowledgement—We thank the West Virginia Department of Environmental Protection, Division of Water and Waste Management, Watershed Branch, Total Maximum Daily Load and Watershed Assessment Section for providing their data. We would also like to recognize and thank the numerous field personnel for collecting samples throughout the state. Without their effort, this research would not be possible. We also thank M. McManus, D. Petersen, H. Lattimer, and three anonymous reviewers. The work was supported by the U.S. Environmental Protection Agency. The views expressed in the present paper are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency or any other persons or groups.

REFERENCES

1. Merriam ER, Petty JT, Merovich GT Jr, Fulton JB, Strager MP. 2011. Additive effects of mining and residential development on stream conditions in a Central Appalachian watershed. *J N Am Benthol Soc* 30:399–418.
2. U.S. Environmental Protection Agency. 2011. A field-based aquatic life benchmark for conductivity in Central Appalachian streams. EPA 600/R-10/023F. Office of Research and Development, National Center for Environmental Assessment, Washington, DC.
3. U.S. Environmental Protection Agency. 2011. The effects of mountaintop mines and valley fills on aquatic ecosystems of the Central Appalachian coalfields. EPA 600/R-09/138F. Office of Research and Development, National Center for Environmental Assessment, Washington, DC.
4. Palmer MA, Bernhardt ES, Schlesinger WH, Eshleman KN, Foufoula-Georgiou E, Hendryx MS, Lemly AD, Likens GE, Loucks OL, Power ME, White PS, Wilcock PR. 2010. Environmental and human health consequences of mountaintop removal mining. *Science* 327:148–149.
5. Pond GJ, Passmore ME, Borsuk FA, Reynolds L, Rose CJ. 2008. Downstream effects of mountaintop coal mining: Comparing biological conditions using family- and genus-level macroinvertebrate bioassessment tools. *J N Am Benthol Soc* 27:717–737.
6. Lindberg T, Bernhardt E, Bier R, Helton A, Merola R, Vengosh A, Di Giulio R. 2011. Cumulative impacts of mountaintop mining on an Appalachian watershed. *P Natl Acad Sci USA* 108:20929–20934.
7. Remane A. 1971. Ecology of brackish water. In Remane A, Schlieper C, eds, *Biology of Brackish Water*, 2nd ed. John Wiley and Sons, New York, NY, USA.
8. Bradley TJ. 2009. *Animal Osmoregulation*. Oxford University Press, New York, NY, USA.

9. Cormier SM, Suter GW II, Pond GJ, Zheng L. 2012. Assessing causation of the extirpation of stream macroinvertebrates by a mixture of ions. *Environ Toxicol Chem* 32:277–287 (this issue).
10. Cormier SM, Suter GW II, Zheng L. 2012. Derivation of a benchmark for freshwater ionic strength. *Environ Toxicol Chem* 32:263–271 (this issue).
11. Pond GJ. 2004. Effects of surface mining and residential land use on headwater stream biotic integrity in the eastern Kentucky coalfield region. Kentucky Department of Environmental Protection, Division of Water, Frankfort, KY, USA.
12. Ziegler R, Suter GW II, Kefford BJ, Schofield K, Pond GJ. 2007/2010. Ionic strength. In Suter GW II, ed, *Causal Analysis/Diagnosis Decision Information System (CADDIS)*. Vol 2: Sources, Stressors and Responses. U.S. Environmental Protection Agency, Washington, DC.
13. Environment Canada and Health Canada. 2001. Canadian Environmental Protection Act, 1999. Priority substances list assessment report: Road salts. En40-215/63E, Ottawa, Ontario.
14. Evans M, Frick C. 2002. The effects of road salts on aquatic ecosystems. Contribution No 02 308. National Water Research Institute, Saskatoon, Saskatchewan.
15. Cormier SM, Suter GW, II., 2012. A method for deriving water quality benchmarks using field data. *Environ Toxicol Chem* 32:255–262 (this issue).
16. Suter GW II, Cormier SM. 2012. A method for assessing the potential for confounding applied to ionic strength in Central Appalachian streams. *Environ Toxicol Chem* 32:288–295 (this issue).
17. Cormier S, Suter GW II. 2012. A method for assessing causation of field exposure-response relationships. *Environ Toxicol Chem* 32:272–276 (this issue).
18. Omernik J, Hughes R, Griffith G. 2011. Common geographic frameworks. EPA/100/R 11/002. U.S. Environmental Protection Agency, Washington, DC.
19. Woods AJ, Omernick JM, Brown DD, Kiilsgaard CW. 1996. Level III and IV ecoregions of Pennsylvania and the Blue Ridge Mountains, the Central Appalachian Ridge and Valley and the Central Appalachians of Virginia, West Virginia, and Maryland. EPA/600/R-96/077. U.S. Environmental Protection Agency, Office of Research and Development, Corvallis, OR, USA.
20. West Virginia GIS Technical Center. 2011. General West Virginia Universities GIS data repository locations. University of West Virginia, Morgantown, WV.
21. West Virginia Department of Environmental Protection. 2011. General WVDEP's data repository locations. WVDEP GIS data sets. State of West Virginia, Charleston, WV, USA.
22. West Virginia Department of Environmental Protection. 2006. Total maximum daily loads for selected streams in the Coal River Watershed. State of West Virginia, Charleston, WV, USA.
23. West Virginia Department of Environmental Protection. 2005. TMDLs for Selected Streams in the Upper Kanawha Watershed. State of West Virginia, Charleston, WV, USA.
24. West Virginia Department of Environmental Protection. 2008. Total Maximum Daily Loads for Selected Streams in the Gauley River Watershed. State of West Virginia, Charleston, WV, USA.
25. West Virginia Department of Environmental Protection. 2008. Total maximum daily loads for streams in the New River Watershed. State of West Virginia, Charleston, WV, USA.
26. Shank M. 2004. Development of a mining fill inventory from multi-date elevation data. *Advanced Integration of Geospatial Technologies in Mining and Reclamation Conference*. December 7-9, 2004. Atlanta, GA.
27. Herlihy AT, Stoddard JL, Johnson CB. 1998. The relationship between stream chemistry and watershed land cover data in the mid-Atlantic region, U.S. *Water Air Soil Poll* 105:377–386.
28. Timpano T, Schoenholtz S, Zipper CD, Soucek D. 2011. *Levels of Dissolved Solids Associated with Aquatic Life Effects in Headwater Streams of Virginia's Central Appalachian Coalfield Region*. Virginia Tech, Blacksburg VA, USA.
29. Bernot MJ, Tank JL, Royer TV, David MB. 2006. Nutrient uptake in streams draining agricultural catchments of the Midwestern United States. *Freshwater Biology* 51:499–509.
30. Callaghan T, Brady K, Chisholm W, Sames G. 2000. Hydrology of the Appalachian bituminous coal basin. In Kleinmann R, ed, *Prediction of Water Quality at Surface Coal Mines*. Acid Drainage Technology Initiative. West Virginia University, Morgantown, WV, USA, pp 36–72.
31. Carey RO, Migliaccio KW. 2009. Contribution of wastewater treatment plant effluents to nutrient dynamics in aquatic systems: A review. *Environ Manage* 44:205–217.
32. Forman RTT, Alexander LE. 1998. Roads and their major ecological effects. *Annu Rev Ecol Syst* 29:207–231.
33. Mulrennan ME, Woodroffe CD. 1998. Saltwater intrusion into the coastal plains of the Lower May River, Northern Territory, Australia. *J Environ Manage* 54:169–188.
34. U.S. Department of Energy. 2004. A white paper describing produced water from production of crude oil, natural gas, and coal bed methane. National Energy Technology Laboratory, Pittsburgh, PA.
35. West Virginia GIS Technical Center. 2002. Land cover (WV GAP). WV GAP analysis program. University of West Virginia, Morgantown, WV.
36. West Virginia GIS Technical Center. 2001. Land cover (NLCD 2001). West Virginia National Land Cover [database]. University of West Virginia, Morgantown, WV, USA.
37. West Virginia GIS Technical Center. 2004. Watershed boundary data sets (8, 10, and 12 digits). University of West Virginia, Morgantown, WV, USA.
38. West Virginia GIS Technical Center. 2010. Streams – National hydrography data sets. University of West Virginia, Morgantown, WV, USA.
39. West Virginia GIS Technical Center. 1996. Mining – Abandoned mine lands. University of West Virginia, Morgantown, WV, USA.
40. West Virginia Department of Environmental Protection. 2011. Mining permits, point locations. Division of Mining and Reclamation Data. State of West Virginia, Charleston, WV, USA.
41. West Virginia Department of Environmental Protection. 2011. Mining related fills, Southern West Virginia as of September 2003. Division of Mining and Reclamation Data, State of West Virginia, Charleston, WV, USA.
42. U.S. Census Bureau. 2000. 2000 TIGER/Line GIS and WV roads shapefiles. Washington, D.C.
43. U.S. Census Bureau. 2000. TIGER/Line GIS shapefiles aerial photographs and topographic maps Washington, D.C.

Environmental Toxicology and Chemistry, Vol. 32, No. 2, pp. 277–287, 2013

Printed in the USA
DOI: 10.1002/etc.2059

Water Quality

ASSESSING CAUSATION OF THE EXTIRPATION OF STREAM MACROINVERTEBRATES BY A MIXTURE OF IONS

SUSAN M. CORMIER,† GLENN W. SUTER II,*† LEI ZHENG,‡ and GREGORY J. POND§
†National Center for Environmental Assessment, U.S. Environmental Protection Agency, Cincinnati, Ohio
‡Tetra Tech, Owings Mills, Maryland, USA
§U.S. Environmental Protection Agency, Region III, Wheeling, West Virginia, USA

(*Submitted* 15 *June* 2012; *Returned for Revision* 23 *July* 2012; *Accepted* 14 *September* 2012)

Abstract—Increased ionic concentrations are associated with the impairment of benthic invertebrate assemblages. However, the causal nature of that relationship must be demonstrated so that it can be used to derive a benchmark for conductivity. The available evidence is organized in terms of six characteristics of causation: co–occurrence, preceding causation, interaction, alteration, sufficiency, and time order. The inferential approach is to weight the lines of evidence using a consistent scoring system, weigh the evidence for each causal characteristic, and then assess the body of evidence. Through this assessment, the authors found that a mixture containing the ions Ca^{+}, Mg^{+}, HCO_3^{-}, and SO_4^{-}, as measured by conductivity, is a common cause of extirpation of aquatic macroinvertebrates in Appalachia where surface coal mining is prevalent. The mixture of ions is implicated as the cause rather than any individual constituent of the mixture. The authors also expect that ionic concentrations sufficient to cause extirpations would occur with a similar salt mixture containing predominately HCO_3^{-}, SO_4^{2-}, Ca^{2+}, and Mg^{2+} in other regions with naturally low conductivity. This case demonstrates the utility of the method for determining whether relationships identified in the field are causal. Environ. Toxicol. Chem. 2013;32:277–287. © 2012 SETAC

Keywords—Epidemiology Conductivity Cause–effect Weight of evidence Macroinvertebrate

INTRODUCTION

Benchmark values that are analogous to conventional laboratory-based water quality criteria can be derived from field studies. An example is the benchmark for conductivity in streams contaminated by leachates of crushed rocks [1,2]. However, because observed relationships between exposure and response are not necessarily causal, a method has been developed for assessing their causality [3]. The method uses a criterion-guided weight-of-evidence process that includes a set of characteristics of causation and a formal weighting method applied in six steps. In the present study, we apply that method to demonstrate its utility and to determine the causal nature of the exposure–response relationship used to derive the conductivity benchmark. Other potentially causal factors and their potential to confound the relationship are assessed in a companion article [4]. In the article evaluating confounding, we found that the model used to determine the conductivity benchmark is not appreciably affected by other co-occurring stressors. Our concern in the present study is whether elevated ionic concentrations are a cause of the extirpation [5] of macroinvertebrates, and not whether an ionic mixture is the cause in a particular stream or the only cause occurring in the streams represented in the data sets. (Note that the concentration of a defined mixture of ions is the cause, but specific conductivity, hereafter referred to as conductivity, is the exposure metric.) By extirpation, we mean the depletion of a population of a genus to the point that it is no longer a viable resource or is unlikely to fulfill its function in the ecosystem [5].

Our specific hypothesis is that increasing the concentration of ions of Ca^{+}, Mg^{+}, HCO_3^{-}, and SO_4^{-} causes the loss of sensitive invertebrate genera, especially mayflies, from streams. This concern originated from studies of streams below valley fills that contained impaired biotic communities where the degree of impairment was most strongly correlated with conductivity [6,7]. Our analysis uses general knowledge from laboratory experiments and also field observations from streams in Appalachia. Although the effects of elevated ionic concentration on freshwater organisms are well established, most studies have focused on Na^{+} and Cl^{-}, which are associated with marine ecosystems or salts from marine deposits [8–11]. However, ionic constituents can be quite different when land disturbance increases ionic concentration. In coal mining areas, the leaching of calcareous overburden results in ionic mixtures containing more HCO_3^{-}/CO_3^{2-} plus SO_4^{-} than Cl^{-} [1,6]. The physiological challenges for organisms are thus quite different [9,11] compared to ionic regulation in Na^{+} and Cl^{-}-rich waters. Furthermore, freshwater streams are naturally very dilute; background conductivities are often less than 100 μS/cm [1], and species have evolved to occupy that niche [9,10], perhaps at the expense of other compensatory mechanisms.

CASE STUDY METHODS

Causal assessment methodology

This causal assessment uses epidemiological methods to assess the general causal hypothesis that increased ionic strength has caused extirpation of stream benthic invertebrates. The method uses all relevant and good-quality evidence in a weight-of-evidence process. The evidence is organized by six characteristics of causation: co-occurrence, preceding causation, interaction, alteration, sufficiency, and time order [12].

All Supplemental Data may be found in the online version of this article.
* To whom correspondence may be addressed (suter.glenn@epa.gov).
Published online 12 November 2012 in Wiley Online Library (wileyonlinelibrary.com).

The causal assessment process is described in a companion article [3] and involves six steps that generate and evaluate whether causation is or is not supported by the body of evidence. A key feature of the process is the weighting and weighing of evidence [3,13]. The evidence is weighted using a system of plus (+) for supporting conductivity as a cause, minus (−) for weakening, and zero (0) for no effect. (Both neutral evidence and ambiguous evidence have no effect on the inference.) A single score is applied to register the logical implication of the relevance of good-quality evidence. Especially strong evidence receives an additional score, based on logical properties (e.g., the effect is inconsistent with the mode of action of the agent) or the quantitative strength of the evidence (e.g., high correlation coefficients). An additional score is lodged if there is consistency among independent studies. A convincing body of evidence requires strong evidence for several characteristics of a causal relationship [3,12].

Assessment endpoints

The entities of concern are benthic macroinvertebrates. The effect is extirpation of genera from streams in their natural range as defined previously [5]. Because the endpoint is the extirpation of multiple genera, a single measurement endpoint is sometimes needed to represent those multiple individual responses. Depending on the type of evidence, different biological measurement endpoints are used. In particular, the number of ephemeropteran genera is used in many of the quantitative analyses because many Ephemeroptera appear to be sensitive (Fig. 1). Also, to the extent that replacement does not occur, the total number of genera is a summary of the consequences of extirpation. The assessment is for general causation [3] of the absence of genera, not for any specific time or location.

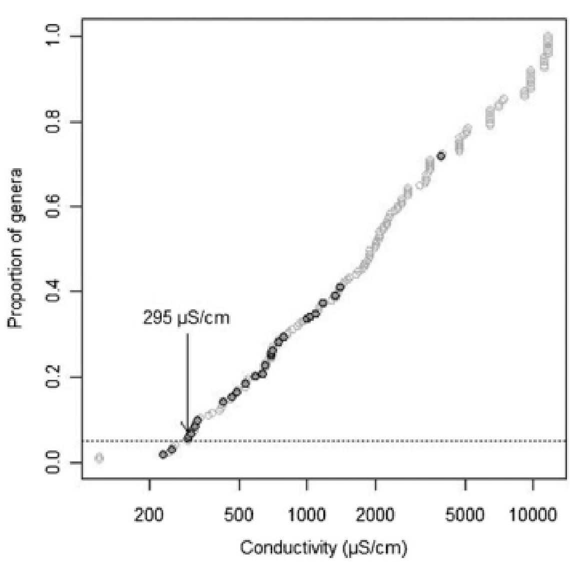


Fig. 1. The genera in the order Ephemeroptera, as a group, are extirpated at lower conductivity levels than many other taxonomic groups. The plot is a species sensitivity distribution (SSD). Open circles represent the 95th percentile extirpation concentration (XC_{95}) for a genus. The closed circles are XC_{95} values for the genera of the order Ephemeroptera. The genus at 230 μS/cm is *Cinygmula* and at 3,923 μS/cm is *Caenis*. Other orders represented in the lower fifth of the SSD include Plecoptera, Trichoptera, and Diptera. Data source: Watershed Assessment Data Base (WABbase).

Data sets

Several sources of field data were used to develop evidence. Field data sets were obtained for West Virginia and Kentucky, USA [2] in Appalachia including ecoregions 68 (Southwestern Appalachia), 69 (Central Appalachia), and 70 (Western Alleghany Plateau) [14]. The Watershed Assessment Database (WABbase), which was obtained from the West Virginia Department of Environmental Protection, is described by Cormier et al. [1] and was used to derive the conductivity benchmark. Additional information sources were used, including (1) toxicity tests from peer–reviewed literature [7]; (2) information on the effects of ionic mixtures on freshwater invertebrates from standard texts and physiological reviews [8–11,15–23]; (3) a U.S. Environmental Protection Agency (U.S. EPA) Region 3 data set from Gregory J. Pond, which includes the original data found in Pond et al. [6] and data collected for a Programmatic Environmental Impact Assessment [24]; (4) data on the composition of Marcellus shale brine from Amy Bergdale, U.S. EPA Region 3, based on analyses by drilling operators; (5) data from the Kentucky Division of Water database [2]; and (6) geographic and related information from the West Virginia Department of Environmental Protection and public sources [1,2].

RESULTS

Pieces of evidence for each characteristic of causation are presented, followed by a summary and scores for evidence of each characteristic.

Co–occurrence

Because causation requires that causal agents interact with unaffected entities, they must co–occur in space and time.

Co–occurrence between conductivity and extirpation of genera. All 163 benthic invertebrate genera appearing in the West Virginia species sensitivity distribution (SSD) list are observed at some sites below 100 μS/cm except *Hydroporus* (lowest occurrence at 168 μS/cm); therefore, low conductivity is not a limiting factor [1,2]. However, 24.5% of genera are never observed at >1,500 μS/cm (Table 1). Among the 10% most susceptible genera are representatives of Trichoptera, Plecoptera, Ephemeroptera, and Diptera, highlighting that many groups are affected.

Scoring—This evidence supports the causal relationship (+); extirpation of 40 genera in West Virginia and 46 in Kentucky in streams with conductivity >1,500 μS/cm is a strong effect (+). The two independent data sets and analyses corroborated one another (+). The total score assigned is + + + (Supplemental Data, Table S1).

Co–occurrence of cause and Ephemeroptera. We constructed a contingency table of the presence/absence of any individual of the order Ephemeroptera at sites near background conductivity (≤200 μS/cm) and high conductivities (>1,500 μS/cm; Table 2). It shows that Ephemeroptera co–occur with low conductivity but that all ephemeropteran species are absent from more than 55% of sites where conductivity is high. This analysis emphasizes the difference between high- and low-conductivity sites with respect to a clear effect endpoint, the absence of all individuals of the order Ephemeroptera.

We repeated the analysis with the U.S. EPA Region 3 data set and the Kentucky data set, with similar results despite the differences in sampling and identification protocols for the Kentucky data set (Table 2). To ensure a sufficient number of samples in these smaller data sets, the low-conductivity

Table 1. After mining or reclamation, specific conductance is greater, and selected metric values are less even though total rapid bioassessment protocol (RBP) [6] habitat scores are similar to unmined conditions[a]

Stream	Sampling year	Specific conductance (μS/cm)	Total genus richness	EPT genus richness	Ephemeropteran genus richness	Total RBP habitat score
Unmined[a]						
Rushpatch	1999	60	42	17	9	147
	2006	70	40	19	7	144
Spring	1999	51	33	17	8	163[b]
	2006	66	37	21	8	149
White Oak	1999	64	32	17	9	161
	2006	88	30	20	8	163
Reclaimed mined[a]						
Ballard	1999	1,201	33	12	3	148
	2006	1,195	20	9	3	149
Stanley Fork	1999	1,387	14	2	0	145
	2006	2,010	28	6	0	155
Sugartree	2000	1,854	22	4	0	141
	2007	1,910	20	4	0	154
Unmined[c]						
Ash Fork	1998	44[a]				
	2003	39[b]	41	20	6	
	2006	51[a]	24	14	4	
	2007	37[a]	27	22	9	
MTM-Valley Fill permit awarded 1994, 1996[c]						
Boardtree	1998	1,396[d]				
	2003	3,015[e]	20	5	2	
	2007	3,390[d]				
MTM-Valley Fill permit awarded 1996[c]						
Stillhouse	1998	511[d]				
	2003	3,199[e]	8	3	0	
	2007	3,970[d]				

[a] Based on Pond et al. [6]. Empty cells indicate data not available.
[b] RBP from Spring 2000.
[c] Identified for this study from the West Virginia Department of Environmental Protection.
[d] Single measurement.
[e] Mean value.
EPT = Ephemeroptera, Plecoptera, Trichoptera; MTM-Valley Fill = mountaintop mining overburden; WABbase = Water Analysis Database; GLIMPSS = genus–level index of most probable stream status; WVSCI = West Virginia Stream Condition Index.

Table 2. Presence of Ephemeroptera contingent on stream conductivity from three data sets[a]

	Ephemeropteran present[b]	Ephemeropteran absent[c]	Total
WVDEP data set—WABbase[d]			
Near background conductivity (≤200 μS/cm)	852 (99.2)	7 (0.8)	859
High conductivity (>1,500 μS/cm)	50 (45)	61 (55)	111
Total	902	68	970
U.S. EPA Region 3 data set[e]			
Conductivity ≤300 μS/cm	7 (100)	0 (0)	7
Conductivity >1,500 μS/cm	4 (19)	17 (81)	21
Total	11	17	28
Kentucky data set[f]			
Conductivity ≤300 μS/cm	150 (97.4)	4 (2.6)	154
Conductivity >1,500 μS/cm	9 (69.2)	4 (30.8)	13
Total	159	8	167

[a] Percentage in parentheses is calculated from the ratio of total observations in a dataset within the designated conductivity range. For example, at least one individual of the order Ephemeroptera was observed in 99.2% (852/859) of sites <200 μS/cm in the WABbase data set.
[b] Sites with >1 ephemeropteran individuals.
[c] Sites with 0 ephemeropteran individuals.
[d] Sampling methods [1].
[e] Sampling methods [6].
[f] Sampling methods [1,37].
WVDEP = West Virginia Department of Environmental Protection; WABbase = Water Analysis Database.

category was <300 μS/cm, and the high-conductivity category was >1,500 μS/cm. In each case, high-conductivity sites were much more likely to lack Ephemeroptera (Table 2).

Scoring—This evidence supports the causal relationship between conductivity and extirpation of genera (+). Where conductivity is high, individuals of the order Ephemeroptera are less likely to occur. A change of 50% or more is large (+). The evidence is corroborated in three independent data sets collected from different streams at different times by different researchers using different sampling protocols (+). The total score assigned is + + + (Supplemental Data, Table S1).

Co–occurrence in nearby catchments. Studies of matched mined and unmined streams provide another way to examine co-occurrence. Pond et al. [6] compared sites in three unmined watersheds with three nearby reclaimed mined watersheds below valleys filled with mountaintop mining overburden (MTM-Valley Fill; Table 3). The conductivity is lower in the unmined sites compared to the reclaimed mined sites, and all of the biological metrics are greater in the unmined sites, even though habitat scores are similar. The number of ephemeropteran genera is two- to threefold greater in the unmined sites. From the WABbase we identified two other valley–filled tributaries, Boardtree and Stillhouse Branch, and one unmined tributary, Ash Fork, in the Twentymile Creek Watershed, West Virginia, USA. The conductivity is lower, and all of the biological metrics are greater in the unmined sites compared to mined sites (Table 3). In a study of 28 watersheds with excellent habitat quality, streams with valley fills had

Table 3. Number of genera contingent on stream conductivity[a]

	West Virginia		Kentucky	
	Genera present	Genera absent	Genera present	Genera absent
Near background conductivity (<150 μS/cm)	162 (99.9)	1 (0.01%)	104 (100%)	0 (0%)
High conductivity ($\geq$1,500 μS/cm)	123 (75.5%)	40 (24.5%)	58 (55.8%)	46 (44.2%)

[a] When conductivity is low, all genera but one are observed, but at high concentrations 24% are absent when 200 specimens are identified (West Virginia, USA) and 44% when all individuals are identified (Kentucky, USA) in a sample. This shows that many genera are affected by ionic stress, not just mayflies, and that low conductivity is not a limiting factor. Percentage in parentheses is calculated from the ratio of total observations in a data set within the designated conductivity range. For example, at least one individual of a genus was observed in 99.9% (162/163) of sites <150 μS/cm in the Watershed Assessment Database (WABbase). Data from WABbase and Kentucky Division of Water database.

greater conductivity and reduced diversity and fewer sensitive genera [25]. These studies show that where conductivity is greater, the biological diversity is less.

Scoring—This evidence supports the causal relationship (+); the number of genera is two to three times greater at the low-conductivity sites for most metrics; few or no Ephemeroptera were observed at three-fourths of the sites (+). The results are consistent and independently corroborated (+). Total score assigned is + + + (Supplemental Data, Table S1).

Preceding causation

Each causal relationship is a result of a web of preceding cause and effect relationships that begins with sources and includes pathways of transport, transformation, and exposure. Evidence of sources of a causal agent increases confidence that the causal event actually occurred and was not a result of a measurement error, chance, or hoax [26].

Complete source-to-cause pathway from the literature. Because exposure to dissolved ions does not require transport or transformation (i.e., organisms are directly exposed to ions in water immediately below sources), only evidence of the occurrence of sources of ionic inputs is assessed for this type of evidence. Potential sources for a mixture of ions HCO_3^-/CO_3^{2-} plus SO_4^- greater than Cl^- in the region include surface and underground coal mining, effluent from coal preparation plants and associated slurry impoundments, effluent from coal fly ash impoundments, scrubbers at coal-fired electric plants, and demineralization of crushed rock [7,23,24,27]. In particular, high-conductivity leachate has been shown to flow from valley fills created during coal mining operations [6,18,24]. In contrast, mixtures are more likely to be dominated by Cl^- when they are associated with winter road maintenance [28,29], brines from natural gas and coalbed methane operations [30], treatment of wastewater [31], and human and animal waste [23,32]. Ecological studies have shown that conductivity increases only slightly following clear–cutting and burning. Dissolved mineral loading may be increased slightly by timber harvesting but also declines quickly as vegetation reestablishes [33]. Golladay et al. [34] and Arthur et al. [35] found increases in nitrogen and phosphorus export in logged catchments in Appalachia but minor differences in calcium, potassium, or sulfate concentrations between logged and undisturbed watersheds. Likens et al. [36] actually found sulfate concentrations to decrease following clear-cutting and experimental suppression of forest growth by herbicides.

Scoring—This evidence from the literature indicates that there are sources of the mixture of dissolved ions that are widespread in the region and can be differentiated from sources of other mixtures (+). Multiple studies are consistent in the description of the ion types associated with different sources (+). Strength is not scored. Total score is + + (Supplemental Data, Table S2).

Co–occurrence of sources and conductivity from the region. Conductivity is shown to increase after the construction of valley fill coal mining operations in two catchments, Boardtree and Stillhouse (Table 3). Conductivity is elevated where surface mining operations occur in a watershed and not in an adjacent unmined watershed (Table 3), and overall concentration of ions is greater in mined watersheds with valley fills than in unmined watersheds [6]. Similar results are reported in mined and unmined sites in Kentucky and in Virginia [25,37]. Principal component analysis sorted mined and residential sites from reference sites primarily on the basis of specific conductance and pH [37].

Scoring—This evidence supports the causal relationship (+). The conductivity at mined sites is 10 to 50 times greater than at unmined sites (+). The source of increased conductivity is independently corroborated and consistent (+). Total score is + + + (Supplemental Data, Table S2).

Characteristic composition of identified sources. Correlation and regression analyses suggest that, in ecoregions 69 and 70, conductivities above 500 μS/cm contain high levels of the ions of Ca^{2+}, Mg^{2+}, HCO_3^-, and SO_4^{2-} [7] (scatterplots in Supplemental Data, Fig. S1a–e), which is consistent with surface coal mining and valley fill sources [6,37]. In the WABbase data set, 98% of the sample sites were characterized by anions with $(HCO_3^- + SO_4^{2-})/Cl^- \geq 1$ [1], and conductivity is less correlated with Cl^- than with the other ions (Table 4). In mined and unmined sites, the dominant cations are Ca^{2+} and Mg^{2+}, and anions are HCO_3^- and SO_4^{2-} [6,38] from largely calcareous geology. This excludes sources dominated by NaCl including saline effluents from human and livestock wastes [23,31,32], road salt [28,29], and produced brines from gas extraction (A. Bergdale, personal communication U.S. EPA, Wheeling WV; Supplemental Data, Table S3) [2,30]. The median difference is very large; 99% of anions are HCO_3^- and SO_4^{2-} in both mined and unmined sites, whereas >99% of the anions are Cl^- in brines from gas extraction in Marcellus shales (Supplemental Data, Table S3). Therefore, this causal assessment relates primarily to mixtures of ions typical of alkaline coal mine drainage and associated valley fill discharges rather than sources that are not coal-related.

Scoring—This evidence supports the causal relationship (+) by showing that there are sources of high conductivity with a consistent matrix of ions. Both mined and unmined sites have similar proportions of Ca^{2+}, Mg^{2+}, HCO_3^-, and SO_4^{2-} but very different concentrations. The difference between the ionic composition of mined watersheds and watersheds with other

Table 4. Spearman rank correlation of stream parameters in WABbase data set[a]

	Conductivity	Alkalinity	Sulfate	Chloride	Hardness	Magnesium	Calcium
Conductivity	1	0.78	0.89	0.64	0.95	0.93	0.92
Alkalinity	0.78	1	0.6	0.56	0.78	0.7	0.79
Sulfate	0.89	0.6	1	0.41	0.85	0.9	0.8
Chloride	0.64	0.56	0.41	1	0.5	0.43	0.5
Hardness	0.95	0.78	0.85	0.5	1	0.96	0.99
Mg	0.93	0.7	0.9	0.43	0.96	1	0.91
Ca	0.92	0.79	0.8	0.5	0.99	0.91	1

[a] This shows that in this region conductivity is most highly correlated with ions other than chloride.
[b] Data set as described in [2] for sites with all seven measurements, $n = 1{,}118$,
WABbase = Water Analysis Database.

sources of ions such as brines is very large (+). The evidence from the WABbase data set and two other Appalachian studies consistently supported the ionic makeup associated with land disturbance, especially surface mining (+). The data for mined and unmined watersheds are from a peer–reviewed publication [6], and the brine values are from reports from extraction permittees in West Virginia (A. Bergdale, personal communication U.S. EPA, Wheeling WV). Although the brine analyses are not peer reviewed, the findings are qualitatively similar to other non–peer–reviewed reports of the makeup of such brines. Total score is + + + (Supplemental Data, Table S2).

Correlation of conductivity with sources. In ecoregion 69 [14], conductivity increased with particular sources based on scatter plots of conductivity for proportions of nine land cover classifications [38]. Data were analyzed from 190 small (<20–km^2) catchments draining to the Coal, Upper Kanawha, Gauley, and New Rivers, West Virginia, USA [38]. The two land-use types, percentage area in valleys filled with mountaintop mining overburden ($r = 0.66$) and the summed percentage area in valley fill, abandoned mine land, and mine land ($r = 0.57$), are most strongly and positively correlated with conductivity. In contrast, percentage area in forest is negatively correlated with ion concentrations ($r = -0.55$). Percentage area in urban/residential ($r = 0.10$) is not well correlated and in this region is confounded somewhat by mining land uses. The ions that are more strongly correlated with percentage area in valley fill are total Ca^{2+} and Mg^{2+} (also captured together as hardness [$r = 0.70$]), HCO_3^- measured as alkalinity ($r = 0.47$), and SO_4^{2-} ($r = 0.70$). Noticeably, Cl^- is not strongly correlated with any land use variable, apparently due to the low range of Cl^- concentrations, except at one site [38].

Only mining, especially associated with valley fills, is a substantial source of the ions that are measured as conductivity [38]. Disturbances associated with agriculture and human habitation may also contribute, but the densities of agricultural and urban land cover are relatively low, and a clear pattern of increasing conductivity and increasing land use is not evident. Furthermore, despite the bedrock of limestone, dolomite, shale, and calcareous cemented sandstone, natural background conductivity is exceedingly low [1] apparently because the native geology is intact and not crushed.

Although conductivity typically increases with increasing land use [29], at relatively low urban land use, conductivity is highly variable [38]. This may be caused by unknown mine drainage, deep mine break–outs, road applications, poor infrastructure conditions (e.g., leaking sewers or combined sewers), or other practices. In contrast, there are clear patterns of increasing conductivity as percentage of area in valley fill increases and decreasing conductivity with increasing forest cover [38].

Scoring—This evidence supports the causal relationship (+). The correlations for percentage area in mountaintop mining with valley fill ($r = 0.66$), all mining minus valley fill and abandoned mine lands ($r = 0.42$), and forestry ($r = -0.55$) [38] are moderately strong based on our a priori scoring criteria [3,39]. The present study has not been independently corroborated, although it is consistent with the findings of Pond et al. [6] and Lindberg et al. [40]. The association seems to be specific for extensive geologic disturbances, which in these regions are from mining and valley fills. The total score is + (Supplemental Data, Table S2).

Interaction and physiological mechanisms

Causal agents alter affected entities by interacting with them through a physical mechanism. Evidence that a mechanism of interaction exists for a proposed causal relationship strengthens the argument for that relationship.

Evidence of mechanism of exposure. Aqueous salts are dissolved ions that are readily available for uptake by aquatic organisms as they pass over their respiratory and other permeable surfaces [8,9,11,17,20,41–43]. Benthic invertebrates that inhabit naturally low-conductivity streams (Table 1) may be subjected to waters that have a greater concentration of ions due to local effluents [2,7]. Therefore, the pollutant is present in a form that is consistent with a well-established mechanism of exposure for aquatic animals.

Scoring—Evidence of a mechanism of exposure is from knowledge that the ions are present in streams [2,7] and from general knowledge of animal physiology and the anatomy of insects and other aquatic invertebrates [8,9,11,17,20,41–43] (+). Because the exposure is by the same mechanism that provides respiration (i.e., maintenance of water flow over permeable membranes), it is strong (+). Many studies support this inference (+). The total score is + + + (Supplemental Data, Table S4).

Biochemical mechanism of effect. Living cells and the organisms they comprise must maintain a relatively narrowly defined internal composition of ions that varies with function and that is different from their environment. Maintaining homeostasis involves osmotic and ionic regulation by cells and tissues. Freshwater organisms, including mayflies, are known to use various physical structures and physiological mechanisms to maintain water content, charge balance, and specific ionic concentrations [8,9,11,17,20,21,41–43]. Many freshwater invertebrates, including mayflies, have mitochondrion–rich chloride cells on gills and other surfaces that take up chloride and other ions [11,17]. Exclusion of ions is insufficient to maintain homeostasis, which requires coordinated anion, cation, and proton transport by passive, active, uniport, and cotransport processes [19].

The clearest mechanism of effects is the inhibition of membrane–transport pathways by excessive ambient concentrations of bicarbonate, which interfere with the uptake and balance of necessary chloride and sodium ions. The processes by which bicarbonate interferes with ion exchange by chloride cells on the gills of Ephemeroptera are illustrated in Supplemental Data, Figure S2.

Scoring—This mechanism supports the causal relationship by providing evidence that the bicarbonate ion matrix in the region can create ionic gradients that interfere with proper homeostasis (+). However, direct observations of the ionic regulatory processes or membrane potential measurements are not described in the literature for affected or tolerant species studied in Appalachia. Evidence from the literature about mitochondrion–rich chloride cells in epithelia of insects (particularly in ephemeropterans), amphibians, and fish, logically leads to disruption of ionic regulation in organisms highly dependent on passive ionic regulation by an HCO_3^-/Cl^- antiport anion exchange. Other ion transport systems are also affected by increases in the concentration of the ion mixture, which is measured as increased conductivity in the region of concern. A large body of peer–reviewed physiological studies [8,9,11,17,20,21,41–43] supports this inference (+). The total score is + + (Supplemental Data, Table S4).

Physiological mechanism of effect. In aquatic systems, organisms are capable of coping with different environmental challenges presented by different concentrations of dissolved ions. However, the extent and rate of adaptation to changes of ionic composition and concentration varies depending on the physiological potential of a particular species [9–11]. As noted previously, osmotic and ionic cellular mechanisms involve selectively permeable membranes. However, it is the disruption of the ionic gradients throughout a physiological system of specialized tissues and organs with specialized functions that determines whether a genus will occur at a location. Some examples include slight or large differences in ionic composition between cell compartments, cells, or external media that are used to release energy from food; transcribe and translate RNA into proteins; regulate pH and water volume; excrete metabolic waste (ammonia and CO_2); enable secretion of enzymes, hormones, and neurotransmitters; guide embryonic development [9,11]; and propagate action potentials in nerves and muscles, thus enabling complex behaviors, and activation of fertilized eggs [9,15,22]. These physiological functions enable organisms to develop, grow, move, and sense their environment. When the pH or ionic gradients are disrupted, stream invertebrates emigrate or die.

Scoring—This evidence supports the causal relationship (+) by demonstrating that the loss of ionic regulation can affect an animal's physiology leading to severe effects. Studies of the physiology of affected species and tolerant species from Appalachia are not available. The effects of ionic disruption are supported by a large body of peer–reviewed physiological studies, some of which are presented above (+). The total score is + + (Supplemental Data, Table S4).

Alteration

A cause alters or changes a susceptible entity. In this case, the alteration is failure to maintain viable populations of sensitive species. Documenting that a change occurs is evidence of causation, but that evidence is much stronger if a specific effect of a cause is characterized. If the specific effect of a cause has no other causes, it can be diagnostic of that cause. Extirpation has many causes, so evidence of alteration is not diagnostic in this assessment but can provide evidence of specificity.

Change of occurrence of genera. Ephemeroptera and Plecoptera do not occur in mesohaline waters, whereas other insect orders do occasionally occur in brackish water [10] (Fig. 1). In an article focusing on Ephemeroptera [6], a nonmetric multidimensional scaling model strongly associated *Cinygmula*, *Drunella*, *Ephemerella*, *Epeorus*, and *Ameletus* with the low-conductivity reference sites and *Stenonema*, *Isonychia*, *Baetis*, and *Caenis* with the higher conductivity sites. These results are consistent with estimated 95th percentile extirpation concentration (XC_{95}) values for those genera [1,2]. The low-conductivity ephemeropteran group of Pond et al. [6] has XC_{95} values between 230 and 591 μS/cm, and the high-conductivity ephemeropteran group has XC_{95} values between 745 and 3,923 μS/cm [1,2]. Another study using data from Kentucky showed similar results [37]; however, there is more uncertainty in this study because habitat alteration may have confounded the relationship with conductivity in that data set. Nevertheless, the relative frequency of the sensitive genera identified in the West Virginia study [6] decreased by more than half at mined sites in Kentucky and, except for *Baetis*, a tolerant genus that was relatively unchanged, the relative frequency of the insensitive genera increased at mined sites with high conductivity. This evidence indicates that some specific genera tend to be consistently less tolerant and other are consistently more tolerant of increased ionic concentrations occurring in the region.

Both the XC_{95} values and the SSD [1,2] demonstrate that a characteristic set of genera, including many Ephemeroptera, was extirpated at relatively low conductivities and another characteristic set was resistant. The relative sensitivities are consistent with the findings of Pond et al. [6] and Pond [37], and with our analyses of data from Kentucky ([2] Appendix G of that report). Genera that are sensitive to high conductivity are similar in Kentucky and West Virginia [2]. Genera that began to decrease in occurrence at levels <500 μS/cm were identified from the fitted lines on generalized additive model plots for West Virginia and for Kentucky [2]. In the WABbase data set, 14 genera with XC_{95} values less than 500 μS/cm also occur in the Kentucky data set. Among these 14 genera, nine (64.3%) have XC_{95} values less than 500 μs/cm in the Kentucky data set. A total of 88 (85%) of the 104 genera in Kentucky used to develop the SSD was also used in the West Virginia SSD. Of these 104 genera, 54 showed declines below 500 μS/cm in at least one data set (44 declined in both data sets, four only in Kentucky, and six only in West Virginia). Therefore, the West Virginia and Kentucky data sets had 44 of 54 genera (81.5%) in common that showed declines below <500 μS/cm.

Scoring—This evidence supports the causal relationship (+) by demonstrating that conductivity greater than background levels causes a consistent set of sensitive genera to be extirpated. The number of genera with similar XC_{95} values (less than 10% difference) in Kentucky and West Virginia with $XC_{95} < 500$ μS/cm is 71.4% and for those with a similar pattern of decline it is 81.5% (+). Multiple studies and data sets confirmed the evidence (+). The total score is + + + (Supplemental Data, Table S5).

Models of change of genera. Empirical models based on macroinvertebrate assemblage composition were used to identify probable causes of biological impairments in a case study in Clear Fork Watershed in West Virginia [44]. Eight weighted averaging regression models were developed and tested using four groups of candidate stressors based on genus–level abundance. The strongest predictive models were for acidic metals (dissolved aluminum) and conductivity, $r^2 = 0.76$ and $r^2 = 0.54$, respectively. These appear to correspond to effects of acidic and calcareous mine drainages, respectively. This study shows that there is a distinct assemblage associated with neutral to alkaline high conductivity that is different from other causes such as those with acid mine sites with toxic levels of dissolved aluminum.

In another approach [44], nonmetric multidimensional scaling and multiple responses were used to examine the separation of dirty reference groups from clean reference groups based on the biological communities observed in the two groups. Four dirty reference groups were identified consisting of sites primarily affected by one of the following stressor categories: dissolved metals (Al and Fe), excessive sedimentation, high nutrients and organic enrichment (using fecal coliform as a surrogate measure of wastewater and livestock runoff), and increased ionic concentration (using sulfate concentration as a surrogate measure). Of the dirty reference groups, the dissolved metals group was significantly different from the other three dirty reference groups ($p < 0.001$). The other three dirty reference groups, although overlapping in ordination space to some extent, were also significantly different from one another ($p < 0.05$). Overall, each of the five reference models (the fifth model was clean reference sites) was significantly different from the others ($p < 0.001$), indicating that differences among stressors, including ionic concentration, apparently led to unique macroinvertebrate assemblages.

In another study with a different data set collected in West Virginia, nonmetric multidimensional scaling was applied to invertebrate genera, and sites were sorted into distinct ordination spaces characterized by low, medium, and high conductivities associated with surface mines with valley fills [6]. A study in Kentucky found similar results [37].

Scoring—This evidence supports the causal relationship (+) by demonstrating that conductivity greater than background levels causes a consistent set of sensitive animals to be extirpated. The prediction was statistically strong (+). The effect is specific enough to clearly separate groups by nonparametric statistical methods in two different data sets. Independent data sets and investigators confirmed that different assemblages of invertebrates occur with different stressors, including neutral–to–alkaline waters with increased concentration of ions (+). The total score is +++ (Supplemental Data, Table S5).

Sufficiency

Because many agents are natural components of the environment (e.g., ions), a causal relationship must show that there are thresholds or patterns of the effect to susceptible entities (e.g., mayflies) associated with the changing magnitude of exposure (e.g., conductivity). In this section, we describe evidence that can be credibly used to evaluate whether the level of ionic concentration is sufficient to cause extirpation. The evidence is primarily from field observations. Several laboratory studies were not used to evaluate sufficiency for the following reasons: (1) the ionic constituents were not similar to those in high conductivity waters in the region of concern; (2) the test species are physiologically tolerant of higher concentrations of ions; or (3) only acutely lethal effects were reported [2]. Such toxicity tests serve to show that ionic mixtures are highly toxic at some levels to some test species, but they do not provide evidence that the levels observed in the streams of the regions were sufficient to cause the extirpation of genera. Although available test results were not useful for this causal assessment, such tests are potentially useful for other causal assessments.

Laboratory tests of reconstituted mine discharges. Kennedy et al. [16] tested simulated coal mine discharge waters in Ohio with the ephemeropteran *Isonychia bicolor*. The ionic matrix was dominated by SO_4^{2-}, HCO_3^-, and Na^+. In 7–d lethality tests, the lowest observed effect concentrations for survival of *Isonychia* (mid–to–late instars) at 20°C occurred at 1,562, 966, and 987 μS/cm in three tests. These values bracket the field-derived XC_{95} for *Isonychia* of 1,180 μS/cm [1,2]. However, when the assay was conducted at 12°C, the lowest observed effect concentration was 4,973 μS/cm, suggesting that longer exposures are needed before effects occur at cold temperatures.

Scoring—The laboratory tests by Kennedy et al. [16] establish that the effect for one insensitive ephemeropteran species, *Isonychia bicolor*, in the laboratory, occurred at a similar conductivity level to that in the field. A total score of + was assigned (Supplemental Data, Table S6).

Field exposure–response relationships of composite metrics. As Hill [45] suggested, a biological gradient in the field suggests that the exposures reach levels that are sufficient to cause effects. Evidence from several studies was evaluated.

Our analyses, using the WABbase data sets, show that as conductivity increases, the total number of genera and the

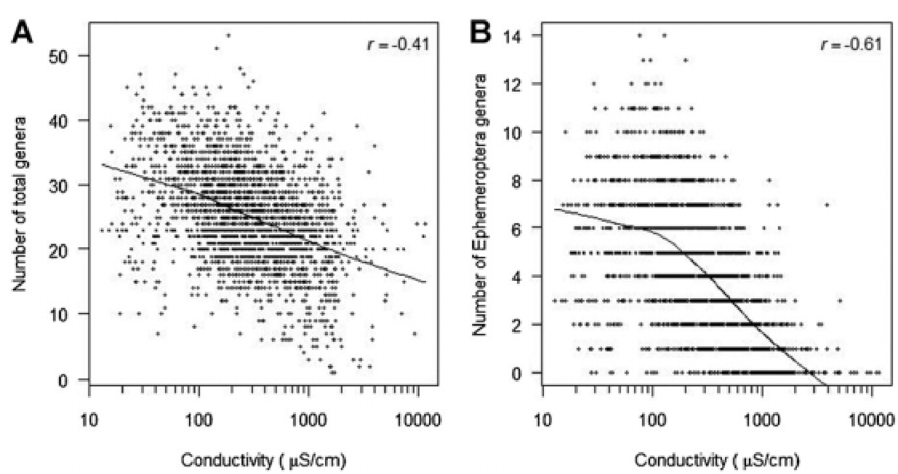


Fig. 2. As conductivity increases, the number of total genera (**A**) and ephemeropteran genera (**B**) decreases. The fitted lines are locally weighted scatterplot smoothing (LOWESS) lines. The LOWESS line fits simple models to subsets of the data, or a span, in this case a change of 0.75 number of taxa or genera. Association with conductivity is evident despite other causes and randomness. Data source: Watershed Assessment Data Base (WABbase).

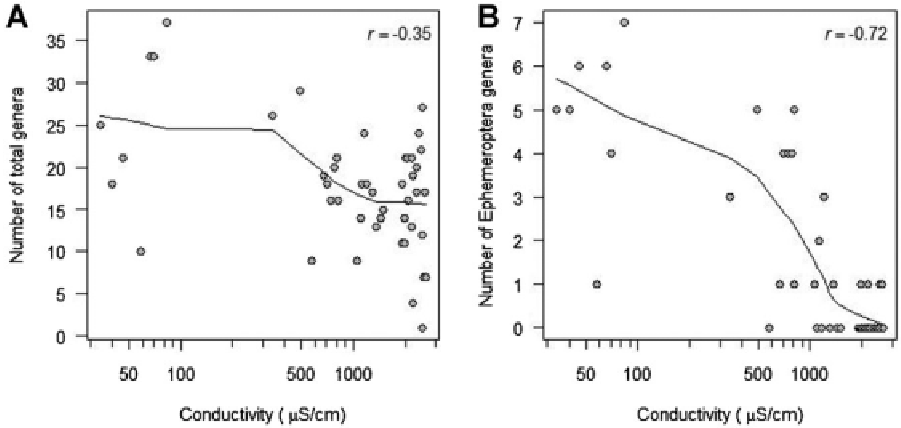


Fig. 3. As conductivity increases, the number of total genera (**A**) and number of Ephemeroptera genera (**B**) decreases. The fitted lines are locally weighted scatterplot smoothing (LOWESS) lines (span = 0.75). Data from EPA Region 3.

number of ephemeropteran genera decrease at conductivity levels shown to extirpate sensitive genera ($r = -0.41$ and -0.61, respectively; Fig. 2). This analysis shows not only the co–occurrence of elevated conductivity and the loss of stream biota but also that there is a regular exposure–response relationship that extends to the lowest observed concentrations (evidence of sufficiency).

In studies of the effects of valley fills in West Virginia by Pond et al. [6], ephemeropteran genera and conductivity were highly negatively correlated ($r = -0.90$) with conductivity and less so with habitat ($r = -0.64$). Pond [37] and Pond et al. [6] also reported that the number of ephemeropteran genera and the number of total genera decrease as conductivity increases. In a recalculation of the Pond et al. [6] data with additional data to create the U.S. EPA Region 3 data set, the ephemeropteran genera and total genera were both moderately negatively correlated with conductivity ($r = -0.72$ and -0.35, respectively; Fig. 3).

In a study in Ohio, Kennedy et al. [46] report that as conductivity increases, the percentage of Ephemeroptera decreases from 23.7% at a mean of 399 μS/cm to 0% at 5,376 μS/cm with intermediate effects between those values. In this study, Na^+ and Ca^{2+} were at similar concentrations at the higher conductivity levels.

In a study of 28 headwater streams in Virginia, Timpano et al. [25] showed that the number of Ephemeroptera, Plecoptera, and Trichoptera (EPT) ($r = -0.70$) and total ($r = -0.64$) genera declined as conductivity increased even when stream habitat was similar.

Scoring—The field observations show that as conductivity increases, the number of Ephemeroptera and total number of genera decrease and, thus, the concentration of ions in streams is sufficient to cause effects (+). The correlation is strong to moderately strong depending on the data set. The effect was specific for the ionic mixture. The correlations were corroborated with independent data sets from different streams sampled by different investigators (+). A total score of + + was assigned (Supplemental Data, Table S6).

Field exposure–response relationships of composite indices. The relationship between conductivity and the West Virginia Stream Condition Index (WVSCI) score, which is a composite of six family level metrics, was also modeled from the WABbase data set [47]. The WVSCI is scored from zero to 100 with a low score indicative of a poorer quality aquatic biological assemblage and poorer stream condition. Mean WVSCI scores from 60 bins were regressed with conductivity (Fig. 4). A strong downward slope of WVSCI was seen with increasing conductivity.

In Pond et al. [6], the genus–level index of most probable stream status and WVSCI scores were strongly correlated with conductivity ($r = -0.90$ and -0.80, respectively). Gerritsen et al. [44] identified 180 μS/cm as a plausible stressor–response threshold and 300 μS/cm as a substantial effects threshold for the association of conductivity and the WVSCI biological index using a data set from the WABbase.

Timpano et al. [25] showed that the Virginia Stream Condition Index score decreased as conductivity increased with ordinary least square regression or quantile regression.

Scoring—This set of evidence indicates that, in multiple data sets and by a variety of biological responses and analytical methods, as conductivity levels observed in the region increase, stream condition decreases, and the assemblage of macroinvertebrates is different from best available reference sites in the region. This is supporting evidence of sufficient ionic concentrations in the streams to cause widespread effects (+). The correlations are strong (+). The correlations were corroborated

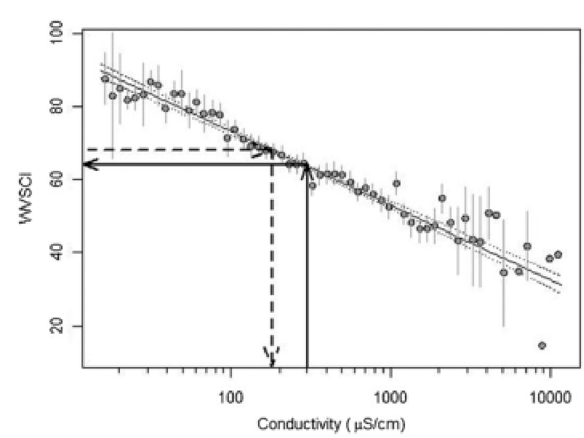


Fig. 4. Stream condition decreases with increasing conductivity. As conductivity increases, the score decreases for a composite index that characterizes stream biota, the West Virginia Stream Condition Index (WVSCI). Points represent mean WVSCI score for conductivity bins. Bars are 90% confidence intervals. The dotted line is the 95% confidence bound for the modeled line. A WVSCI impairment score of 68 intercepts the regression line at 180 μS/cm (dashed arrow). The model estimates a WVSCI value of 64 at 300 μS/cm (solid arrow). Data source: Watershed Assessment Data Base (WABbase).

with different methods in four independent studies (+). A total score of + + + was assigned (Supplemental Data, Table S6).

Field exposure–response relationships: Susceptible genera. As conductivity increases, the occurrence and capture probability decrease for many genera in West Virginia and Kentucky [1,2] at the conductivity levels predicted to cause effects. The loss of these genera is a severe and clear effect.

In the West Virginia data set at 500 μS/cm, 17% of genera (28/163) are extirpated and an additional 68% of genera are declining as conductivity increases at many sites. In the Kentucky data set, 11.5% of genera (12/104) are extirpated at 500 μS/cm, and a total of 67% of genera are in decline. This evidence shows that exposures are sufficient to extirpate susceptible genera in two geographic areas. The associations show that relatively low exposures are sufficient to adversely affect susceptible genera.

Timpano et al. [25] estimated a 20% decline of genera for streams exceeding 652 μS/cm; however, their estimates were based on a small sample size—60 genera with as few as five observations used to estimate the effect. The taxa most sensitive to ionic strength were reported to be ephemeropterans followed by trichopterans.

Scoring—The observed effects logically support the causal relationship between increased conductivity and declining occurrence of susceptible genera and indicate that effects occur at relatively low conductivity levels (+). The effect is strong, with complete extirpation of many genera (+). The results were corroborated with independent data sets from Kentucky and Virginia (+). The total score is + + + (Supplemental Data, Table S6).

Time order

Logically, a causal event occurs before an effect is observed. Evidence of time order could be provided by changes in the invertebrate assemblages after the introduction of a source that increased conductivity.

We could not obtain conductivity and biological survey data collected before and after construction of a valley fill or release of ion-rich effluents from other sources. Hence, this characteristic of causation is scored as no evidence.

Scoring—No evidence.

Conclusions

The evaluation of the body of evidence showed that the available evidence supports a causal relationship between mixtures of matrix ions in streams of ecoregions 68, 69, and 70 and resulting biological impairments. That conclusion is based on evidence showing that the relationship of conductivity to the loss of aquatic genera has the characteristics of causation. The six characteristics of causation are summarized below.

Co–occurrence

Loss of genera occurs when conductivity is high but is rare when conductivity is low (+ + +).

Preceding causation

Sources of the ionic mixture are present and are shown to increase stream conductivity in the region (+ + +).

Interaction

Aquatic organisms are directly exposed to dissolved ions. Based on first principals of physics, ionic gradients in high-conductivity streams would not favor the exchange of ions across gill epithelia. Physiological studies over the last 100 years have documented the many ways that physiological functions of organisms are affected by the relative amounts and concentrations of ions (i.e., combinations of ions that some genera do not have mechanisms or the capacity to regulate; + +).

Alteration

Some genera and other response metrics and assemblages are affected at sites with higher conductivity, whereas others are not. These differences are characteristic of high conductivity (+ + +).

Sufficiency

Laboratory analyses report results of effects for a tolerant species, but test durations and most ionic compositions are not representative of exposure in streams. However, regular increases in effects on invertebrates with increased exposure to ions, based on field observations, indicate that exposures are sufficient (+ + +).

Time order

Conductivity is high and extirpation has occurred after mining permits are issued, but conductivity and biological data before and after mining began are not available (no evidence).

DISCUSSION

We have shown how evidence of causal characteristics was used to determine that increased concentrations of a specific ion mixture are a cause of extirpation of benthic invertebrates in Appalachian streams. Although no analysis can prove that an observed association is causal, the strength of the body of evidence is sufficiently convincing to support action. In particular, it was instrumental for assuring the U.S. EPA that high-conductivity effluents in Appalachia were causing extirpation of aquatic life and that this information provided the best science available for decision making [48,49]. A rigorous assessment using multiple lines of evidence was necessary because assessments that use relationships in field data achieve realism at the potential expense of known causation. No statistical analysis can resolve this problem, because, as we all learned, correlation does not equal causation. That aphorism applies to all statistical analyses of associations between variables because they simply quantify the consistency of the association. Hence, we can only hypothesize causal relationships, refute some, and determine how well the evidence supports others [3]. We believe that this is best done by a consistent and transparent process of weighing the available and relevant evidence [13]. In fact, we believe that most published reports of field studies describe associations and that the present study is one of the few that demonstrates that the relationship is causal.

This causal assessment does not attempt to identify constituents of the mixture that account for the effects. Instead, it shows that the mixture in streams with elevated conductivity and neutral or somewhat alkaline waters in Appalachia can cause and is causing the extirpation of sensitive genera of macroinvertebrates. Laboratory-based physiological evidence suggests that the relative amounts of ions as well as the concentrations of individual ions determine the toxic mechanisms. Failure of HCO_3^--mediated regulation of multiple ions in cells, particularly H^+, Na^+, and Cl^-, is one potential mode of action.

This causal assessment does not compare the relative importance of ionic-induced extirpation of genera in Appalachia with other known stressors in the region such as metal toxicity, stream bed siltation, or eutrophication [7,44]. Instead, it determines that addition of the ionic mixture to streams can and does cause extirpation of aquatic invertebrates [1,2].

Likewise, this assessment does not evaluate how well any model predicts the effects of ionic stress. However, any such model would depend for its causal claims on this assessment.

The causal relationship describes in general how Ephemeroptera and other invertebrates respond to increased concentration of ions in water. A general causal relationship does not require that the species or genera be the same in all applications or at all locations. Therefore, we expect that ionic concentrations sufficient to cause extirpations would occur with a similar salt mixture containing HCO_3^-, SO_4^{2-}, Ca^{2+}, and Mg^{2+} in other regions with naturally low conductivity, because the assessment is of general causation for this salt mixture and susceptible stream invertebrates.

SUPPLEMENTAL DATA

Tables S1–S6.
Figures S1–S2. (957 KB DOC).

Acknowledgement—We thank the West Virginia Department of Environmental Protection for their cooperation and data. Anonymous and named reviewers improved the manuscript: M. Griffith, C. Delos, M. Passmore, J. VanSickle, C. Schmitt, C. Menzie, C. Hawkins, and members of the U.S. Environmental Protection Agency (U.S. EPA) Biological Advisory Committee. The U.S. EPA Science Advisory Board provided review, interdisciplinary insights, and encouragement: D. Patten, E. Boyer, W. Clements, J. Dinger, G. Geidel, K. Hartman, R. Hilderbrand, A. Huryn, L. Johnson, T.W. LaPoint, S.N. Luoma, D. McLaughlin, M.C. Newman, T. Petty, E. Rankin, D. Soucek, B. Sweeney, and R. The article was prepared for publication by D. Kleiser, C. Lewis, S. Moore, and L. Wood of EC Flex, Inc., and L. Kessler, K. Secor, and L. Tackett of IntelliTech Systems. The present study is based on work supported by the U.S. EPA. The views expressed in this article are those of the authors and do not necessarily represent the views or policies of the U.S. EPA.

REFERENCES

1. Cormier SM, Suter GW II, Zheng L. 2013. Derivation of a benchmark for freshwater ionic strength. *Environ Toxicol Chem* 32:263–271 (this issue).
2. U.S., Environmental Protection Agency. 2011. A field based aquatic life benchmark for conductivity in Central Appalachian streams. EPA 600/R-10/023F. Office of Research and Development, National Center for Environmental Assessment, Washington, DC.
3. Cormier S, Suter GW II. 2013. A method for assessing causation of field exposure-response relationships. *Environ Toxicol Chem* 32:272–276 (this issue).
4. Suter GW II, Cormier SM. 2013. A method for assessing the potential for confounding applied to ionic strength in Central Appalachian streams. *Environ Toxicol Chem* 32:288–295 (this issue).
5. U.S., Environmental Protection Agency. 2003. Generic ecological assessment endpoints (GEAEs) for ecological risk assessment. EPA 630/P-02/004B. Risk Assessment Forum, Washington, DC.
6. Pond GJ, Passmore ME, Borsuk FA, Reynolds L, Rose CJ. 2008. Downstream effects of mountaintop coal mining: Comparing biological conditions using family- and genus-level macroinvertebrate bioassessment tools. *J N Am Benthol Soc* 27:717–737.
7. U.S. Environmental Protection Agency. 2011. The effects of mountaintop mines and valley fills on aquatic ecosystems of the Central Appalachian coalfields. EPA 600/R-09/138F. Office of Research and Development, National Center for Environmental Assessment, Washington, DC.
8. Evans DH. 2008. Teleost fish osmoregulation: What have we learned since August Krogh, Homer Smith, and Ancel Keys? *Am J Physiol Reg I* 295:R704–R713.
9. Evans DH. 2008. *Osmotic and Ionic Regulation: Cells and Animals*. CRC, Boca Raton, FL, USA.
10. Remane A. 1971. Ecology of brackish water. In Remane A, Schlieper C, eds, *Biology of Brackish Water*, 2nd ed. John Wiley & Sons, New York, NY, USA.
11. Bradley TJ. 2009. *Animal Osmoregulation*. Oxford University Press, New York, NY, USA.
12. Cormier SM, Suter GW II, Norton SB. 2010. Causal characteristics for ecoepidemiology. *Hum Ecol Risk Assess* 16:53–73.
13. Suter GW II, Cormier SM. 2011. Why and how to combine evidence in environmental assessments: Weighing evidence and building cases. *Sci Total Environ* 409:1406–1417.
14. Woods AJ, Omernik JM, Brown DD, Kiilsgaard CW. 1996. Level III and IV ecoregions of Pennsylvania and the Blue Ridge Mountains, the Ridge and Valley, and the Central Appalachians of Virginia, West Virginia, and Maryland. EPA 600/R-96/077. U.S. Environmental Protection Agency, Office of Research and Development, Corvallis, OR, USA.
15. Hagiwara S, Jaffe LA. 1979. Electrical properties of egg cell membranes. *Annu Rev Biophys Bioeng* 8:385–416.
16. Kennedy AJ, Cherry DS, Currie RJ. 2004. Evaluation of ecologically relevant bioassays for a lotic system impacted by a coal-mine effluent, using Isonychia. *Environ Monit Assess* 95:37–55.
17. Komnick H. 1977. Chloride cells and chloride epithelia of aquatic insects. *Int Rev Cytol* 49:285–328.
18. Merricks TC, Cherry DS, Zipper CE, Currie RJ, Valenti TW. 2007. Coal-mine hollow fill and settling pond influences on headwater streams in southern West Virginia, USA. *Environ Monit Assess* 129:359–378.
19. Nelson DL, Cox MM. 2005. *Lehninger Principles of Biochemistry*, 4th ed. WH Freeman & Co, New York, NY, USA.
20. O'Donnel MJ. 2011. Mechanisms of excretion and ion transport in invertebrates. *Comprehen Physiol.* DOI: 10.1002/cphy.cp130217.
21. Smith DG. 2001. *Pennak's Freshwater Invertebrates of the United States: Porifera to Crustacea*, 4th ed. John Wiley & Sons, New York, NY, USA.
22. Tarin JJ, Cano A. 2000. *Fertilization in Protozoa and Metazoan Animals: Cellular and Molecular Aspects*. Springer Verlag, Berlin, Germany.
23. Ziegler CR, Suter GW II, Ben JK. 2010. Candidate cause: Ionic strength. *CADDIS*, Vol 2: *Sources, Stressors, and Responses*. U.S. Environmental Protection Agency, Washington, DC.
24. Bryant G, Mcphilliamy S, Childers H. 2002. A survey of the water quality of streams in the primary region of mountaintop/valley fill coal mining, October 1999 to January 2001. *Draft Programmatic Environmental Impact Statement on Mountaintop Mining/Valley Fills in Appalachia—2003 Appendix D*. U.S. Environmental Protection Agency, Region 3, Philadelphia, PA, USA.
25. Timpano A, Schoenholtz S, Zipper C, Soucek D. 2011. Levels of dissolved solids associated with aquatic life effects in headwater streams of Virginia's Central Appalachian coalfield region. Virginia Tech, Blacksburg VA, USA.
26. Bunge M. 1979. *Causality and Modern Science*, 3rd revised ed. Dover, New York, NY, USA.
27. Hem JD. 1985. *Study and Interpretation of the Chemical Characteristics of Natural Waters*, 3rd ed. USGS Water Supply Paper 2254. U.S. Government Printing Office, Washington, DC, pp 263.
28. Forman RTT, Alexander LE. 1998. Roads and their major ecological effects. *Annu Rev Ecol System* 29:207–231.
29. Herlihy AT, Stoddard JL, Johnson CB. 1998. The relationship between stream chemistry and watershed land cover data in the mid-Atlantic region, U.S. *Water Air Soil Pollut* 105:377–386.
30. U.S., Department of Energy. 2004. A white paper describing produced water from production of crude oil, natural gas, and coal bed methane. U.S. Department of Energy, National Energy Technology Laboratory, Morgantown, WV.
31. Carey RO, Migliaccio KW. 2009. Contribution of wastewater treatment plant effluents to nutrient dynamics in aquatic systems: A review. *Environ Manage* 44:205–217.
32. Bernot MJ, Tank JL, Royer TV, David MB. 2006. Nutrient uptake in streams draining agricultural catchments of the Midwestern United States. *Freshwater Biol* 51:499–509.
33. Swank WT, Douglass JE. 1977. Nutrient budgets for undisturbed and manipulated hardwood ecosystems in the mountains of North Carolina. In Correll DL, ed, *Watershed Research in Eastern North America: A Workshop to Compare Results*. Smithsonian Institution, Edgewater, MD, USA, pp 343–363.
34. Golladay SW, Webster JR, Benfield EF, Swank WT. 1992. Changes in stream stability following forest clearing as indicated by storm nutrient budgets. *Arch Hydrobiol* 1:1–33.
35. Arthur MA, Coltharp GB, Brown DL. 1998. Effects of best management practices on forest streamwater quality in eastern Kentucky. *J Am Water Resour Assoc* 34:481–495.

36. Likens GE, Bormann FH, Johnson NM, Fisher DW, Pierce RS. 1970. Effects of forest cutting and herbicide treatment on nutrient budgets in the Hubbard Brook watershed ecosystem. *Ecol Monogr* 40:23–47.
37. Pond GJ. 2010. Patterns of Ephemeroptera taxa loss in Appalachian headwater streams (Kentucky, USA). *Hydrobiologia* 641:185–201.
38. Cormier S, Wilkes S, Zheng L. 2012. Relationship of land use and elevated ionic strength in Appalachian watersheds. *Environ Toxicol Chem* 32:296–303 (this issue).
39. Cormier SM, Suter GW II. 2012. A method for assessing the potential for confounding applied to ionic strength in Central Appalachian streams. *Environ Toxicol Chem* 32:288–295 (this issue).
40. Lindberg T, Bernhardt E, Bier R, Helton A, Merola R, Vengosh A, Di Giulio R. 2011. Cumulative impacts of mountaintop mining on an Appalachian watershed. *Proc Natl Acad Sci U S A* 108:20929–20934.
41. Sutcliffe DW. 1962. The composition of Haemolymph in aquatic insects. *J Exp Biol* 39:325–343.
42. Thorp JH, Covich AP. 2001. *Ecology and Classification of North American Freshwater Invertebrates*, 2nd ed. Academic, New York, NY, USA.
43. Wood CM, Shuttleworth TJ. 2008. *Cellular and Molecular Approaches to Fish Ionic Regulation*. Academic, San Diego, CA, USA.
44. Gerritsen J, Zheng L, Burton J, Boschen C, Wilkes S, Ludwig J, Cormier SM. 2010. Inferring causes of biological impairment in the Clear Fork Watershed, West Virginia. EPA 600/R-08/146. U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH, USA.
45. Hill AB. 1965. The environment and disease: Association or causation. *Proc R Soc Med* 58:295–300.
46. Kennedy AJ, Cherry DS, Currie RJ. 2003. Field and laboratory assessment of a coal processing effluent in the Leading Creek Watershed, Meigs County, Ohio. *Arch Environ Contam Toxicol* 44:324–331.
47. Gerritsen J, Burton J, Barbour MT. 2000. A stream condition index for West Virginia wadeable streams. Prepared for the U.S. Environmental Protection Agency by Tetra Tech Inc, Owings Mills, MD, USA.
48. Stoner N, Giles C. 2011. Improving EPA review of Appalachian surface coal mining operations under the Clean Water Act, National Environmental Policy Act, and the Environmental Justice Executive Order. In July 21, 2011 memorandum to Garvin S, Fleming GK, Hedman, S. U.S. Environmental Protection Agency, Washington, DC.
49. Ward KJ. 2011. Big news: EPA finalizes water quality guidance to reduce impacts of mountaintop removal mining. *Coal Tattoo*. The Charleston Gazette, Charleston, WV, USA.
50. Pond GJ, Bailey JE, Lowman B. 2008. West Virginia GLIMPSS (genus-level index of most probable stream status): A benthic macroinvertebrate index of biotic integrity for West Virginia's wadeable streams. West Virginia Department of Environmental Protection, Division of Water and Waste Management, Watershed Branch, Charleston, WV, USA.

00/3-77-042
1977

Ecological Research Series

SUSPENDED AND DISSOLVED SOLIDS EFFECTS ON FRESHWATER BIOTA
A Review

UNITED STATES · ENVIRONMENTAL PROTECTION AGENCY ·

Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Corvallis, Oregon 97330

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into nine series. These nine broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The nine series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies
6. Scientific and Technical Assessment Reports (STAR)
7. Interagency Energy-Environment Research and Development
8. "Special" Reports
9. Miscellaneous Reports

This report has been assigned to the ECOLOGICAL RESEARCH series. This series describes research on the effects of pollution on humans, plant and animal species, and materials. Problems are assessed for their long- and short-term influences. Investigations include formation, transport, and pathway studies to determine the fate of pollutants and their effects. This work provides the technical basis for setting standards to minimize undesirable changes in living organisms in the aquatic, terrestrial, and atmospheric environments.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

EPA-600/3-77-042
April 1977

SUSPENDED AND DISSOLVED SOLIDS EFFECTS

ON FRESHWATER BIOTA: A REVIEW

by

Darwin L. Sorensen
Margaret M. McCarthy
E. Joe Middlebrooks
Donald B. Porcella

Utah State University Foundation
and the
Utah Water Research Laboratory
College of Engineering
Utah State University
Logan, Utah 84322

Project Officer

Jack H. Gakstatter, Chief
Special Studies Branch
Corvallis Environmental Research Laboratory
Corvallis, Oregon 97330

CORVALLIS ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U. S. ENVIRONMENTAL PROTECTION AGENCY
CORVALLIS, OREGON 97330

DISCLAIMER

FOREWORD

Effective regulatory and enforcement actions by the Environmental Protection Agency would be virtually impossible without sound scientific data on pollutants and their impact on environmental stability and human health. Responsibility for building this data base has been assigned to EPA's Office of Research and Development and its 15 major field installations, one of which is the Corvallis Environmental Research Laboratory (CERL).

The primary mission of the Corvallis Laboratory is research on the effects of environmental pollutants on terrestrial, freshwater, and marine ecosystems; the behavior, effects and control of pollutants in lake systems; and the development of predictive models on the movement of pollutants in the biosphere.

This report presents a review of the recent literature describing the effects of suspended and dissolved solids on aquatic organisms.

A. F. Bartsch
Director, CERL

ABSTRACT

It is widely recognized that suspended and dissolved solids in lakes, rivers, streams, and reservoirs affect water quality. In this report the research needs appropriate to setting freshwater quality criteria or standards for suspended solids (not including bedload) and dissolved solids are defined by determining the state of our knowledge from a critical review of the recent literature in this field. Common literature sources and computer searching routines were used as an initial source of information followed by detailed journal searches. Although some 185 journal articles, government reports, and other references were cited herein (about 45 percent published since 1974) and many other reports (about 300 citations) were reviewed, there is a dearth of quantitative information on the response of freshwater biota, especially at the community level, to suspended and dissolved solids.

Consequently, the major research need was defined as the development and/or application of concepts of community response to suspended and dissolved solids concentrations and loads. These concepts need to be applied especially to the photosynthetic level and the microfauna and macrofauna levels. Fish studies are of lower priority since more and better research has been reported for these organisms.

In addition, the role of suspended solids in transporting toxic substances (organics, heavy metals), aesthetic evaluation of suspended solids in aquatic ecosystems and dissolved solids in drinking water, and economic aspects of dissolved solids in municipal-industrial water were defined as research needs.

This report was submitted in fulfillment of Purchase Order No. CC6991630-J by the Utah State University Foundation and the Utah Water Research Laboratory under sponsorship of the U. S. Environmental Protection Agency. This report covers a period from July, 1976 to December, 1976 and work was completed as of January, 1977.

TABLE OF CONTENTS

TABLE OF CONTENTS (CONTINUED)

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGMENTS

Many persons have contributed to the successful completion of the literature review reported herein. The innovation and direction of the project by Jack H. Gakstatter, the project officer, is gratefully acknowledged. The support and extra effort by the Utah Water Research Laboratory staff has helped greatly in the performance of the work. Project business management by the Utah State University Foundation has been expeditiously performed. We gratefully acknowledge the expert assistance of the staff of the Merrill Library at Utah State University. Special recognition is due Mary Cleave who has often volunteered her time and expertise to locate materials included in the review.

SECTION I

CONCLUSIONS

Generally, the review indicates that considerable effort has been directed toward determining the freshwater ecosystem effects of dissolved and suspended solids. However there is a significant gap at the freshwater community level in our understanding of the impacts of these pollutants and research needs are principally related to developing concepts about community response to dissolved and suspended solids.

Specific major conclusions about biological effects of dissolved and suspended solids gained from the reviews were:

1. Acute effects on specific organisms were difficult to demonstrate; succession and/or adaptation can allow communities to be maintained even though specific organisms may differ.

2. The total quantity of dissolved salts and the composition of the ions are both important in terms of organism type selection and productivity. The mode of action of dissolved salts is primarily due to osmotic interactions. Cation and anion ratios seem to have important roles in succession of certain organisms.

3. Dissolved organic compounds frequently increase the availability (and toxicity or biostimulation) of specific elements.

4. Although fishes adapt somewhat to gross changes in salinity, life cycle effects may prevent specific fish from being maintained in a specific aquatic habitat. Osmoregulation is an important aspect of adaptation and biochemical changes (e.g., protein and glucose levels in blood) are evidence of salinity changes.

5. Suspended solids have significant effects on community dynamics when they interfere with light transmission because of turbidity (shading).

6. Suspended solids may have significant effects on succession due to shading, abrasive action, habitat alteration, and sedimentation. Avoidance reactions of fish, selection of species and shading impacts on community stability have been demonstrated.

7. The role of sediments in serving as a reservoir of toxic chemicals has been demonstrated but the quantitative and directional aspects of toxicant transfer are largely unknown and whether the sediments are a sink or a source of toxicants needs to be studied.

8. Relatively high suspended solids were needed to cause behavioral reactions (20,000 mg/l) or death (200,000 mg/l) in a short time in fish. Recovery is fairly rapid when fish were returned to clear water.

SECTION II

INTRODUCTION

SCOPE OF THE REVIEW

The current literature on the effects of suspended and dissolved solids on aquatic living systems is reviewed in this report. Attention was directed to the literature published since 1971 with occasional reference to especially important work on reviews published prior to 1971. The effects of suspended and dissolved solids on freshwater organisms and their habitat was emphasized. Works concerning estuarine or marine life were reviewed only when they were directly relevant or appeared to be the only work available on a given topic.

The effects of suspended and dissolved solids on the physical or chemical environment are included in the review as supportive material for the effects on biological systems. Here again the review was directed primarily toward the literature published since 1971, but was further limited to major or review type publications which had direct reference to the topic. An important recent review on methodologies for assessing streamflow requirements was not included because that report dealt only peripherally with suspended solids and salt effects on biota (Stalnaker and Arnette, 1975). However the reader should be aware that streamflow integrally affects dissolved and suspended solids and that relationship needs consideration.

DEFINITIONS OF SUSPENDED AND DISSOLVED SOLIDS

Natural surface or groundwater is never found as pure H_2O. Separation of the impurities of natural water into particulate and dissolved fractions is, in practice, made on the basis of working definitions such as those found in Standard Methods (APHA, 1975). Suspended solids are the residue in a well mixed sample of water which will not pass a standard (glass fiber) filter. The residue trapped on the filter is dried (103-105C) and reported in units of weight per volume (mg/l). Suspended solids usually impart an optical property to water called turbidity. Particulate matter causes light to be scattered and absorbed rather than transmitted in straight lines. This property (turbidity) can be measured by standardized methods but it cannot be related to weight concentrations of suspended solids because of the effects of size, shape, and refractive index of the particles. However, turbidity measurements do give an indication of the relative abundance of suspended material in a water sample.

Dissolved solids (filterable residue) are the material that pass through a standard (glass fiber) filter and remain after the water has been evaporated and the material dried (180C or 103 to 105C). Salinity is the filterable solids in water after all carbonates have been converted to oxides, all bromine and iodide have been replaced by chloride, and all organic matter have been oxidized. Salinity measurements are usually numerically smaller than dissolved solids measurements (APHA, 1975). Total dissolved solids (TDS) and salinity terminology are often used interchangeably in practice and are not distinguished as to biological or chemical-physical effects in this review.

The ionization of substances dissolved in water allows water to conduct an electric current. The numerical expression of this property is referred to as conductivity. The mobility, valence, and actual and relative concentrations of each of the dissolved ions affect conductivity. Most inorganic acids, bases and salts (e.g., HCl, Na_2CO_3, NaCl, $MgSO_4$) in solution are good conductors. Organic compounds that do not dissociate in aqueous solution are not good conductors. Conductivity is a good method for determining the degree of mineralization of water, for assessing the effect of diverse ions on chemical equilibria, and for determining physiological effects of dissolved ions on plants or animals. The dissolved ionic matter in water may be estimated by multiplying the conductivity (in μmhos/cm) by an empirically determined factor which usually ranges from 0.55 to 0.9 (APHA, 1975).

SOURCES OF SUSPENDED AND DISSOLVED SOLIDS

Natural Sources

Natural weathering and decomposition of rocks, soils, and dead plant materials and the transport or dissolution of the weathered products in water contributes a natural ''background'' of suspended and dissolved materials to natural waters. Even rain and snowfall contain such contaminants which are washed from the atmosphere. Snyder et al. (1975) observed that gross precipitation in a forest in northern Idaho contained a mean suspended solids concentration of 21.8 mg/l. Likens et al. (1970) report an annual mineral dissolved solids export from an undisturbed northern hardwood ecosystem watershed of 13.9 metric tons/km^2. In the Colorado River Basin natural diffuse sources of salt are estimated to contribute 60.5 percent and mineral springs 8 percent of the 36,393 tons (33,084 metric tons) of salt/day exported via that river (USU, 1975, part one). Geologic formations (e.g. exposed marine shales) and other factors of the watershed contribute greatly to natural salt loading of streams (Blackman et al., 1973).

Erosion of soil materials depends greatly on many watershed factors (Bennett, 1974). The protection of undisturbed forest canopies and their mats of detrital material make such forests very resistant to erosion (EPA, 1973; Debyle and Packer, 1972). Snyder et al. (1975) report natural suspended solids concentrations in a northern Idaho forest stream as 2.7 to 9.0 mg/l. Erosion and sedimentation from rangelands is expertly reviewed by Branson et al. (1972). They point out that approximately 40 percent of the world's land surface is classified as rangeland, 80 percent of which is within arid and semi-arid zones. These areas are especially subject to erosion due to extremes in the hydrologic cycle and limited plant cover. Concentrations of suspended solids in the Colorado River have been reported as high as 38,700 mg/l (USU, 1975, part four).

Fletcher et al. (in press) have reviewed the processes contributing to or controlling erosion in arid areas of the western U.S. along with the potential effect that the erosional process has on nitrogen fertility of soils in these areas.

Rural and Agricultural Sources

Sixty-four percent of the land in the U.S. is used for agriculture and silviculture. The major pollutant of water in the U.S. is sediment and it has been estimated that 50 percent or more of the sediment deposited in streams and lakes of the U.S. is contributed by cropland. This amounts to 1.8 billion metric tons of sediment annually. Local values of sediment loading vary widely as to rainfall and rainfall intensity, type of crop, soil characteristics, topography, type of tillage and conservation practices (EPA, 1973). Bowen (1972) points out the pressing need to address water pollution problems associated with runoff and to develop technology for their control. He points out that contrary to the expected dilution effect expected during periods of high flow associated with rainfall, that pollution is often worse during high flows. This suggests a large contribution of pollution due to runoff from land.

A study conducted in eastern South Dakota (Dornbush et al., 1974) measured annual soil losses from agricultural land ranging from < 10 to 1000 lb/acre/yr (< 11 to 1120 kg/ha/yr). Runoff due to rainfall accounted for 93.7 percent of the sediment losses. Most of the sediment loss was from cultivated fields. The bulk of soil losses occurred during short duration, high intensity rain storms. Feedlot runoff waters have been found to contain from 1,000 to 13,400 mg/l suspended solids as well as high levels of other pollutants (Middlebrooks, 1974). Filip and Middlebrooks (1976) observed suspended solids concentrations of approximately 20,000 mg/l in a study to evaluate the eutrophication potential of cattle feedlot runoff. In describing the nonpoint rural sources of pollution in Illinois, Lin (1972) identified suspended solids loading from feedlots as a problem. In her work in the South River Basin in Virginia, Southerland (1974) observed a suspended solids contribution ranging from 3.35 to 29.5 lb/acre/day (3.76 to 33.1 kg/ha/day) during a storm event. She estimated that agriculture, forests, and urban runoff contributed 99.99 percent of the suspended solids during periods of storm flows.

Literature published prior to 1969 dealing with dissolved and suspended solids contributions as well as other pollution problems of irrigation return flows has been reviewed by the USU Foundation (1969). Law and Skogerboe (1972), Blackman et al. (1973), and Branson et al. (1975) have reviewed the effect of irrigation usage of water on dissolved solids content of water. Irrigation water often dissolves mineral salts and organic matter as its flows over and through soils and adds these materials to the stream as it returns as tailwater (runoff) or as groundwater. Oster and Rhoades (1975) have modeled the gain in salt burden of irrigation drainage water due to mineral dissolution by waters from eight rivers used for irrigation in the western U.S. Hagius et al. (in press) observed that irrigation return flows can be inhibitory to algal growth under bioassay conditions.

The Sevier River in central Utah undergoes seven complete stream diversions for irrigation along its 200 mile course, and in the process increases in salinity 20 fold (Law and Skogerboe, 1972). It has been estimated that 15,809 tons/

day (14,372 metric tons/day) or 30.5 percent of the total salt load of the Colorado River is due to irrigation (USU, 1975, part one). Sorensen et al. (1976) have estimated that from zero to more than 35 percent of the salt loading in various subbasins of the Bear River Basin, Idaho-Utah-Wyoming is due to irrigation. Also irrigation can serve to concentrate salinity by removing diluting water from the stream by consumptive use (e.g. evapotranspiration).

King and Hanks (1975) conducted field and laboratory research to determine the effects of irrigation management and fertilizer use upon the quality and quantity of irrigation return flow. The total seasonal discharge of salts from the tile drainage system was directly related to the quantity of water discharged, because the solute concentration of the groundwater was essentially constant over time. Under such conditions, reduction of salt content of return flow is accomplished by reduced drain discharge. Field studies and computer models showed that salts may be stored in the zone above the water table over periods of several years without adversely affecting crop yields on soils with high ‘‘buffering’’ capacity. However, over the long term, salt balance must be obtained. Appreciable amounts of nitrate moved into drainage water at depths of at least 106 cm when commercial fertilizer and dairy manure were applied to the ground surface. Submergence of tile drains in the field reduced nitrate concentrations in the effluent, especially under heavy manure applications.

Urban Runoff and Stormwater Sources

Runoff waters from urban and suburban areas have been observed to contain significant amounts of pollutants. Bryan (1971) found that an urban drainage basin in North Carolina produced runoff that contained an annual load of total organic matter in excess of the load from the sewage treatment plant for the same area. This area produced 43.6 lb/acre/day (49.0 kg/ha/day) of total solids. Sartor et al. (1974) calculated that for a hypothetical city of 100,000 persons and 14,000 acres (5,666 ha) with 400 curb miles (644 km) that street runoff following a one-hour storm would yield 560,000 pounds (254,500 kg) of settleable plus suspended solids/hour. He found that the major constituent of street surface contaminants was inorganic, mineral material similar to common sand and silt. Another study (Whipple et al., 1974) estimated that suspended solids concentration doubled (from 36 mg/l to 74 mg/l) due to runoff from an urban area in New Jersey.

Sources from Forestry Practices

Undisturbed forests are virtually free of erosion, but poorly managed lumbering or forest fires can lead to significant contributions of suspended sediments from forests (EPA, 1973). Deforestation and herbicide treatment of a northern hardwood forest ecosystem (Likens et al., 1970) caused a four fold increase in particulate matter output over that of undisturbed forest. Inorganic materials in the particulates increased from a normal 50 percent to 76 percent. Negligible increases in turbidity were associated with this increase in particulate matter.

Debyle and Packer (1972) working in a Larch-Douglas Fir forest in northern Idaho on plots which had been clearcut and the logging debris broadcast burned, observed a maximum soil erosion of 168 lb/acre/year (189 kg/ha/year). In the third year of study after logging and burning of slash, erosion had been reduced to 15 lb/acre/year (17 kg/ha/year). In four years, vegetal recovery returned conditions to near prelogging status. In one steep denuded area rainfall exceeding two inches (5.1 cm) in 10 hours (0.4 inches [1.0 cm]/hour during one two-hour period) produced ''much'' (no numbers given) of the total of 1,507 pounds (685 kg) of erosion occurring on that plot in the first year after treatment.

Working in the same forest ecosystem in northern Idaho, Synder et al. (1975) found increases in suspended solids in streams on clearcut and burned plots of from 4 to 14 times higher than undisturbed areas. Buffer strips of unlogged areas between the logged and burned area and the stream effectively reduced sediment loading to the streams.

Likens et al. (1970) found a significant increase (from 13.9 to as high as 97 metric tons/km^2) in dissolved solids being exported from the disturbed forest ecosystem at Hubbard Brook. High rates of nitrification of nitrogen from decaying organic matter resulted in increased availability of hydrogen ions which replaced cations on the various exchange sites on the soil making them susceptible to leaching. Since this high rate of salt loss was the result of mining the nutrient capital of the ecosystem (nitrification) it could not be expected to continue indefinitely.

Snyder et al. (1975) found significant increases in electrical conductivity and in most major ions in streams draining clearcut and burned plots in northern Idaho. Here, high runoff yielded low concentrations and low runoff yielded high concentrations of dissolved solids.

Construction and Mining Sources

Construction and mining activities occupy 0.6 percent of the land area of the U.S. Construction activities are responsible for 99.5 percent of the sediment eroded from construction sites (EPA, 1973). Glancy (1973) found that annual sediment yields ranged from 620 to 7,600 tons/mi^2 (218 to 2,670 metric tons/km^2) from developed areas whereas undeveloped areas yielded 60 to 930 tons/mi^2 (21 to 326 metric tons/km^2) in the Lake Tahoe-Incline Village area, Nevada. Goldman (1974) has shown that bacteria associated with these sediments can be important in cycling nutrients which can lead to eutrophication. Construction activities in a development area in Florida disturbed a marsh and lake, and increased suspended solids in water draining from the area (Anderson and Ross, 1975). Here, a 0.28 inch (0.71 cm), 15 minute storm produced 0.178 lb/acre (0.20 kg/ha) of suspended solids. A recent report by the Utah Water Research Laboratory (UWRL, 1976) reviews erosion problems associated with highway construction in the U.S. Methods in use for erosion control during highway construction are reviewed and evaluated, and research needs are identified in the UWRL report.

Dissolved mineral pollutants are of primary importance to the mining industry. Acid mine drainage contributes large amounts of toxic materials to surface waters that have a devastating effect on a local basis. Neutralized acid mine drainage can also be a serious local source of salinity (EPA, 1973).

Dredging and Disposal Sources

In 1972 dredging transferred over 380 million cubic yards of dredge spoils from freshwater and marine sediments (Slotta and Williamson, 1974). A great deal of concern over the effects of the suspended and relocated sediments has been raised, and considerable research has been directed toward assessing potential hazards and developing criteria for reducing the impacts of dredging and disposal operations (Hansen, 1971; Fulk et al., 1975; Lee et al, 1975; Blom et al., 1976; Chen et al., 1976).

Municipal and Industrial Wastewater Sources

Contributions of dissolved and suspended solids from municipal and industrial sources are of concern primarily because of their local impact and composition. Southerland (1974) found that suspended solids contributions from wastewater effluents in the upper South River Basin in Virginia were always overshadowed by loads from runoff sources. However, suspended solids from municipal and industrial effluents such as those from the sugar industry (EPA, 1971), paper manufacture (EPA, 1972), and fish hatcheries (Liao, 1970) are often composed of oxidizable organic matter which can, through biodegradation, reduce the oxygen content of receiving water making it unfit for desirable aquatic life. A large paper manufacturing plant discharging 29 million gal/day (111,000 m^3/day) of treated wastewater discharged approximately 5,000 pounds (2,300 kg) of suspended solids per day (EPA, 1972). Cane sugar manufacture at one plant in Hawaii produced 1,850 pounds (841 kg) of suspended solids for each ton (0.91 metric ton) of sugar produced (EPA, 1971).

Dissolved solids from municipal and industrial effluents are of concern primarily due to their special, often toxic, composition. Biochemical oxygen demand (BOD) due to dissolved organic materials is the problem of most widespread concern. Heavy metals and other dissolved toxic materials also draw special attention to municipal and industrial wastes (McGauhey and Middlebrooks, 1972a; 1972b). Salt loading from municipal and industrial sources is usually not of great importance in a river basin. Municipal and industrial salinity loading in the Colorado River Basin contribute less than 1.7 percent of the total daily salt load (USU, 1975, part one). Consumptive use by municipalities and industries can serve to concentrate salt loads (Blackman, 1973).

COMPOSITION OF DISSOLVED SOLIDS

Inorganic dissolved solids is considered the combination of dissolved salts found in natural water. A summation of the concentrations of the major ions found in water can be and sometimes is used to approximate total dissolved solids (TDS) (APHA, 1975). These major ions are as follows: Sodium (Na^+), potassium (K^+), calcium (Ca^{++}), magnesium (Mg^{++}), carbonate ($CO_3^=$), bicarbonate (HCO_3^-), sulfate ($SO_4^=$), and chloride (Cl^-). The relative abundance of these ions in natural water and the way in which they are contributed varies widely (Hem, 1970; Likens et al., 1970; Snyder et al., 1975).

Organic matter dissolved in water varies greatly as to composition and concentration. Probably of greatest importance on a large scale is the macromolecular humic and fulvic acids and similar compounds which persist in the environment as degradation products of plant materials. These compounds can serve as chelating or complexing agents for metals and nutrients, and have been shown to be effective in solublizing chlorinated hydrocarbons (Wershaw et al., 1969; Blom et al., 1976). Dissolved organic compounds which exert a BOD are of serious local importance to aquatic life. Currently, great effort is being made to remove these compounds from wastewater effluents. However, urban run-off often goes untreated even though it is a significant source of oxygen demanding organic matter (Whipple et al., 1974). Low molecular weight organic compounds are in certain instances very important. V. D. Adams et al. (1975) have found high concentrations of low molecular weight dissolved organic compounds (i.e., acetaldehyde, methanol, ethanol, propanol, acetone, and 2-propanol) in a eutrophic reservoir in northern Utah. Possible sources of these compounds include algal by-products and algal decomposition products.

TYPES OF SUSPENDED SOLIDS

Eroded soils are the most important type of suspended solids on a large scale. Sand, silt, and clay are dislodged by rainfall and overland flow and carried into streams and lakes from rural and agricultural areas, forests, and urban areas (Likens et al., 1970; Bryan, 1971; Lin, 1972; Glancy, 1973; Sartor et al., 1974). Sediment resuspended in the course of the stream (bed load) is also an important type of suspended solids but will not be addressed in this review. A review of bed load effects is being currently prepared for EPA, Region X, by the University of Washington.

Organic suspended particulates compose an important part of suspended solids in most natural waters. Natural detrital material can be dislodged from the soil surface and enter a stream or lake. Likens et al. (1970) reported that 50 percent of the suspended solids being exported from the undisturbed area at Hubbard Brook were organic in nature. Often the less dense organic fraction of soil will be preferentially removed in runoff causing the organic fractions of the suspended solids to actually be enriched (Debyle and Packer, 1972). This organic fraction is often higher in nutrients than the inorganic fraction of the soil (Fletcher et al., in press). The suspended solids washed from feedlots are primarily organic material (Miner et al., 1966). Much of the suspended matter in urban runoff is organic (Bryan, 1971). The organic nature of suspended solids in municipal and industrial effluents has been discussed above.

SECTION III

PHYSICAL-CHEMICAL EFFECTS OF DISSOLVED SOLIDS

EFFECTS ON IRRIGATION WATER QUALITY

A great amount of research dealing with the effects of irrigation water salinity on soils and crops has been accomplished. It is beyond the scope of this report to deal extensively even with the more recent literature pertaining to the subject, but some description of the problems and management solutions is included.

The Committee on Water Quality Criteria (1973) have prepared a good review of water quality considerations for irrigation including crop tolerance to salinity and effects on soils. Methods for dealing with saline and alkaline soils (Richards, 1954) have been reviewed. Problems related to usage of high dissolved solids water in irrigation are usually found in arid and semi-arid areas such as the western U.S. and the middle east. Repeated irrigation with high salinity water in these areas increases the concentration of soluble salts in the soil due to large portions of the applied water being removed by evaporation, leaving the salts behind. High concentrations of salts in the soil solution results in high osmotic pressures which make it difficult for plants to extract water. Soil salinity is usually measured as electrical conductivity of a saturation extract. Salinity levels that may produce yield-limiting soil salinity have been calculated (Branson et al., 1975) and are shown in Table 1. These values are applicable to areas with a climate similar to southern California where soil-solution salinity levels in the active part of the rootzone are commonly threefold more than in the irrigation water due to evapotranspiration.

High ratios of sodium to calcium and magnesium in irrigation water can lead to excessive exchangeable sodium percentages in the soil. Sodium-sensitive plants can be limited in production in even slightly affected soils. Soil structure can be destroyed by excessive exchangeable sodium leading to permeability and aeration problems (Branson et al., 1975). Accumulated salts in the soil solution of soils receiving high dissolved solids irrigation water can be removed by leaching the soil with an excess of irrigation water above that required for evapotranspiration and plant growth. Soil salinity can be leached by rainfall in areas such as India where monsoon rains occur (Lal and Singh, 1973). Drainage waters containing surplus salts leached from irrigated soils may have a several fold increase in salt concentration over that of the irrigation water (Branson et al., 1975).

Bernstein and Francois (1973) have found that crop yield response for alfalfa (*Medicago sativa* L. cv. Sonora) appears to be related to the mean

TABLE 1. SOIL SALINITY LEVELS (EC_e) ASSOCIATED WITH VARIOUS YIELD DECREMENTS (%) AND THE CALCULATED CORRESPONDING IRRIGATION WATER ELECTRICAL CONDUCTIVITIES (EC_w).*

Crop	Yield decrements: 0% EC_e †	0% EC_w †	10% EC_e	10% EC_w	25% EC_e	25% EC_w	50% EC_e	50% EC_w
	mmhos/cm							
Field Crops								
Barley (*Hordeum vulgare*)	8	5.3	12	8	16	10.7	18	12
Sugarbeets (*Beta vulgaris*)	6.7‡	4.5	10	6.7	13	8.7	16	10.7
Cotton (*Gossypium hirsutum*)	6.7	4.5	10	6.7	12	8	16	10.7
Safflower (*Carthamus tinctorius*)	5.3	3.5	8	5.3	11	7.3	14	8
Wheat [*Triticum aestivum* (*T. vulgare*)]	4.7	3.1	7	4.7	10	6.7	14	9.3
Sorghum (*Sorghum vulgare*)	4	2.7	6	4	9	6	12	8
Soybean (*Glycine max*)	3.7	2.5	5.5	3.7	7	4.7	9	6
Sesbania [*Sesbania exaltata* (*S. macrocarpa*)]	2.7	1.8	4	2.7	5.5	3.7	9	6
Rice (Paddy) (*Oryza sativa*)	3.3	2.2	5	3.3	6	4	7	4.7
Corn (*Zea mays*)	3.3	2.2	5	3.3	6	4	7	4.7
Broadbean (*Vicia faba*)	2.3	1.5	3.5	2.3	4.5	3	6.5	4.3
Flax (*Linum usitatissimum*)	2	1.3	3	2	4.5	3	6.5	4.3
Beans (Field) (*Phaseolus vulgaris*)	1	0.7	1.5	1	2	1.3	3.5	2.3
Vegetable Crops								
Beets (*Beta vulgaris*)	5.3	3.5	8	5.3	10	6.7	12	8
Spinach (*Spinacia oleracea*)	3.7	2.5	5.5	3.7	7	4.7	8	5.3
Tomato (*Lycopersicon esculentum*)	2.7	1.8	4	2.7	6.5	4.3	8	5.3
Broccoli (*Brassica oleracea*)	2.7	1.8	4	2.7	6	4	8	5.3
Cabbage (*Brassica oleracea*)	1.7	1.1	2.5	1.7	4	2.7	7	4.7
Potato (*Solanum tuberosum*)	1.7	1.1	2.5	1.7	4	2.7	6	4
Sweet Corn (*Zea mays*)	1.7	1.1	2.5	1.7	4	2.7	6	4
Sweet Potato (*Ipomoea batatas*)	1.7	1.1	2.5	1.7	3.5	2.3	6	4
Lettuce (*Lactuca sativa*)	1.3	0.9	2	1.3	3	2	5	3.3
Bell Pepper (*Capsicum frutescens*)	1.3	0.9	2	1.3	3	2	5	3.3
Onion (*Allium cepa*)	1.3	0.9	2	1.3	3.5	2.3	4	2.7
Carrot (*Daucus carota*)	1	0.7	1.5	1	2.5	1.7	4	2.7
Beans (*Phaseolus vulgaris*)	1	0.7	1.5	1	2	1.3	3.5	2.3
Cantaloupe (*Cucumis melo*)	2.3	1.5	3.5	2.3	No Data		No Data	
Watermelon (*Citrullus lanatus*)	2	1.3	No Data		No Data		No Data	
Forage Crops								
Bermuda Grass (*Cynodon dactylon*)	8.7	5.8	13	8.7	16	10.7	18	12
Tall Wheat Grass (*Agropyron elongatum*)	7.3	4.9	11	7.3	15	10	18	12
Crested Wh. Grass (*Agropyron cristatum*)	4	2.7	6	4	11	7.3	18	12
Tall Fescue (*Festuca arundinacea*)	4.7	3.1	7	4.7	10.5	7	14.5	9.7
Barley (hay) (*Hordeum vulgare*)	5.3	3.5	8	5.3	11	7.3	13.5	9
Perennial Rye (*Lolium perenne*)	5.3	3.5	8	5.3	10	6.7	13	8.7
Harding Grass (*Phalaris tuberosa stenoptera*)	5.3	3.5	8	5.3	10	6.7	13	8.7
Birdsfoot Trefoil (*Lotus corniculatus*)	4	2.7	6	4	8	5.3	10	6.7
Beardless Wild Rye (*Elymus triticoides*)	2.7	1.8	4	2.7	7	4.7	11	7.3
Alfalfa (*Medicago sativa*)	2	1.3	3	2	5	3.3	8	5.3
Orchard Grass (*Dactylis glomerata*)	1.7	1.1	2.5	1.7	4.5	3	8	5.3
Meadow Foxtail (*Alopecurus pratensis*)	1.3	0.9	2	1.3	3.5	2.3	6.5	4.3
Clover (*Trifolium repens*)	1.3	0.9	2	1.3	2.5	1.7	4	2.7

TABLE 1. Continued.

Crop	Yield decrements 0% EC_e †	EC_w †	10% EC_e	EC_w	25% EC_e	EC_w	50% EC_e	EC_w
	mmhos/cm							
				Fruit Crops				
Date Palm (Phoenix dactylifera)	5.3	3.5	8	5.3			16[§]	10[§]
Fig (Ficus carica) Olive (Olea europaea) Pomegranate (Punica granatum)	2.7-4.0	1.8-2.7	4.6	2.7-4.0			9[§]	6[§]
Grape (Thompson) (Vitis venifera)	2.7	1.8	4	2.7			8[§]	5.3[§]
Grapefruit (Citrus paradisi) Orange (Citrus sinensis) Lemon (Citrus limon)	1.7	1.1	2.5	1.7			5[§]	3.3[§]
Apple [Malus pumila (Pyrus malus)] Pear (Pyrus communis)	1.7	1.1	2.5	1.7			5[§]	3.3[§]
Almond (Prunus amygdalus) Apricot (Prunus armeniaca) Peach (Prunus persica) Prune (Prunus domestica)	1.7	1.1	2.5	1.7			5[§]	3.3[§]
Walnut (Juglans regia)	1.7	1.1	2.5	1.7			5[§]	3.3[§]
Blackberry (Rubus sp.) Boysenberry (Rubus ursinus) Raspberry (Rubus sp.)	1.0-1.7	0.7-1.1	1.5-2.5	1.0-1.7			4[§]	2.7[§]
Avocado (Rubus idaeus)	1.3	0.9	2	1.3			4[§]	2.7[§]
Strawberry (Fragaria sp.)	1.0	0.7	1.5	1.0			3[§]	2.0[§]

* From Univ. of Calif. Committee of Consultants report to California State Water Resources Control Board, March 1974, based on USDA-Ag. Inf. Bull. 283 and personal communication with Dr. Leon Bernstein, U.S. Salinity Laboratory, Riverside, Calif.

† EC_e is electrical conductivity of saturation extract in millimhos per centimeter (mmho/cm): EC_w is electrical conductivity of irrigation water (in mmho/cm).

NOTE: Conversion from EC_e to EC_w assumes a threefold concentration of salinity in soil solution (ECsw) in the more active part of the root zone due to evapotranspiration. $EC_w \times 3 = ECsw$; $ECsw \div 2 = EC_e$.

‡ Tolerance during germination (beets) or early seedling stage (wheat, barley) is limited to EC_e about 4 mmho/cm.

§ Calculated values, assuming 50% decrease in yield results from doubling of salinity values for 10% yield decrement.

salinity of the soil water, and that this mean salinity is influenced more by the salinity of the irrigation water than by the salinity of the drainage water. Alfalfa and presumably other plants are affected relatively little when the plants concentrate the soil solution to nearly the limits of tolerance. This indicates that leaching requirements may be reduced from 25 percent to 40 percent of the previously recommended levels depending on salt tolerance of individual species. This would reduce irrigation drainage volume making it more easily treated or diverted from a receiving water. Since the allocation of water between irrigation and leaching can have important bearing on policy decisions in water limited areas, methodologies of reducing the leaching water requirement are important (McFarland, 1975).

EFFECTS OF SALINITY ON THE QUALITY OF DRINKING WATER FOR ANIMALS

The effects of high salinity in livestock drinking water is well reviewed in Water Quality Criteria, 1972 (CWQC, 1973). Effects on animals ranges from mild diarrhea and increased or decreased water consumption in some animals at relatively low concentrations of salt (e.g. 4,000 mg/l total salts) to severe anorexia, anhydremia, and collapse at high concentrations (e.g. 20,000 mg/l NaCl). Table 2 presents a guide to the use of saline waters for livestock and poultry (CWQC, 1973). Effects of salinity in the drinking water of domestic animals would be expected to be similar for wild animals of similar physiology.

Recent work by A. W. Adams et al. (1975) showed that 4,000 ppm sulfate as Na_2SO_4 or $MgSO_4$ significantly depressed feed consumption and hen-day production of laying hens. They also found that Na_2SO_4 significantly increased water consumption and fecal moisture content, while $MgSO_4$ decreased water consumption. Mortality data suggested that lethal levels of Na_2SO_4 and $MgSO_4$ for laying hens were between 16,000 and 20,000 ppm.

Digesti and Weeth (1976) found increased methemaglobin and sulfhemaglobin in beef heifers given water containing 1,250 and 2,500 mg/l sulfate (as Na_2SO_4). Test animals would discriminate against 21 mM ($\sim$ 2000 mg/l) sulfate and reject 34.5 mM ($\sim$ 3300 mg/l) sulfate. Based on these data and the finding that no adverse effects were noted at 2,500 mg sulfate/l Digesti and Weeth (1976) placed the ''safe'' concentration for sulfate in drinking water for cattle at 2,500 mg/l. They also found that cattle would discriminate against 45.6 m chloride and reject 115.6 m chloride.

EFFECTS ON PUBLIC WATER SUPPLY

The effects of high dissolved solids in public water supplies are primarily physiological, aesthetic (taste), and economic. High levels of mineralization in drinking water may have a laxative effect especially on transients (CWQC, 1973).

The ''California Mineral Taste Study'' conducted primarily by W. H. Bruvold has provided a functional relation between mineral content of drinking water and consumer attitude toward taste. In accomplishing this, the ''California Mineral Taste Study'' may be unique in assessing aesthetic effects of water quality.

TABLE 2. GUIDE TO THE USE OF SALINE WATERS FOR LIVESTOCK AND POULTRY (CWQC, 1973).

Total Soluble Salts Content of Waters (mg/1)	Comment
Less than 1,000	Relatively low level of salinity. Excellent for all classes of livestock and poultry.
1,000-2,999	Very satisfactory for all classes of livestock and poultry. May cause temporary and mild diarrhea in livestock not accustomed to them or watery droppings in poultry.
3,000-4,999	Satisfactory for livestock, but may cause temporary diarrhea or be refused at first by animals not accustomed to them. Poor waters for poultry, often causing water feces, increased mortality, and decreased growth, especially in turkeys.
5,000-6,999	Can be used with reasonable safety for dairy and beef cattle, for sheep, swine, and horses. Avoid use for pregnant or lactating animals. Not acceptable for poultry.
7,000-10,000	Unfit for poultry and probably for swine. Considerable risk in using for pregnant or lactating cows, horses, or sheep, or for the young of these species. In general, use should be avoided although older ruminants, horses, poultry, and swine may subsist on them under certain conditions.
Over 10,000	Risks with these highly saline waters are so great that they cannot be recommended for use under any conditions.

Using methods of psychometric scaling, taste panel studies rated general taste quality of natural waters. Waters were carefully selected which had no detectable odor, nor history of odor due to anything but common minerals. The water samples, with the exception of one, had not been chlorinated. The results show an inverse linear relationship between general taste quality and mineral content. It was also found that persons may accept water of less than neutral quality. Potability (palatability) grades for various levels of TDS were suggested as follows: Excellent, < 300 mg/1; Good, 301-600 mg/1; Fair, 601-900 mg/1; Poor, 901-1100 mg/1; unacceptable, > 1101 mg/1 (Bruvold et al., 1967; Bruvold and Ongerth, 1969). The study casts doubt on the usefulness of threshold testing of aesthetics for setting standards by finding that clearly detectable mineral taste may be unacceptable for daily drinking.

A public survey of six California communities using water ranging from 50 to 1401 mg TDS/1 confirmed that attitude scale scores became more negative as TDS increases. The least offensive taste was found in sulfate and bicarbonate solutions while chloride and carbonate solutions have the most offensive taste. Synergistic or inhibiting effects of ions were not observed. Each ion appeared to make a straightforward contribution to the ratings according to its concentration. Dissolved oxygen variations did not seem to have a significant effect on mineral taste. Chlorine additions at 0.8 mg/l could be detected by a special panel and did not remove mineral taste. Temperature did not have a profound effect on taste either, even though cooler water was liked a little more. Contrary to common belief, consumers did not habituate or adjust to the mineral taste with time. Distilled water was less liked than water with a low mineral content. Beverages (coffee, tea, grape, and orange) made with mineralized water showed the same taste effects as for the individual ions ($SO_4^=$ and HCO_3^- had better taste than Cl^- anc $CO_3^=$). Increasing salinity in natural water decreased the palatability of these beverages (Bruvold, 1975).

Theoretically, any water can be processed into high quality water--for a price. Desalination appears to be as much as 50 or more times the cost of typical water treatment in existing water treatment plants including softening (Hartung and Tuepker, 1969).

Lawrence (1975) has developed estimating functions for the indirect costs imposed by high TDS on urban water use including industrial water supply. He listed the principal effects of high TDS as: (1) increased potentials for corrosion of vulnerable ferrous metals, (2) dezincification of vulnerable copper alloys and (3) industrial imposition (maintenance and treatment costs) to cooling waters and critical process waters. Low levels of salinity are not undesirable since distilled water itself is corrosive generally.

Water heater life is shortened about one year for every 200 mg/l additional TDS. Water hardness causes wear and tear on laundered fabrics, increased consumption of soaps, detergents, cleaners, chelating agents or combinations of these. Estimated total impact cost curves (penalty cost) were developed for the Los Angeles River planning area for 1974. These curves show the penalty cost estimate to range between about \$25/acre-ft (2.0¢/m^3)/100 mg/l TDS at the low ($\sim$ 200 mg/l) TDS range and about \$35/acre-ft (2.8¢/m^3)/100 mg/l TDS at the high ($\sim$ 800 mg/l) TDS range. These costs do not include bottled water use to avoid mineral taste problems since this was considered as a non-uniformly applied cost and not of significant magnitude in the study area.

EFFECTS ON INDUSTRIAL WATER SUPPLY

Water used by the manufacturing industry in 1973 totaled approximately 15,000 billion gallons (57 billion m^3) per year. Of this large quantity of water 81.2 percent is freshwater, 9.3 percent brackish water, and 9.5 percent salt water. Of the freshwater used 62.3 percent is used for cooling and condensing, 30.9 percent is used as process water, and 5 percent as boiler feed water. Brackish and salt waters are used almost entirely (93.7 percent and 91.6 percent respectively) for cooling and condensing water (U.S. Department of Commerce, 1975). Cooling water withdrawal by steam-electric plants in 1973 totaled 273,000 cfs (64,000 billion gallons/year or 244 billion m^3/year). Saline water use accounted for 28.3 percent of the total. The approximately 46,000 billion gallons (174 billion m^3/year) of fresh water used represents 11 percent of the total streamflow of the conterminous U.S., and when this is combined with manufacturing cooling water use the total is nearly 14 percent of the total streamflow (Federal Power Commission, 1976).

The dissolved solids characteristics of water that has been used for industrial water supplies varies greatly according to the requirements of individual industries and process. Concentrations of dissolved solids in water used in industry are reported to range from 150 to 35,000 mg/l (CWQC, 1973). Boiler feed, cooling tower makeup, and industrial process waters usually require specific treatments such as softening, dealkalizing, demineralization, or ameliorative additives in order to meet specific needs. Therefore, individual industries incur different costs from a given quality water. In metropolitan San Diego, California, the average industrial costs for water treatment were slightly over \$5/acre-ft (0.405¢/m^3)/100 mg TDS/l (Lawrence, 1975). Approximately 7,400 billion gallons (27.9 billion m^3) of water received treatment prior to industrial use in the manufacturing process in 1973. The number of manufacturing establishments which employed some sort of intake water treatment totaled 5,549. Treatments used included physical treatment, coagulation, softening, ion exchange, pH control, aeration, filtration, chlorination, and others (U.S. Department of Commerce, 1975).

SECTION IV

PHYSICAL-CHEMICAL EFFECTS OF SUSPENDED SOLIDS

RESERVOIR FILLING

The loss of reservoir capacity through the accumulation of sediment is a problem with serious economic consequences. A bibliographical review of the subject for the period 1964 to December 1975 which contains 105 abstracts has been compiled by R. J. Brown (1975) of the National Technical Information Service. It is beyond the scope of this review to discuss this literature in detail.

Generally, the factors contributing to the rate of sedimentation are erosion, sediment delivery rates, trap efficiency of the reservoir, and bulk density of the sediment (Paulet et al., 1972). They found that reservoir sedimentation is significantly associated with the characteristics of the contributing watershed, particularly the soils and geomorphology. Features of reservoir sedimentation can be estimated from stream characteristics and textural properties of the soil. Sedimentation rates were greater with finer texture, more uniform particle size, and lesser clay content in the soil. Similarly, greater sedimentation rates per unit of drainage area occurred with smaller drainage areas and shorter main stream length, lower order of the main stream, and smaller stream length ratio (i.e. the ratio of the mean length of the stream segment of the order of the stream on which the reservoir is located to the mean length of the segments of the next lower order). Lund et al. (1972) found that sedimentation rate predictions using the model of Paulet et al. (1972) could not be improved by including sediment clay mineralogical parameters.

TOXIC SUBSTANCE TRANSPORT

Halogenated Organics

A great deal of research has been and is being conducted on the release to the environment and ultimate fate of halogenated organic compounds. Several of these types of compounds have been associated with ecological damage and are deleterious to human health. Selected western U.S. streams (Brown and Nishioka, 1967; Manigold and Schulze, 1969) were surveyed for pesticides (i.e., aldrin, DDD, DDE, DDT, dieldrin, endrin, heptachlor, heptachlor epoxide) and herbicides (i.e., 2,4-D; 2,4,5-T; silvex). Both classes of compounds were found but not at all sampling stations. Herbicides were the most infrequently encountered (possibly due to degradation). DDT and its metabolites were the most commonly found. The highest concentrations of insecticide were found in samples having the highest sediment load.

Pfister et al. (1969) used liquid-liquid extraction methods on Lake Erie water and found no detectable chlorinated pesticides in the water. However, they found lindane associated with the small (size) inorganic fraction, and aldrin and endrin associated with the less dense fraction (mostly organics, detritus, and microorganisms) of the microparticulates in the water. They pointed out that not including particulates in water analysis is inadequate for pesticides.

Wershaw et al. (1969) found that DDT was 20 times more soluble in 0.5 percent sodium-humate than in water alone, and that humic acid strongly sorbs 2,4,5-T from solution. DDT was found to be concentrated 15,800 times in coloring colloidal material by Poirrier et al. (1972). This coloring colloidal material was described as allochthonous polymeric hydroxy carboxylic acids complexed with varying quantities of iron which were less than 10 μm in size. These colloids may stay in suspension for long periods of time, but may precipitate with changes in environment. It is possible that they may be transported to estuaries where contact with seawater may cause them to precipitate and adsorb to plants and/or be used by estuarine organisms as food.

The intimate association of clay and organic matter (organoclay complexes) can modify clay adsorption properties. Kahn (1974) found that 2,4-D adsorption by a fulvic acid-montmorillonite complex was smaller than previously reported values for humic acid, but was much higher than for montmorillonite alone. Low heats of adsorption by these complexes are on the order of van der Waals-type adsorption. Pierce et al. (1974) described the adsorption of DDT to clay as electrostatic attraction between the net negative charge on clay surfaces and hydrogen atoms on the aromatic rings of the DDT. Adsorption of DDT to humic acid has been attributed to hydrophobic bonding to portions of the humic polymer. Pierce et al. (1974), noting the increased adsorption capacity of humic matter, pointed out the need for knowledge of the transport and distribution of humic substances as related to the transport and distribution of chlorinated hydrocarbons in the environment.

Rizwanul et al. (1974) found that the higher the chlorine content of a PCB (polychlorinated biphenyl) the greater its solubility in water. He also found that sand and silica gel adsorbs very little PCB 1254, while kaolinite clay, montmorillonite clay, illite clay, and woodburn soil (Corvallis, Oregon) adsorbed increasingly more, respectively. The organic content of the soil was suspected as being the reason for higher adsorption.

A linear relationship has been found to exist between the concentration of chlorinated hydrocarbons and total organic carbon (as well as humic and fulvic acid material) in marine sediments (Choi and Chen, 1976). This study also found organoclay complexes to be important in adsorption of chlorined hydrocarbons. Chlorinated paraffins (suggested substitutes for PCB in many applications) have been tested for uptake by juvenile Atlantic salmon by Zitko (1974). He found that the juvenile salmon accumulated a relatively large amount of PCB (144 mg/g/144 hours) but little if any chlorinated paraffins from suspended solids. Feeding of the chlorinated paraffins to the fish did not result in accumulation, but some indications of toxicity were found.

Dredging and dredge spoil disposal operations have been suspected of freeing toxic chlorinated hydrocarbons from contaminated sediments. Slotta and

Williamson (1974) point out that the cause-effect relationship between dredging and toxic organic matter release is not well documented. Transfer of PCB and pesticide material to the water column from resuspended sediments collected near Chicago, IL; Green Bay, WI; Fall River, MA; Houston, TX; and Memphis, TN; was found to be negligible (Fulk et al., 1975). Chlorinated hydrocarbon concentrations associated with the suspended solids reached ''background'' levels after settling periods of 5 to 24 hours. The oil and grease content of the water was most important in ''describing'' the concentration of pesticides remaining in solution. Chen et al. (1976) assayed the release of chlorinated hydrocarbons from settled dredge spoil under reducing conditions and were unable to detect any after 3 months incubation. Here again the concentration of chlorinated hydrocarbons was closely correlated with macromolecular organic compounds in the sediments and to particles of 8 μm or smaller. Lee et al. (1975) have refined methods used for evaluating the hazard of toxic substance which may be released from sediments scheduled for dredging.

Toxic halogenated organic compounds may enter surface waters already adsorbed to soil or organic material. Lin (1972) suggested that agricultural erosion may be an important source for pesticides in water. The very low concentrations of pesticides found in agricultural runoff in eastern South Dakota by Dornbush et al. (1974) suggest that the contribution from agriculture may be quite variable and site specific. The processes involved in transport and distribution of toxic substances through or over a watershed have been mathematically modeled by Frere (1975). Such modeling efforts help understanding of the processes affecting loss of pesticides (or other toxics) from land to which they are applied.

Metals

Heavy metals may be adsorbed by, coprecipitated with, or complexed by suspended solids. Thus, heavy metals may be translocated or deposited with the sediment load of a natural waterway. Changes in biological, electrochemical, or physiochemical conditions in sediments such as those experienced during dredging and disposal operations could conceivably cause the release of toxic metals to the water. Slotta and Williamson (1974) pointed out that heavy metals may not be released during dredging operations due to adsorption on or coprecipitation with iron (III) oxides and iron (II) sulfides which are exposed during dredging. Blom et al. (1976) investigating the effect of sediment organic matter on the migration of chemical constituents during disposal of dredged material, found that significant amounts of heavy metals were released, but that concentrations remained below water quality criteria. They also found that oxygenation of the dredged material decreases metal release except for manganese in seawater, and to a lesser extent cadmium in both sea and freshwater. There was no evidence found that sediment or soluble carbon controls the release of metals or nutrients even in the presence of ligands.

Chen et al. (1976) found that during dredge spoil disposal, concentrations of silver, cadmium, and mercury were basically unchanged under all experimental conditions. Concentrations of chromium, copper, and lead were found from 3 to 10 times over background seawater levels. Iron, manganese, and zinc were released in even larger quantities. They also found that the release of metals from freshwater sediment in a seawater environment is somewhat larger than the release from marine sediments, but since the concentrations (except for iron) were in the sub-ppb to ppb range this was not considered to be a significant hazard. Extracted macromolecular organics such as humic and fulvic substances were found to contain from 2 to 15 times higher concentrations of trace metals than total sediment on a weight basis.

NUTRIENT TRANSPORT

Suspended solids often contain adsorbed or complexed plant nutrient compounds which, if made available for biological uptake and use, can lead to accelerated eutrophication of lakes and streams. Rural and agricultural runoff waters and their associated eroded material often contain considerable quantities of nutrients. Much of these nutrients may have been applied as fertilizer to the land. Phosphorus especially is tightly bound to soil particles and removed as sediment (Lin, 1972). With increasing trends in fertilizer application, good soil conservation practices are needed to minimize this source of pollution. Runoff water from animal feedlots contains particulate matter which is high in nutrients (Miner, 1966; Middlebrooks, 1974). Laboratory and field investigations which characterize suspended sediments from varied hydrologic, soil, and land use characteristics showed that agricultural activities in the dryland wheat region of eastern Washington contributed large amounts of sediment and dissolved nitrogen during heavy runoff periods. Urban activities provided substantial amounts of nitrogen and phosphorus during the remaining months. In excess of 90 percent of the orthophosphate exposed to the sediments was adsorbed (Carlile et al., 1974).

Anderson and Ross (1975) monitored a suburban development site and observed a significant increase in suspended solids and nutrients, especially phosphorus, associated with construction activities. Nutrient losses have been associated with increased erosion in clearcut forest areas in northern Idaho (Debyle and Packer, 1972; Snyder et al., 1975). Nutrient and suspended sediment production are greatly dependent on the patterns and magnitude of water drainage in the forested areas draining to the Lake Tahoe area, and disturbances, such as development construction activities, increase sediment production (McGauhey et al., 1971; Brown et al., 1973; Skau and Brown, 1974). Goldman (1974) reported that bacteria associated with particulate matter and nutrients eroded from the watershed into Lake Tahoe facilitate nutrient regeneration and may contribute to eutrophication. Huang and Hwang (1973) have shown that from 0 to 38 percent of the inorganic and from 63 to 89 percent of the organic phosphorus in sewage is associated with the suspended and colloidal particulates.

Nutrients released from sediments resuspended during dredging operations have given mixed results as to their algal growth stimulation ability. Larsen et al. (1975) have shown that sediment release of phosphorus has a great impact on the phosphorus budget of Shagawa Lake. Slotta and Williamson (1974) suggest that the localized nature of dredging operations, large dispersion factors, and the decrease in light penetration due to turbidity from the dredging, lower the algal bloom potential. Blom et al. (1976) observed the release of ammonia and low levels of orthophosphate from marine and freshwater dredged sediments. The numerical product of sediment organic content and the sediment organic nitrogen content was useful in predicting the release of ammonia nitrogen from dredged material. The release of other nutrients or metals from sediments was not related to any measured sediment parameter.

Chen et al. (1976) found that nitrogen and phosphorus were released in sub-ppm, and silicate in 10-20 ppm concentrations from suspended dredged sediments. Clay type sediments released nitrogenous compounds 2 to 10 times higher than silty and sandy sediments. Ammonia and organic nitrogen was released from settled spoil material under anaerobic conditions while nitrate and nitrite were released under aerobic conditions at about the same concentrations (10 ppm—N). Orthophosphate from settled sediments was released at concentrations between 0.1 to 0.8 ppm under both aerobic and anaerobic conditions.

Chemical analyses of certain systems have been interpreted to show that clays and sediments are effective in adsorbing organic compounds (heterotrophic substrates and vitamins) from solution. Button (1969) has shown that clays added to solutions of thiamine and glucose do not make these compounds unavailable to microorganisms or remove them to a significant degree from solution. Thus, it is not likely that suspended sediments influence significantly the populations of suspended microorganisms by sorbing vitamins or organic substrates.

AESTHETIC EFFECTS OF TURBIDITY

Turbidity, the optical property given to water by suspended solids, affects human perception visually. The clarity of natural water is seldom perceived alone, but is a component of the total field of vision or landscape. Most persons would probably agree that a clear mountain stream as part of an alpine landscape is pleasing and that a turbid stream in the same setting would be objectionable. However, the majestic appearance of the Green River in Utah flowing over large rapids is greatly enhanced when the river is laden with silt. Generally, however, high turbidities are considered to be unpleasing. For example, Buch (1956) described turbid reservoirs as unpleasing in the aesthetic sense, and implies that fewer anglers visited a reservoir for that reason. Forshage and Carter (1973) described the turbidity caused by gravel dredging in the Brazos River, Texas, as aesthetically unpleasing for several miles below the dredging site.

Little research on the effects of turbidity on the aesthetics of natural waters has been done. Methodologies for aesthetic measurement are still in the developmental stages. Reports of aesthetic evaluations which include turbidity in water quality assessment provide little if any data relating to the actual user or public opinion. Leopold (1969) and Hamill (1974) have used scales of ''evaluation numbers'' ranging from one to five, of water quality parameters which include turbidity. Selected panels of persons which may or may not have represented user group opinions are used in these studies. Hamill's (1974) work used a method which derived the highest aesthetic value by summing evaluation numbers from 31 environmental factors, 7 water quality factors (of which turbidity was one), 10 physical factors, and 14 human use factors. Turbidities of > 5,000 ppm were ranked ''5'' on the scale, the highest evaluation possible, with turbidities of < 25 ppm ranked ''1,'' or the lowest evaluation possible.

In identifying social goals, Gum (1974) listed water ''clarity'' as a subgroup of ''aesthetic opportunity.'' Measures of water clarity were defined as suspended silt load (ppm) and BOD (ppm). Masteller et al. (1976) reviewed current methodologies of assessing aesthetic values of streams and landscapes including those incorporating water quality factors. They state that aesthetic measuring techniques are generally inadequate, often being too judgmental and relying on panels of experts.

EFFECTS ON WATER SUPPLY

Modern water supply treatment plants are designed to remove suspended solids within the range commonly experienced in the raw water supply. Of course, as the suspended solids load that must be removed from the raw water increases, the expense of removal increases and the water supply value decreases. This is reflected in the ranges of standards promulgated for raw water resources of domestic water supply (McKee and Wolf, 1963). An excellent source of water supply, requiring only disinfection as treatment, would have a turbidity range of from 0 to 10 units. A good source of water supply, requiring usual treatment such as filtration and disinfection would have a turbidity range of 10 to 250 units. Waters with turbidities over 250 units are poor sources of water supply requiring special or auxiliary treatment and disinfection.

Robeck (1969) pointed out that waters of higher turbidity (30 JTU vs. 5 JTU) may be more easily coagulated and clay is sometimes added to raw water to give this effect. Surface area, charge density, and exchange capacity of clay mineral particles all have an effect on treatability. He also calls for protection of high quality (low turbidity) waters and states that effort should be made to minimize sudden changes in raw water turbidity since these affect coagulation, chlorine demand, and filterability of the water. The maximum contaminant level for turbidity in finished drinking water is one turbidity unit (EPA, 1975). An excellent review of 49 papers dealing with human perception and evaluation (aesthetics) of taste, odor, color, and turbidity in drinking water by Bruvold (1975) is recommended as a thorough treatment of this subject. Also Bruvold (1975) cites work in which the combined 1962 Public Health Service limits for turbidity, color, and odor were judged acceptable by only 48 percent of the respondents. He suggests that no more than 10 percent of the users should call a public water unacceptable, indicating that these standards need to be reconsidered.

SECTION V

EFFECTS OF DISSOLVED SOLIDS ON AQUATIC BIOTA

As will be seen in the review of the literature that follows, only occasionally do dissolved or suspended solids have drastic acute effects on the biology of most freshwater systems. Effects of these water quality parameters are usually subtle, seldom serving to completely eliminate (or to extremely stimulate) biological systems in streams or lakes. In assessing the impacts of a marine disposal outfall high in suspended and dissolved solids, Harville (1971) points out that it is not feasible to use the simple presence or absence of organisms as an indicator of pollution, since some resistant form of life will always be present.

EFFECTS ON PHYTOPLANKTON, PERIPHYTON, AND VASCULAR PLANTS

Dissolved solids consist of both organic and inorganic molecules and ions that are in true solution in water. Reid (1961) defined the most conspicuous materials which are found in varying quantities in natural waters to include carbonate, chlorides, sulfates, phosphorus, and nitrates. These anions occur in combination with such metallic cations as calcium, sodium, potassium, magnesium and iron to form ionized salts. Many of these dissolved materials are essential for growth and reproduction of aquatic organisms. The presence and the success of an organism in the environment is controlled by the quality and quantity of inorganic and organic nutrients; deficiency or excess or both may be limiting. When these various salts are present in suitable proportion, the different cations counteract each other, and the solution is physiologically balanced. Warren (1971) reports that the harmful effects of increased salt concentrations are caused, not by toxicity of its individual components, but by high osmotic pressure. When establishing criteria for dissolved solids in water, the importance of osmotic stress associated with increases in major cation and anion species must be considered (Provasoli, 1969).

Because unrooted aquatic plants depend entirely on dissolved solids for nutrients, any change in the nutrient level of a lake is reflected in its biota (Wetzel, 1973-1974). Algae as a group, however, are physiologically, as well as morphologically, very heterogenous. This heterogeneity makes generalization about their nutrition difficult. Therefore, when dealing with specific ecological problems it is dangerous to extrapolate data from one species to another. Ruttner (1952) reports that a limitation of the number of species in an environment begins at salt concentration exceeding that of the sea (35 g/l or 3.5 percent). Specht (1975) reports inhibition of _Selenastrum_ at salinities in excess of 9 parts per thousand whereas Cleave et al. (1976) report inhibition of Selenastrum at salinities of between 250 and 500 mg/l. [The effects of increased salinity on mangroves and submerged marine plants will not be covered here, the reader is referred to Reimold and Queen (1972) for an introduction to this topic.]

The first report of Na as an essential nutrient for blue-green algae came from Allen and Arnon (1955) who stated that 5 ppm suffices for optimal growth of Anabaena cylindrica. Brownell and Nicholas (1967), working with this same species, found that Na deficiency led to depressed N_2 fixation. It has also been shown that Anabaena variabilis and Synechocystis aquatilis tolerate NaCl up to 23.5 percent (w/v) and Microcystis firma tolerates NaCl up to 60 percent (w/v) (Schiewer, 1974). Provasoli (1969) proposed that monovalent ions might, with other factors, be responsible for tipping the balance in favor of blue-greens. Blue-green algae have an absolute need for Na as well as K which is a pattern apparently not shared by other fresh water algal groups.

Pearsall (1932) reported that a monovalent to divalent (M/D) cation ratio below 1.5 was favorable to diatoms in oligotrophic waters and Provasoli, et al. (1954) report Synura petersenii to prefer low total solids (60-100 ppm) and M/D above 2. This would appear to explain why eutrophic lakes affected by civilization often have blue-green blooms. Urbanization adds not only organic matter but also Na and P.

Zafar (1967) concluded that dissolved organic matter directly influenced the periodicity of blue-greens. While Seenayya (1973) suggested that an increase in chlorophyll a generally coincided with increasing TDS, Kerekes and Nursall (1966) reported a definite correlation of seston biomass to an increase in TDS. They hypothesized that as TDS increased, more nutrients became available thereby increasing the productivity of the water to a certain point (Figure 1). Continued increase in TDS tended to inhibit organoproduction, so that the productivity of the water decreased. In the study lakes, the TDS and alkalinity for maximum productivity were about 1,400 ppm and 450 ppm, respectively. However, the study lakes were not corrected for different nutrient levels and this confounding prevents confirmation of their hypothesis. The need to consider nutrient level as well as other limnological variables than TDS in natural field conditions is illustrated by the results of other workers. Topping (1975) found that the maximum standing crop in British Columbia lakes occurred at about 8,200 ppm. Topping (1975) also reported that increased concentrations of TDS become osmotically limiting. Larson (1970) reported that dissolved solids in Odell Lake (Oregon) were about 1/3 of Crater Lake (Oregon) yet production in Odell Lake was 8-10 times greater. Seventy-five percent of the total dissolved solids of Crater Lake were made up of six elements suggesting that, although total dissolved solids were relatively high, certain essential ions may have been deficient and therefore limiting.

Batterton and van Baalen (1971), working with blue-green algae, reported that 1 mg NaCl/l satisfied requirements for growth and higher concentrations of NaCl apparently inhibited growth. They, however, reported that inhibition was caused more by ionic (Na^+) stress than by osmotic stress. Most of the literature, however, supports the conclusion that the osmotic pressure of the solution is responsible for the observed changes in productivity following an increase in salt concentration (Schmidbauer and Ried, 1967).

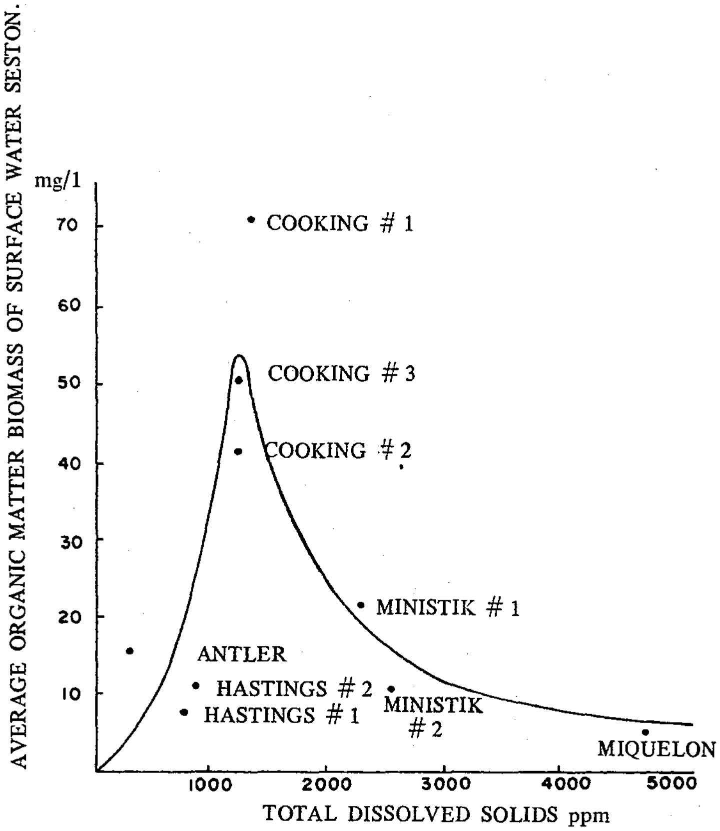


Figure 1. Relationship of organic matter biomass of surface water seston (productivity) to the total dissolved solids in nine bodies of water in central Alberta. (Kerekes and Nurshall(1966),reprinted by permission of the International Association of Theoretical and Applied Limnology.)

Dissolved organic substances can function directly as growth factors or essential micronutrients for algae. Doig and Martin (1974) report that iron associated with dissolved organic material may well cause the onset of logarithmic growth in Gymnodinium breve, the red tide alga.

The addition, in any amount of substances which cause shifts in population composition of the primary producers could adversely affect aquatic organisms farther up the food chain. Because of the close association between dissolved solids, nutrient availability and the growth of aquatic organisms, standards set forth to prescribe limits on TDS must take into account biological effects to insure maximum use of the water.

EFFECTS ON ZOOPLANKTON

Crustacean plankton populations have increased with increased total dissolved solids (TDS) and eutrophication of lakes in the Okanagan Valley, British Columbia (Patalas, 1973; Patalas and Salki, 1973). TDS in Lake Okanagan had increased by 19 mg/l between (July 4 to August 26) 1935 and 1969. The zooplankton abundance had increased from 2.8 mm^3/cm^2 in 1935 to 13.3 mm^3/cm^2 on

September 9, 1969, and to 7.8 mm^3/cm^2 on August 27, 1971. This represents a 4.8 and 2.8 fold increase respectively. There had been an 8 fold increase in bottom organisms. The increase in zooplankton populations probably are the result of increased eutrophication (related to phosphorus loading) which was reflected by the increase in TDS. No significant changes in species of plankton since 1935 were observed.

The chronic toxicity of NTA (nitrilotriacetate) to Daphnia magna was reduced with increasing water hardness (a major component of dissolved solids) up to 438 mg/l total hardness (Biesinger et al., 1974). Dissolved polyelectrolytes used as flocculants or coagulant aids in solids removal treatment of water were toxic to Mysis and Daphnia at concentrations ranging from 0.06 mg/l to 16.5 mg/l. Two polyelectrolytes (Superfloc 330 and Calgon M-500) impaired reproduction of Daphnia at low concentrations (Biesinger et al., 1976).

Faucon and Hummon (1976) found that the life expectancy, reproductive rate, and intrinsic rate of natural increase of the parthenogenic gastrotrich Lepidodermella squammata were maximal at pH 7.1 and total conductivity of 465 μmho/cm. Life expectancy was reduced to zero when acid mine waters were added to make the pH 4.6 and conductivity 825 μmho/cm. It was concluded that L. squammata is capable of living and reproducing at pH 6.0 to 6.5 under field conditions low in carbonates, providing non-carbonates are not abundant, or under field conditions high in non-carbonate ions, providing sufficient carbonates are present. This implies a dependence on anion ratios for survival of this organism.

EFFECTS ON MACROINVERTEBRATES

Five species of Odonatan nymphs, four species of Heteroptera, and three species of Coleoptera which had been adapted to freshwater, were tested by Shirgur and Kewalramani (1973) for their tolerance to various dilutions of seawater and to 3.5 percent solutions of major constituents of seawater. In general, Odonatan nymphs which survived longer than 360 hrs in dilutions of seawater below 30 percent were considered to be the least tolerant organisms, and Coleopterans, surviving greater than 360 hrs in dilutions below 60 percent were the most tolerant. The most sensitive species tested was Anisops barbata, which survived only 134.5 hrs in 10 percent seawater. The most tolerant species, Cybister cognatus, survived beyond 360 hrs in 50 percent seawater. Potassium chloride (KCl) was found to be the most toxic constituent and $MgSO_4$ the least toxic constituent of seawater.

Wichard and Komnick (1974) have shown that damselfly larvae (Zygoptera) osmoregulate against hypotonic salt solutions by virtue of rectal chloride epithelia which adsorb electrolytes from solution. Dills and Rogers (1974) observed increases in dissolved solids in streams subject to acid mine drainage in which macroinvertebrate community structure was adversely affected. However, hydrogen ion concentration was the only parameter highly correlated with species diversity.

The invertebrate fauna of two low salinity (25 to 40 mg/l), low pH (4.8-6.0) lakes on Stradbroke Island, Australia has been described (Bensink and Burton, 1975). Ninety-seven percent of the 1401 ppm TDS in one lake was due to dissolved organic (humic) matter, while the 124 ppm TDS in the other lake was only 48 percent dissolved organic matter. Littoral fauna species composition was very different in these lakes, probably due to differences in chemical-physical factors. Interference with light penetration in the brown colored humic lake may have been an important factor affecting faunal community structure.

EFFECTS ON SALMONID FISHES

McKim et al. (1973, 1974, 1975, 1976) have prepared extensive reviews which include the effects of salinity on freshwater fish. Eisler (1973), and Eisler and Wapner (1975) have also reviewed the literature dealing with salinity effects on fish in both marine and freshwater environments.

Bergström (1971) found an increase in blood glucose concentration correlated with a decrease in plasma sodium concentration in young salmon (*Salmo salar*) which had been placed in deionized water. It is possible that the increase in glucose may be of osmoregularity significance. Oxygen consumption rates were lowest in rainbow trout (*Salmo gairdneri*) maintained in a salinity of 7.5 ppt (Rao, 1971). This salinity is isosmotic with the fish plasma and the reduced oxygen requirement probably reflects a reduction in the osmotic load cost for the fish. The slope of a regression line relating fish weight to oxygen consumption, increased with increasing salinity at 15C, but no significant effect on the oxygen consumption-fish weight relationship was observed at 5C. Fish activity was not different in freshwater and 15 ppt salinity. Maximum oxygen consumption was observed at 30 ppt salinity (except for smaller fish at 15C)(Rao, 1971).

Zeitoun et al. (1973) found an increased protein requirement in rainbow trout (*Salmo gairdneri*) fingerlings raised in elevated (20 ppt) salinities. They related this requirement to the protection of the internal environment of the fish against a hypertonic external environment. However, the osmoregulatory capabilities of euryhaline coho salmon (*Oncorhynchus kitutch*) smolts did not require extra protein at 20 ppt (Zeitoun et al., 1974b). Water salinity and dietary protein concentration in rainbow trout (*S. gairdneri*) fingerlings did not influence serum protein (Zeitoun et al., 1974a). Hematocrit increased with increased salinity but was not affected by dietary protein levels. Leduc (1972) found that Atlantic salmon retain the same osmoregulation whether from ocean stock or from freshwater hatcheries. Block (1974) found that rainbow trout acclimated to 100 percent seawater had elevated levels of erythrocytes and tissue lipids when held at 1C, while plasma cholesterol and glucose levels remained unchanged. Seawater adapted rainbow trout accumulated urea in their plasma when held at 1 and 10C. This may have been due to the inability to excrete ammonia against the higher exterior concentration of sodium; then the ammonia would be converted to urea at colder temperatures, a less toxic substance.

Lack of oxygen brought about complete breakdown in osmoregulatory ability in the rainbow trout which was manifested by elevated levels of plasma electrolytes. Rainbow trout can be put directly into seawater cages if salinity is reduced to 22 ppt with mortalities of only one to eight percent (Landless, 1976).

EFFECTS ON OTHER FISH

With concern about the effects of impending degradation in water quality due to decreases in freshwater flows and increases in waste discharges, Turner and Farley (1971) studied the effects of temperature, salinity, and dissolved oxygen on the survival of striped bass (Morone saxitalis, Walbaum) eggs and larvae. Egg survival in salinities greater than approximately 1,000 ppm TDS is greatly reduced especially at higher temperatures unless they are hardened in freshwater. Dissolved oxygen levels of from four to five mg/l adversely affect egg and larval survival. Turner (1976) collected striped bass eggs and larvae from the Sacramento and San Joaquin Rivers in California during the period 1963 to 1972 and found that most spawning in the Sacramento-San Joaquin Delta occurred where salinities during spawning had been below 200 mg/l TDS with occasional maximum of 1,500 mg/l due to seawater intrusion. This high salinity level did not adversely affect egg survival. Turner (1976) pointed out, however, that although the ranges of salinities encountered (200 to 71,400 mg/l TDS) had a limited short term effect on egg survival and spawning, long term effects such as accumulative effects of small differences in survival or migratory preferences may reduce spawning in high total dissolved solids waters. Increased sodium chloride concentrations in freshwater hatchery ponds increased mean survival of striped bass fry to 7.65 percent as opposed to 1.7 percent survival in control ponds. The large variability in survival found in both pond types makes it difficult to determine if this difference (5.95 percent) in survival is significant.

Common carp (Cyprinus carpio) lived at salinities of 12 ppt for 10 weeks, but higher salinities were unfavorable (Al-Hamed, 1971). Fertilized carp eggs hatched at salinities from two to ten ppt, but had 'favorable' hatching success only up to 6.6 ppt.

Umminger (1971) found elevated levels of serum glucose in killifish (Fundulus heteroclitus) held at 0.1C. There was a 30 percent loss of serum sodium, a 42 percent loss of serum chloride, but only a 15 percent decrease in serum osmolarity in these fish. The relatively low decrease in osmolarity was due to a 1,967 percent increase in serum glucose. Osmoregulation ability by inorganic ion concentration adjustment is apparently inhibited at low temperatures. The turnover of sodium by the killifish (Fundulus kansae) is sharply increased by transfer to low calcium seawater from normal seawater. Mortality brought on by this phenomenon can be prevented by dilution to 80 percent (v/v) of the low-calcium seawater (Fleming et al., 1974). Rao (1974) found that incubation salinities over the range of five to 14 ppt produced the shortest incubation period, maximum yolk-conversion efficiency, largest larval size at hatching, and the maximum viable hatch of the California killifish (Fundulus parvipinnis). Lower salinities at fertilization resulted in shorter incubation periods and larger larvae at hatching indicating increased growth rates under the low salinity conditions.

Lutz (1972) studied the effect of osmotic and ionic stress on plasma, tissue, and whole body electrolyte composition of the perch (Perca fluviatilis). The perch showed a good degree of adaptive ionic regulation as it was able to survive up to one-third seawater with only potassium, magnesium, and chloride showing moderate significant rises in plasma. Attempts to acclimate perch to one-half seawater led to a total breakdown of the ionic controlling mechanisms. Osmotic rather than ionic considerations determined the lethality of the medium.

Peterka (1972), and Burnham and Peterka (1975) have studied the effects of salinity on eggs and larvae of the fathead minnow (Pimephales promelas) and other fishes. Peterka (1972) found that hatching success and sac fry survival was most successful for fathead minnow eggs fertilized in water with a conductivity of 1300 μmho/cm and held in water of 500, 1,300, 4,000, or 6,000 μmho/cm. Much lower success was found for eggs fertilized in 500 or 4,000 μmho/cm water and held in the above concentrations. Sac fry survival was similar in trend. There was no hatch of walleye (Stizostedion vitreum vitreum), approximately one percent hatch of northern pike (Esox lucius), and 22 to 93 percent hatch of fathead minnow eggs held in 4,000 μmho/cm water. No sac fry of northern pike survived 6,000 μmho/cm, while approximately one percent of the fathead minnow sac fry survived 12,000 μmho/cm water. All of the surviving fry at this concentration had physical abnormalities. The literature reviewed by Peterka (1972) indicated that ionic composition of the water seemed more important to tolerance by the fathead minnow than did TDS. The fathead minnow was unable to survive TDS > 2,000 ppm in the $NaHCO_3$, Na_2CO_3, and K_2CO_3 saline lakes of Nebraska, but survived approximately 15,000 ppm TDS in the Na_2SO_4 and $MgSO_4$ lakes of Saskatchewan and North Dakota. In the field, a North Dakota saline lake with 7,000 ppm TDS was not detrimental to reproduction and growth of the fathead minnow. The fathead minnow grew faster in lakes of 3,250 ppm TDS than at 1,060 ppm TDS.

Chittenden (1973) found that young American shad (Alosa sapidissima) could tolerate an abrupt as well as a gradual change from freshwater (five ppt) to salinities of about 30 ppt without mortality. Complete mortality occurred when the fish were abruptly transferred from 30 ppt to 0 ppt salinity but not with gradual decrease from 5 ppt to zero ppt salinity. Since these fish are euryhaline, they can use both brackish and freshwater nurseries. The American shad was formerly one of the most abundant anadromous fishes in the United States.

Digestive rates of the mosquitofish (Gambusia affins) generally increased with increasing salinity (Shakuntala, 1975).

Channel catfish (Ictalurus punctatus) and blue catfish (Ictalurus furcatus) have been collected from Gulf of Mexico waters with salinities of 11.4 ppt. Hybrids of these catfish were studied for salinity tolerance by Stickney and Simco (1971). They found that the hybrids were able to tolerate salinities between 14 and 15 ppt for periods of 96 hours. Allen and Avault (1971) found that blue catfish were more tolerant to 14 ppt salinity than were channel catfish. Size of the fish did not seem to affect the tolerance of either species. Both species of fish showed signs of distress early in the experiments, but showed some signs of recovery near the middle or end of the experiment. All the test fish lost weight indicating that neither species was able to adapt to 14 ppt salinity. Transfer of the fish from the 14 ppt to freshwater did not cause adverse effects. White catfish (I. catus) seemed to tolerate 14 ppt salinity better than blue catfish. Block (1974) found that 30 percent seawater did not change hematocrit values in channel catfish as compared to freshwater values. Tissue water of fish in freshwater and 2C was three percent above the level in freshwater and 30C. In 30 percent seawater at 2C the tissue water of the channel catfish was increased only one percent compared to 30C fish.

Osmoregulation by the catfish may be lost at low temperatures (2C) as evidenced by decreases in plasma osmolarity, sodium, and chloride levels in freshwater adapted fish. Davis and Simco (1976) observed increases in plasma sodium and chloride levels of channel catfish after five days exposure to 10 and 12 g/l sodium chloride in July (27C); at the same time there was a plasma electrolyte concentration increase for 4.8 hours after which the concentration leveled off. Catfish exposed similarly in March (9C) had a slow increase in plasma electrolyte throughout the 13 day experiment.

Hollander and Avault (1975) studied the salinity tolerance of buffalo fish (*Ictiobus cyprinellus*, and *I. niger*). They found that eggs of all fish types tolerated salinities as high as 15 ppt and hatched in 72 days. Emerging normal fry could tolerate only 9 ppt. Fry of both species of buffalo fish had the best survival time at 9 ppt and the poorest at zero ppt. Fingerlings had an upper salinity tolerance of 12 ppt, and yearlings tolerated 10 ppt salinity. Perry (1976) repored the successful spawning of black buffalo and bigmouth buffalo in ponds with salinities ranging from 1.6 to 1.8 ppt and 1.4 to 2.0 ppt respectively.

Leatherland et al. (1974) studied the regulation of plasma sodium (Na^+) and potassium (K^+) in African Tilapia fishes. Upon comparing plasma levels of Na^+ and K^+ in fishes from concentrated ‘‘soda’’ lakes to fishes from freshwaters, they found that generally Na^+ and K^+ were more concentrated in species from soda lakes. The Na^+/K^+ ratio in the serum was not related to ambient salinity. One species (*Tilapia alcalica*) from a saline lake tolerated a loss of plasma Na^+ in fresh water, while another saline adapted species (*T. grahami*) was better able to maintain plasma Na^+ levels. Fresh water species (*T. zilli* and *T. nigra*) could not tolerate salinities in excess of 2.5 percent NaCl. Mucopolysaccharide cells in *Tilapia mossambica* may be converted to chloride cells active in osmoregulation under conditions of hyperosmotic stress. The adsorptive surface of the intestine also increases, possibly to facilitate adsorption of water for hypoosmotic regulation in the hyperosmotic media (Narasimham and Parvatheswararao, 1974). Adaptation to osmotic stress in *T. mossambica* has been shown to follow a regular time course involving two phases (Bashamohideen and Parvatheswararao, 1976). There is a rapid rise in oxygen consumption in proportion to the magnitude of stress imposed by transfer of the fish into higher saline media, followed by a gradual decrease in oxygen usage which stabilizes at a new level almost equal to the original normal (freshwater) medium.

The recreational fishery of the Salton Sea, California, a terminal lake receiving irrigation return flows, presents an unusual case for salinity management in inland fisheries. Marine fish species such as sargo (*Anisotermus davidsoni*), orangemouth corvina (*Cynoscion xanthulus*), and bairdiella (*Bairdiella icistia*) have been introduced successfully into the saline waters which have about 36 ppt salinity. Increasing salinities seriously threaten this fishery through adverse effects on the eggs and larvae of these fish (Lasker et al., 1972; May 1976). It has been shown that bairdiella egg and larvae survival are severely inhibited in 40 ppt Salton Sea water. The unusually harmful effects of Salton Sea water may be attributed to its higher proportions of calcium and sulfate which are approximately threefold higher (percentage of total salinity) than seawater. In particular, divalent cations (e.g. Ca^{++}) may have adverse physiological effects (May, 1976).

There is considerable evidence that the pituitary gland (*pars intermedia*) plays a vital role in the osmoregulation of euryhaline fishes (Chidambaram et al., 1972). The bullhead (*Ictalurus melas*) was unable to survive longer than seven days in freshwater after removal of the pituitary gland. Prolactin treatment, isosmotic saline maintenance, or autografted pituitary glands prolonged freshwater survival. Harrison et al. (1974) immersed goldfish (Carassius auratus L.) in a graded series of sodium chloride solutions up to a concentration of 15 g/l and found that the rising osmolarity induced cytophysiological changes (staining reaction) in specialized cells of the pituitary gland. Singley and Chavin (1975) observed increases in cortisol and ACTH titers in goldfish subjected to saline stress.

Subramanyam (1974) studied the succinic dehydrogenase activity of the freshwater teleost, Heteropneustes fossilis during acclimation to elevated salinities. He found that the enzyme activity increased in the liver but not in the kidney, reflecting the metabolic response to osmotic stress. This would indicate that the effect of salinity stress varied from tissue to tissue.

SECTION VI

EFFECTS OF SUSPENDED SOLIDS ON AQUATIC BIOTA

EFFECTS ON PHYTOPLANKTON, PERIPHYTON, AND VASCULAR PLANTS

When establishing criteria concerning suspended solids it must be kept in mind that the concentration of suspended solids in natural waters is influenced by such factors as topography, geology, soil conditions, intensity, and duration of rainfall, type and amount of vegetation in the drainage basin, and man's activity in the drainage basin. Most flowing waters have considerable variation in the suspended solids concentration from day-to-day; therefore, loading of suspended solids in lakes from streams will vary from day-to-day. Since natural variation in suspended solids is so great, it is not desirable to have fixed rigid standards. For this reason, Cairns (1967) in reviewing the ecological effects of suspended solids, suggests that the effects upon aquatic organisms living in the system be used to determine the suspended solids standard.

Plants adapted to the aquatic environment include floating and benthic macroscopic plants, phytoplankton, and periphyton. The role of phytoplankton in the environment includes oxygenation of the water, conversion of inorganic material to organic material, a source of food for zooplankton and, after death, a nutrient source. Macrophytes also play an important role in nutrient cycling in addition to a major role in forming habitats for other organisms. These habitats include surfaces for attachment of bacteria, periphyton, and aquatic insects as well as providing protection and nesting sites for fish. Consequently, perturbation of the system that would adversely affect the phytoplankton, periphyton, or macrophyte community would also adversely affect other members of the food chain. Suspended solids concentration standards based on the response of this community would insure that maximum use be made of a drainage basin without impairing its ability to function beneficially in the ecosystem.

The major ecological parameters of suspended solids which would affect photosynthetic systems includes reduction in light penetration, sedimentation, and habitat alteration, abrasive action, and effects of adsorbed toxins. The importances of these effects may vary, some species being affected more than others.

Since photosynthetic organisms form the basis of the food chain, any reduction in the availability of light (regardless of nutrient concentration) which causes a decrease in photosynthetic productivity, has a widespread effect on other organisms dependent on them for food. Swale (1964) working on the River

Lee, emphasized that for most of the year, fluctuation in the concentrations of phosphorus and nitrogen could not be the factors determining the number of algae. She placed emphasis on rates of flow and detrital turbidity as major factors limiting algal production. Lund (1969), also working on the River Lee, reported that even a reduction of phosphorus and nitrogen to a tenth of their concentration could still permit very large phytoplankton populations to develop if light intensity were not limiting. Increases in suspended solids brings about reduction in light penetration and this greatly reduces the primary producers except for those species that are planktonic or living on floating debris. This reduction causes a shift from herbivores to those that are primarily detritus feeders (Patrick, 1972). Not only does reduction in light penetration restrict photosynthesis, it may also alter oxygen relationships in surface waters (Oschwald, 1972). Angino and O'Brien (1968) suggest that reduction in oxygen production due to excess turbidity may be critical in some large streams.

Light penetration is important not only with respect to productivity but also with respect to community composition. Wetzel and McGregor (1968) reported that low light intensity inhibits germination of Najas flexilis and Chara and would, therefore, eliminate these two species from the community.

Sedimentation, due to suspended solids, results in habitat destruction and abrasive action. These two effects can severely alter the photosynthetic population. Many species of plants are confined to one or a very few types of substratum because they need a special surface for attachment. Destruction of specific habitats will not only eliminate one part of the populations but may also introduce a new population to the area. Hynes (1970) reported that fairly even discharge containing silt can create great stable areas of weed development which can completely alter the substratum (directly and indirectly) and with it the animal population.

Adsorption of chemicals by suspended solids is particularly important if it leads to a build-up of toxic substances in a limited area with the possibility of sudden release. For some trace elements, especially copper, the limits between need and toxicity may be extremely narrow. Low concentrations of copper ($\leq 10^{-7}$ M) are essential for Chlorella while concentrations $\geq 10^{-7}$ M are toxic (Green et al., 1939; Greenfield, 1942).

EFFECTS ON ZOOPLANKTON AND AUFWUCHS PROTOZOANS

Published research concerning the direct effect of suspended solids on minute invertebrates is limited. It could be assumed that as turbidity limits light penetration and hence aquatic algae and plant productivity (Oschwald, 1972), the grazing microfauna would also be limited. In addition, the abrasive action of suspended sediments would be expected to have an adverse effect on attached protozoans and micrometazoans.

Response of Daphnia magna in suspensions of several kinds of solids was reviewed by EIFAC (1965). Harmful levels of kaolinite and montmorillonite were 102 and 82 ppm respectively. Charcoal was harmful at 82 ppm. Pond sediment was not lethal to Daphnia up to 1458 ppm. Toxicity of suspended solids to Daphnia appeared to be type specific. The reproduction rate of Daphnia seemed to increase at lower concentrations of suspended solids (e.g. 39 ppm kaolinite, 73 ppm pond sediment). The review also cited work in which it was found that the production of Daphnia in the Mondsee in Austria was reduced from 400,000 kg/year to 80,000 kg/year due to high clay turbidities caused by road construction. This reduction in plankton severely affected the production of whitefish (Coregonus).

Spoon (1975) found a doubling in the number of protozoan or micrometazoan species colonizing artificial substrates in the upper Potomac estuary below the Blue Plains sewage treatment plant in 1974 as contrasted to 1971. Water quality in 1974 showed an improvement over 1971 in turbidity as well as dissolved oxygen, phosphorus, nitrogen and organic carbon. It is not clear whether turbidity directly affected the colonizing protozoans and metazoans. An increase in algae was also observed in 1974 (see also Spoon, 1976). Research is needed to determine the mode and extent of the effect of suspended solids on protozoa and related organisms.

EFFECTS ON MACROINVERTEBRATES

Work by Gammon (1970) includes a review of the literature published prior to 1970 on the effect of inorganic sediment on stream macroinvertebrates (Table 3). Stream substrate may be altered by suspended silt deposition and this can have important effects on the macroinvertebrate community. Using a scale ranging from one to 452, various substrates mixed with silt rated no higher than 27. A substrate combination of moss, gravel, rubble, and Elodea rated over 400 while shifting sand supported the fewest macroinvertebrates thus rating only one. Hynes (1970) has also commented on the importance of substratum to selection and diversity of aquatic insect populations.

Field monitoring and experimental work by Gammon (1970) in a stream below a limestone quarry where the average suspended solids load was increased approximately 40 mg/l showed that there was considerable impact on the macroinvertebrate population. Suspended solids concentrations ranged from 13 to 52 mg/l above the quarry and 21 to 250 mg/l below the quarry. Species of the Tricorythoides increased somewhat below the quarry as opposed to the area above the quarry due to their preference for silt or mud substrate while net spinners (Cheumatopsyche) were reduced during periods of heavy sediment input. Drift rates of macroinvertebrates from an impacted riffle increased approximately linearly with increasing suspended solids up to 160 mg/l. There was a 25 percent increase in drift at an increase of 40 mg/l suspended solids above normal and a 90 percent increase in drift at an increase of 80 mg/l suspended solids above normal. Drift rates seemed to be more closely related to suspended solids than to settled sediment but both settled and suspended sediment reduced invertebrate populations. Drifting species were the same as those in the riffle. It appeared that the effect of suspended solids on invertebrates in the studied system was equal, i.e. there was no species selection by suspended solids.

Stream faunal recovery after strip mine reclamation has been studied by Hill (1972). He found that the pollutant limiting to populations of fish and bottom organisms in reclaimed and partially reclaimed streams was inorganic silt, and that complete reclamation of spoil areas reduces the levels of siltation and turbidity which in turn allows recovery of stream faunal communities.

TABLE 3. SUMMARY OF SUSPENDED SOLIDS EFFECTS ON AQUATIC MACROINVERTEBRATES (DATA COLLECTED FROM GAMMON, 1970; HILL, 1972; AND ROSENBERG AND WIENS, 1975).

Organism(s)	Effect	Suspended Solid Concentration	Source of Suspended Solids	Comment
Mixed Populations	Lower summer populations		Mining area	
Mixed Populations	Reduced populations to 25%	261-390 ppm (Turbidity)	Log dragging	
Mixed Populations	Densities 11% of normal	1000-6000 ppm		Normal populations at 60 ppm
Mixed Populations	No organisms in the zone of settling	>5000 ppm	Glass manufacturing	Effect noted 13 miles downstream
Chironomus & Tubificidae	Normal fauna replaced by (Species Selection)		Colliery	Reduction in light reduced submerged plants
Cheumatopsyche (Net spinners)	Number reduced	(High concentrations)	Limestone Quarry	Suspended solids as high as 250 mg/l
Tricorythoides	Number increased		Limestone Quarry	Due to preference for mud or silt
Mixed Population	90% increase in drift	80 mg/l	Limestone Quarry	
Mixed Populations	Reduction in numbers	40-200 JTU	Manganese Strip mine	Also caused changes in density and diversity
Chironomidae	Increased drift with suspended sediment		Experimental sediment addition	
Ephemoptera, Simuliidae, Hydracarina	Inconsistant drift response to added sediment		Experimental sediment addition	

Turbidities in unreclaimed streams ranged between 40 and 200 JTU with maximum levels of 32,000 JTU having been recorded. Turbidity and siltation caused an overall reduction in the number of bottom organisms which resulted in changes in density, diversity, and community structure. Six years after reclamation in one stream, faunal recovery was complete. Gravel dredging on the Brazos River, Texas, limited macroinvertebrates by causing a loss of gravel habitat which was replaced by a sand-silt bottom (Forshage and Carter, 1973). Increased turbidity may also have had an effect on macroinvertebrate populations.

Rosenberg and Wiens (1975) added bankside sediment to the Harris River in northern Canada in order to study the mode of action of suspended and settled sediments and the responses of stream fauna. Preliminary results of their study indicated that the number of Chironomidae caused to drift by sediment addition always increased with sediment addition, but that the Ephemeroptera, Simulidae, and the Hydracarina were inconsistent in their drift response to suspended sediment. Based on their data and several assumptions they estimated that it would take as long as 18 days and as short as seven hours for 50 percent of the resident macrobenthic population to leave their experimental riffle area when sediment was added as it was in their experiments (100 and 250 mg/l intended concentrations). McGaha and Steen (1974) in their study of Mississippi flood control reservoirs found that benthic fauna appeared to be more closely related to bottom type, submerged vegetation, and normal life cycles than to turbidity. Reservoir habitats appear qualitatively different with regard to effects on community responses than stream habitats, as would be expected.

EFFECTS ON SALMONID FISHES

The European Inland Fisheries Advisory Commission (EIFAC, 1965) promulgated protective standards on salmonid and other fish types and delineated five ways that finely divided solids may harm freshwater fishes. These are:

(1) by acting directly on the fish swimming in water in which solids are suspended, and either killing them or reducing their growth rate, resistance to disease, etc.;

(2) by preventing the successful development of fish eggs and larvae;

(3) by modifying natural movements and migrations of fish;

(4) by reducing the abundance of food available to the fish; and

(5) by affecting the efficiency of methods of catching fish.

A summary of their results was prepared to illustrate these effects on salmonids (Table 4).

On recommending water quality criteria for the protection of aquatic communities the Committee on Water Quality Criteria (CWQC, 1973) relied strongly on the EIFAC study. Their recommendation is as follows:

TABLE 4. SUMMARY OF EFFECTS OF SUSPENDED SOLIDS ON SALMONID FISH. (DATA TAKEN FROM REVIEW IN EIFAC, 1975).

Fish (Species)	Effect	Concentration of Suspended Solids	Source of Suspended Materials	Comment
Rainbow Trout (Salmo gairdneri)	Survived one day	80,000 ppm	Gravel washing	
	Killed in one day	160,000 ppm	Gravel washing	
	50% mortality in 3 1/2 wks	4,250 ppm	Gypsum	
	Killed in 20 days	1000-2500 ppm	Natural sediment	Caged in Powder River, Washington
	50% mortality in 16 wks	200 ppm	Spruce fibre	70% mortality in 30 wks
	1/5 mortality in 37 days	1,000 ppm	Cellulose fibre	
	No deaths in 4 wks	553 ppm	Gypsum	
	No deaths in 9-10 wks	200 ppm	Coal washery waste	
	20% mortality in 2-6 months	90 ppm	Kaslin and diatomaceous earth	Only slightly higher mortality than control
	No deaths in 8 months	100 ppm	Spruce fibre	
	No deaths in 8 months	50 ppm	Coal washery waste	
	No increased mortality	30 ppm	Kaslin or diatomaceous earth	
	Reduced growth	50 ppm	Wood fibre	
	Reduced growth	50 ppm	Coal washery waste	
	Fair growth	200 ppm	Coal washery waste	
	"Fin-rot" disease	270 ppm	Diatomaceous earth	
	"Fin-rot" disease	200 ppm	Wood fibre	
	"Fin-rot" disease	100 ppm	Wood fibre	Symptons after 8 months exposure
	No "fin-rot"	50 ppm	Wood fibre	
	Reduced egg survival	(Siltation)		Eggs in gravel
	Total egg mortality in 6 days	1000-2500 ppm	Mining operations	Powder River, Oregon (Not specifically rainbow trout eggs)
Pacific Salmon (Oncorhynchus)	Survived 3-4 wks	300-750 ppm (2300-6500 ppm for short periods each day)	Silt	Fingerlings

TABLE 4. Continued.

Fish (Species)	Effect	Concentration of Suspended Solids	Source of Suspended Materials	Comment
	Reduced survivial of eggs	(Silting)		Eggs in gravel
	Supports populations	(Heavy loads)	Glacial silt	Spawn when silt is washed from spawning beds.
	Avoid during migration	(Muddy water)		Yuba River, California
Brown Trout (Salmo trutta)	Do not dig redds	(Sediment in gravel)		Water must pass through gravel
	Reduced populations to 1/7 of clean streams	1000-6000 ppm	China-clay waste	
Cutthroat Trout (Salmo clarkii)	Abandon redds	(If silt is encountered)		
	Sought cover and stopped feeding	35 ppm		Two hours exposure
Atlantic Salmon (Salmo salar)	No effect on migration	Several thousand ppm		River Severn, British Isles
Brook Trout (Salvelinus fontinalis)	No effect on movement	(Turbidity)		

	Maximum Concentration of Suspended Solids
High level of protection	25 mg/l
Moderate protection	80 mg/l
Low level of protection	400 mg/l
Very low level of protection	over 400 mg/l

More recent work by Sykora et al. (1972) showed that suspensions of iron hydroxide of 50, 25, 12, and 6 mg/l iron caused juvenile brook trout (Salvelinus fontinalis, Mitchell) to reach no more than 16 percent, 45 percent, 75 percent, and 100 percent of the weight of control fish, respectively. The turbidity of the water at a theoretical concentration of 50 mg/l iron as $Fe(OH)_3$ (95.5 mg/l $Fe(OH)_3$) averaged 86 JTU (range 130 to 60 JTU) while the average turbidity at a 'theoretical' (prepared) 6 mg/l iron was 23 JTU (range 42 to 14 JTU). It was assumed that impaired visibility due to high turbidity prevented the fish from feeding which in turn resulted in slower growth. The review by Oschwald (1972) pointed out that angler success for most game fish species improved as turbidity decreases.

Williams and Harcup (1974) working on an industrial river in south Wales found that spawning areas for brown trout were limited by industrial and urban developments, sporadically high levels of suspended coal residues and other factors. Native trout produced in the stream showed poor growth. High levels of suspended solids in the lower reaches of the river increased the movement of fish into a downstream river. Suspended solids concentrations ranged from 0 to 22 mg/l at an upstream station and from 7 to 1530 mg/l at the most downstream station. Resuspended harbor sediment (subject to dredging) at concentrations of up to 5 percent wet weight (28.8 g/l dry weight) had no observable effect on coho salmon fry (Oncorhynchus kisutch) or threespine sticklebacks (Gasterosteus aculeatus) in 96 hr bioassays (LeGore and DesVoigne, 1973). The sediments were contaminated with high levels of organic matter, oil and grease, zinc, and lead.

EFFECTS ON OTHER FISHES

The acute direct effects of turbidity on fishes was investigated by Wallen (1951). Using 14 genera and 16 species, he found that behavioral reactions to turbidity did not develop until turbidities neared 20,000 ppm. Most of the experimental fish endured more than 100,000 ppm turbidity for a week or longer, but these same fishes died at turbidities of 175,000 to 225,000 ppm. Lethal turbidities caused death in 15 minutes to 2 hours after exposure was begun. Fishes that were killed by the exposure to the suspended clay developed opercular cavities and clogged gill filaments. Some effects on selected fish used in Wallens' study are listed in Table 5. The tolerance of the test fish for such high suspended solids concentrations compared with known natural concentrations led Wallen to conclude that natural clay turbidity was not a lethal condition in the life of juvenile to adult fishes.

Buch (1956) in reporting work on the effects of turbidity on fish and fishing, stated that young bass were not found in waters with greater than 84 ppm, redear sunfish in greater than 174 ppm, and bluegills in 185 ppm turbidity.

TABLE 5. SOME EFFECTS OF TURBIDITY ON SELECTED FISH SPECIES (DATA FROM WALLEN, 1951).

Species	Turbidity at First Adverse Reaction	Turbidity at First Death
Golden Shinner (*Notemigonus crysoleucas*)	20-50,000 ppm	50-100,000 ppm
Mosquitofish (*Gambusia affins*)	40,000	80-150,000
Goldfish (*Carassius auratus*)	20,000	90-120,000
Carp (*Cyrinus carpio*)	20,000	175-250,000
Red Shinner (*Notropis lutrensis*)	100,000	175-190,000
Largemouth Black Bass (*Micropterus salmoides*)	20,000	101,000 (average)

Clear farm ponds produced from 1.7 to 5.5 times the total weight of fish in turbid ponds. Largemouth bass were most affected by turbidity. Interference with light penetration lowered plankton productivity by 8 to 12.8 times in turbid waters as opposed to clear waters. This reduction in productivity limited the amount of available food for fish. Individual channel catfish grew faster in clear ponds but greater total weights were obtained in muddy ponds due to lack of competition. The presence of carp (which increased turbidities) reduced the growth of bass and bluegills, but led to increased yields of channel catfish and bluegills. A clear reservoir attracted more anglers, yielded greater returns per unit of fishing effort, as well as desirable species, and was aesthetically more attractive.

Smith et al. (1965) found that the mortality of fish exposed to suspensions of wood fibers such as those from pulping plants, depended on the species of fish, type of wood fibre, processing method, dissolved oxygen concentration, and to a lesser degree, water temperature. Using young of the year of fathead minnows (*Pimephales promelas*) and walleyes (*Stizostedion vitreum vitreum*), they found that ground conifer wood was the most lethal and had the greatest effect on walleye fingerlings, and that ground wood pulps were more lethal than chemical pulps.

Gammon (1970) presented an excellent review of the effects of suspended solids on fishes. His review as it pertains to non-salmonid fishes is summarized in Table 6.

TABLE 6. EFFECTS OF SUSPENDED SOLIDS ON NON-SALMONID FISH (DATA COLLECTED FROM GAMMON, 1970).

Fish (Species)	Effect	Concentration of Suspended Solids	Source of Suspended Materials	Comment
Mixed fish populations	Decrease in occurence	Turbidity increase		
Mixed fish populations	Critical levels affecting populations	100-300 ppm	Industrial	England, Scotland, and Wales fisheries
Perch (*Perca flavesiens*)	High egg mortality	(Silting)		
European Pike Perch (*Lucioperca lucioperca*)	High egg mortality	(Silting)		
Zebra (*Brachyolanio rerior*)	Earlier egg hatch and no increase in egg mortality	18,000-30,000 ppm	Limestone dust	Fry died within 4 hours at 74,800
Barbel (*Barbus fluviatilis*)	Decreased migration	(Increasing turbidity)		
European eel (*Anguilla anguilla*)	Increased migration	(Increasing turbidity)		
Smallmouth bass (*Micropterus dolomieui*)	Successful nesting, spawning, hatching	(Sporadic periods of high turbidity)		

In investigating the effects of limestone quarry suspended solids, Gammon (1970) found that most fish were reduced in numbers below the quarry. Carp (Cyprinus carpio) were often seen in very turbid waters, but were seldom more than 50 percent as abundant as above the outfall. Carpsuckers (Carpiodes cyprinus) were the most sensitive to suspended solids but smallmouth bass (Micropterus dolomieni) were also sensitive. Gizzard shad (Dorosoma cepedianum) tolerated lower concentrations but avoided higher concentrations of suspended solids. Spotted bass (Micropterus punctulatus) were unaffected and did not avoid high levels of suspended solids. Golden redhorse (Moxostoma erythrurum) and spotted bass grew at significantly lower rates below the outfall than those above the outfall. Other fish species grew at about the same rate above and below the outfall. This lack of supression of growth was probably due to the tendency for these fish to avoid turbid waters.

Ritchie (1972) reviewed the effects of suspended solids (turbidity) on fish population changes and indicated that the Lake Erie fish community had changed from ciscoes (Coregonus), whitefish, and yellow perch (Perca flavescens) to sauger (Stizostedion canadense), sheepshead (Aplodinotus grunniens), catfish, and carp partly because of sediment.

Hill (1972) observed that the blacknose dace (Rhinichthys atratulus) was the most common fish collected in streams occurring in unreclaimed manganese strip mine areas; these streams were subjected to high levels of turbidity. Sculpins (Cottus sp.) that were otherwise common to the study area were always absent in unreclaimed streams.

Gravel dredging effects on the fauna of the Brazos River, Texas were studied by Forshage and Carter (1973). They concluded that habitat destruction and siltation caused a shift in fish populations from largemouth bass, green sunfish, bluegill, and redear to white crappie, warmouth, channel catfish, and flathead catfish.

Horkel and Pearson (1976) have found that green sunfish (Lepomis cyanellus) did not significantly increase their oxygen consumption rate in bentonite suspensions of as high as 26.7 ppt (2,359-3,750 formazin turbidity units (FTU)). However, ventilation rates increased 50 percent to 70 percent at the same oxygen consumption rate with turbidities above 898 FTU. Opercular movements of the green sunfish returned to the pre-treatment rates by the third day of exposure.

Although these results are sometimes difficult to interpret because of either conflicting conclusions for some fish species at different life stages or confounding due to variation in more than one independent variable, the results do indicate that 1) there are severe effects of suspended solids on species survivability largely through life cycle effects, 2) significant effects of suspended solids on habitat may prevent maintenance of or eliminate a fish species from a specific freshwater ecosystem, and 3) there is a strong relationship between land uses and suspended solids concentrations in streams that manifests its effect directly on the fish community.

SECTION VII

RESEARCH NEEDS RELATED TO STANDARDS ON SUSPENDED AND DISSOLVED SOLIDS FOR PROTECTION OF FRESHWATER BIOTA

While it has been frequently stated that the dissolved solids and suspended materials found in streams, rivers, reservoirs, and lakes affect water quality, little information is available as to just what some of these effects are on the freshwater biota. Angino and O'Brien in a 1968 paper summarizing some of the effects that the suspended load has or may have on determining water quality, recognized that the direct effect of suspended solids on organisms, chemical quality, photosynthesis, temperature and oxygen content is poorly understood. Since then, little information has been added to our knowledge of these effects.

The necessity for establishing water quality standards based on the response of the aquatic community to changes is obvious; the means for doing so are not as readily apparent. More quantitative data concerning direct and indirect effects of changes in dissolved and suspended solids on aquatic life need to be gathered before standards can aid in maintaining the maximum number of uses of the watershed. As Wolman (1971) stated in his paper on ''The Nation's Rivers,'' we are particularly weak in our ability to detect subtle initial changes from a natural to a polluted condition. More research is needed so we can understand changes in biological systems due to changes in environment. This will enable us to prescribe standards which will prevent the onset of ''the polluted condition.''

To ascertain the research needs relevant to the development of water quality standards, it is necessary to relate possible impacts of suspended and dissolved solids on freshwater biota and to prioritize the research needs on the least understood subject areas. Using the information contained in the foregoing review, a classification was developed to relate specific qualities of the suspended and dissolved solids to likely impact on freshwater ecosystems (Table 7). Primary, secondary and tertiary effects on biota of these pollutants would be expected to be observed. For example, primary includes direct life cycle effects (growth, reproduction) or toxicity (acute and chronic); secondary includes chemical effects which in turn cause biological effects, such as, the interaction of dissolved oxygen and fish; tertiary includes the effects of organisms on organisms, such as, decreased light reduces primary productivity which in turn affects the whole food chain.

Standards must reflect these different levels of effect. Because climax communities generally reflect natural conditions, we are usually concerned with changes of condition from what occurs naturally. Thus one important area of

TABLE 7. CLASSIFICATION OF SUSPENDED AND DISSOLVED SOLIDS AND THEIR PROBABLE MAJOR IMPACTS ON FRESHWATER ECOSYSTEMS.

	Biochemical, Chemical, and Physical Effects	Biological Effects*
Suspended Solids		
Clays, silts, sand	Sedimentation, erosion & abrasion, turbidity (light reduction), habitat change	Respiratory interference, habitat restriction, light limitation
Natural organic matter	Sedimentation, DO utilization	Food sources, DO effects
Wastewater organic particles	Sedimentation, DO utilization, nutrient source	DO effects, eutroph.
Toxicants sorbed to particles	All of the above	Toxicity
Dissolved Solids		
Major inorganic salts	Salinity, buffering, precipitation, element ratios	Nutrient availability, succession, salt effects
Important nutrients	DO production	Eutrophication
Natural organic matter	DO utilization	DO effects
Wastewater organic matter	DO utilization	DO effects
Toxicants	Effects on DO	Toxicity

*Some of these effects are a result of direct impacts of pollutant (primary effect) and some are a result of changes due to biochemical, chemical, or physical changes (secondary) or biological interactions (tertiary effects).

research concerns establishing the effects on natural communities of changes from natural suspended solids and dissolved solids concentrations and their patterns and time in space to a new set of conditions caused by human activities (land uses, waste disposal or water consumption and use). Thus, there is a need to develop a quantitative relationship between response parameters (biomass, diversity, growth rates) and the change in pollutant concentration. This should be the overall goal for determining research needs relevant to setting standards for suspended solids and dissolved solids. Specific research needs must be related to this goal.

In the achievement of this goal it is important to stress the need to design experiments carefully so that confounding due to multiple and uncontrolled manipulations do not invalidate the conclusions. This is particularly true for studies on suspended and dissolved solids because 1) the difficulty in isolating secondary and tertiary effects, 2) the problem of other pollutants which are either associated with or carried on suspended solids, and 3) confounding effects in field studies where increased dissolved and suspended solids are associated with increases in other pollutants.

Impacts of dissolved and suspended solids on the physical and chemical parameters are well understood. However, biological responses, particularly at the community level, are only poorly understood but are probably most relevant to setting standards. Thus most of the research needs relate to determining community responses to dissolved and suspended solids concentrations and loads. Concepts relating to community responses either need development or must be applied to the practical problem of setting standards. These concepts include diversity, successional processes, energy transfer and food web relationships and ecosystem modeling. Thus, the understanding and definition of freshwater community response parameters to dissolved and suspended solids are defined as the principal research need.

EFFECTS OF SUSPENDED AND DISSOLVED SOLIDS ON AQUATIC PHOTOSYNTHETIC SYSTEMS

Although the importance of the effects of dissolved and suspended solids on photosynthetic systems has been recognized in the literature, very little quantitative data are available. Therefore, when trying to establish dissolved (DS) and suspended solids (SS) concentration standards based on the response of the aquatic community to changes in its environment, one realizes the need for more research on their effects on photosynthetic systems.

Successional Effects--SS

The effects of reduction in light penetration due to suspended solids has been established in the literature. Very little has been reported, however, concerning levels of suspended solids and their direct effect on the plant population. We need to know what level of increase will cause shifts in populations from desirable species to less desirable species, for example, algae to macrophytes or green to blue-green algae.

Abrasive and Siltation Effects--SS

More research is also needed concerning the direct physical effects of suspended solids. Very little is known about the effects of abrasive action on attached algae and rooted plants. We also need to know what effects sedimentation has on attached and rooted plants. Good quantitative data are needed in all these areas concerning community response before standards insuring maximum use of the watershed can be established.

Successional Effects--DS

Changes in community composition due to increases in dissolved solids must also be quantified before standards dealing with dissolved solids are established.

More studies, such as the one carried out by Kerekes and Nursall (1966) dealing with seston biomass and increase in TDS, need to be done so that standards based on community response can be determined.

Primary Production Effects--DS

The effects of dissolved solids on producer organisms (algae and plants) are needed in terms of photosynthetic rate, nutrient availability and interactions, and successional effects for different concentrations.

EFFECTS OF SUSPENDED AND DISSOLVED SOLIDS ON ZOOPLANKTON AND MACROINVERTEBRATES

There is very little information available on the effects of dissolved solids per se on the microfauna of freshwater. Published information relates almost entirely to the effects of specific constituents of dissolved solids such as nutrients (and resulting primary productivity), heavy metals, and toxic organics. Suspended solids effects on protozoans and micrometazoans are also poorly understood. Therefore, it is difficult to assess the adequacy of water quality standards for protection of these organisms.

Successional Effects--Microfauna

A great deal of research is needed both in the laboratory and under field conditions to assess the tolerances of at least common species of zooplankton, attached protozoans, and micrometazoans to various concentrations and types of suspended and dissolved solids. Population composition changes should also be looked at when trying to determine the effects of changes in suspended and dissolved solids. Any shifts in the zooplankton population could adversely affect other aquatic organisms in the food chain. More knowledge in this area is needed before standards can be set based on the response of this community to changes.

Successional Effects--Macroinvertebrates

The effects of dissolved solids on macroinvertebrates also have not been documented quantitatively in the literature. Here again a great deal of research is needed to assess toxic and sublethal effects of dissolved solids on these organisms. Special attention should probably be directed toward species selection and effects on ecosystem structure. Bioassay techniques and case by case studies will probably be required to set effluent standards for protection of aquatic insect communities which may be impacted by increased dissolved solids levels.

The literature provides a fair understanding of the effects of suspended solids on macrobenthic communities. Increased turbidities cause increased insect drift and may selectively reduce insect populations, hence altering ecosystem structure. The recommended criteria of the CWQC (1973) are probably adequate to protect most aquatic communities.

Macroinvertebrates--Acute Changes in SS

Unusual increases in suspended solids concentrations probably affect established macroinvertebrate communities more than concentrations per se, especially in low suspended solids waters. Research is needed to expand the knowledge of suspended solids effects on macroinvertebrate ecosystem types as related to habitat and climate. Little, if any, information is available on physiological effects of suspended solids on aquatic insects. These effects must be studied to understand their impacts on community dynamics.

EFFECTS OF SUSPENDED AND DISSOLVED SOLIDS ON FISH

Considerable amounts of research have been published on the effects of dissolved and suspended solids on fish, consequently additional research should have a lower priority. Many fish have been shown to be able to tolerate high suspended solids or relatively high salinities for at least a short time. Eggs, larvae, and fingerling fish are generally more susceptible to stress by dissolved or suspended solids than are adult fish. Standards which are similar to the recommended criteria of the CWQC (1973) are adequate for protecting fish against suspended solids.

However, some streams probably naturally exceed the recommended low level of protection afforded by 400 mg/l suspended solids on a regular basis. In these streams, special research will be required to determine safe levels of suspended solids for the native fish population. Standards for protection of fish from dissolved solids should be designed similarly with the recommended criteria promulgated by the CWQC (1973), i.e. bioassays and field studies should be conducted to determine what levels of salinity can be tolerated without damaging ecosystem structure and function, and discharge standards should be designed to protect the water against exceeding these levels.

SECTION VIII

OTHER RESEARCH NEEDS

SUSPENDED SOLIDS TRANSPORT OF TOXIC SUBSTANCES

The fluvial translocation of suspended solids to which toxic organics or toxic metals have been adsorbed poses a significant threat to public health and ecosystems that is not well understood. The findings that humic matter and other organics by themselves or in complexes with inorganic clays can greatly increase the solubility of chlorinated organic compounds and thus increase their mobility in the environment, calls for research into the transport and distribution of humic substances and associated chlorinated organics in the environment. Only limited information is available concerning the nature of the cause-effect relationship of toxic substance release during dredging operations. Some laboratory studies have shown negligible release of toxic materials from dredged sediments, but some field observations conflict with this. Fundamental information is needed on contaminant-to-sediment attachment mechanisms so that conditions under which the contaminants might be released can be better predicted (Chen et al., 1976). Monitoring requirements for dredging operations need to be improved (Slotta and Williamson, 1974).

AESTHETIC EFFECTS OF SUSPENDED SOLIDS

A great deal of emphasis is being placed by human populations on the quality of life including aesthetic opportunity. However, methods of evaluating aesthetic preference and/or acceptance have only begun to be developed. No meaningful information is available concerning the aesthetic perception of suspended solids (turbidity) in water. Sociological research is greatly needed to develop methods of evaluating aesthetic perception of water quality (including turbidity) and then collecting sociological data so that planning efforts for upgrading or maintaining water quality can use this information.

THE EFFECTS OF SUSPENDED AND DISSOLVED SOLIDS ON PUBLIC AND INDUSTRIAL WATER SUPPLY

Bruvold (1975) has evaluated the effects of mineral taste on public acceptance of drinking water in California. This information is very valuable in setting salinity limits for public water supplies. However, more geographically widespread information on mineral taste acceptance is needed. Bruvold (1975) also points out that the presently promulgated standards for turbidity, color, and odor in drinking water may be too high and need reevaluation. Dissolved and suspended solids effects also involve corrosion and wear and tear problems in public water distribution systems, industrial equipment, and individual residence equipment. These effects are primarily of economic concern. Little information is available, however, on the exact nature and extent of this economic impact. Research is needed on a broad geographical scale into the economics of using or treating turbid or mineralized water (including treatment alternatives) for public or industrial water supply.

REFERENCES

Adams, A. W., F. E. Cunningham, and L. L. Munger. 1975. Some effects on layers of sodium sulfate and magnesium sulfate in their drinking water. Poultry Sci. 54(3):707-714.

Adams, V. D., R. R. Renk, P. A. Cowan, and D. B. Porcella. 1975. Naturally occurring organic compounds and algal growth in a eutrophic lake. PRWG137-1, Utah Water Research Laboratory, Utah State University, Logan, Utah 84322. 140 p.

Al-Hamed Mahmoud, I. 1971. Salinity tolerance of common carp: (*Cyprinus carpio*, L). Bull. Iraq Nat. Hist. Mus. 5(1):1-7; Bioabstracts 1972, 53:36403.

Allen, K. O., and J. W. Avault, Jr. 1971. Notes on the relative salinity tolerance of channel and blue catfish. Progressive Fish Culturist 33(3):135-137.

Allen, M. B., and D. I. Arnon. 1955. Studies on nitrogen-fixing bluegreen algae. II. The sodium requirements of *Anabaena cylindrica*. Physiologia Pl. 8:653-660.

Anderson, M. W., and B. E. Ross. 1975. Hydrologic study of a small suburban watershed. PB-249 744, Natl. Tech. Infor. Serv., Springfield, Va. 22161. 89 p.

Angino, E. E., and W. J. O'Brien. 1968. Effects of suspended material on water quality. Int. Assoc. of Scientific Hydrology 78:120-128.

APHA. 1975. Standard methods for the examination of water and wastewater. 14th edition. American Public Health Association, Washington, D.C.

Barwick, D. H. 1973. The effect of increased sodium chloride on striped bass fry survival in freshwater ponds. Proc. 27th Ann. Conf. S.E. Assoc. Game and Fish Comm. p. 415.

Bashamohideen, M., and V. Parvatheswararo. 1976. Adaptations to osmotic stress in the fresh-water euryhaline teleost, *Tilapia mossambica*. I. Time course. Zool. Anz. 196(5/6):323-332.

Batterton, J. C., Jr., and C. van Baalen. 1971. Growth responses of blue-green algae to NaCl concentration. Arch. Mikrobiol. 7:151-165.

Bennett, J. P. 1974. Concepts of mathematical modeling of sediment yield. Water Resources Research 10:485-492.

Bensink, A. H. A., and H. Burton. 1975. North Stradbroke Island a place for freshwater invertebrates. Proc. R. Soc. Queensland 86(7):29-45.

Bergström, E. 1971. Influence of deionized water on blood glucose and plasma sodium ion concentration in young salmon (*Salmo salar* L.). Arch. Internat. Physiol. Biochem. 79:785-792.

Bernstein, L., and L. E. Francois. 1973. Leaching requirement studies: Sensitivity of alfalfa to salinity of irrigation and drainage waters. Soil Sci. Soc. Amer. Proceedings 37(6):931-943.

Biesinger, K. E., R. W. Andrew, and J. W. Arthur. 1974. Chronic toxicity of NTA (Nitrilotriacetate) and metal-NTA complexes to *Daphnia magna*. J. Fish. Res. Board Can. 31(4):486-489.

Biesinger, K. E., A. E. Lemke, W. E. Smith, and R. M. Tyo. 1976. Comparative toxicity of polyelectrolytes to selected aquatic animals. Jour. WPCF 48(1):183-187.

Blackman, W. C., Jr., J. V. Rouse, G. R. Schillinger, and W. H. Shafter, Jr. 1973. Mineral pollution in the Colorado River Basin. Jour. WPCF 45(7):1517-1557.

Block, R. M. 1974. Effects of temperature and salinity on the osmotic adjustment in the euryhaline rainbow trout, *Salmo gairdneri* Richardson and the stenohaline channel catfish, *Ictaluris punctatus* (Rafinesque). Unpublished dissertation, University of North Dakota; Dissertation Abstracts 1975, 35/10:p.4930-B.

Blom, B. E., T. F. Jenkins, D. C. Leggett, and R. P. Murrmann. 1976. Effect on sediment organic matter on migration of various chemical constituents during disposal of dredged material. Dredged Material Research Program. Contract Report D-76-7, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Miss. 39180. 182 p.

Bowen, D. H. M. 1972. Runoff poses next big control challenge. Environ. Sci. & Technol. 6(9):771.

Branson, F. A., G. F. Gifford, and J. R. Owen. 1972. Rangeland hydrology. Range Science Series No. 1. Society for Range Management, Denver, Colorado. 93 p.

Branson, R. L., P. F. Pratt, J. D. Rhoades, and J. D. Oster. 1975. Water quality in irrigated watersheds. J. Environ. Qual. 4(1):33-40.

Brown, E., and Y. A. Nishioka. 1967. Pesticides in selected western streams. A contribution to the national program. Pest. Monit. J. 1(2):38-46.

Brown, J., W. Howe, and C. Skau. 1973. Nutrient and sediment production from forested watersheds. PB-241 524, Natl. Tech. Infor. Serv., Springfield, Va. 22161.

Brown, R. J. 1975. Reservoir and lake sedimentation (a bibliography with abstracts). NTIS/PS-75/886/2ST Natl. Tech. Infor. Serv., Springfield, Va. 110 p.

Brownell, P. F., and D. J. D. Nicholas. 1967. Some effects of sodium on nitrate assimilation and N_2 fixation in *Anabaena cylindrica*. Plant Physiol. 42:915-921.

Bruvold, W. H. 1975. Human perception and evaluation of water quality. CRC Crit. Reviews in Environ. Cont. 5(2):153-231.

Bruvold, W. H., and H. J. Ongerth. 1969. Taste quality of mineralized water. J. Amer. Water Works Assoc. 61(4):170-174.

Bruvold, W. H., H. J. Ongerth, and R. C. Dillehay. 1967. Consumer attitudes toward mineral taste in domestic water. J. Amer. Water Works Assoc. 59(5):547-556.

Bryan, E. H. 1971. Quality of stormwater drainage from urban land. Selected Water Resources Abstracts 5(7),W72-03995.

Buch, D. H. 1956. Effects of turbidity on fish and fishing. Trans. N. Amer. Wildl. Conf. 21:249-261.

Burnham, B. L., and J. J. Peterka. 1975. Effects of saline water from North Dakota lakes on survival of fathead minnow (*Pimephales promelas*) embryos and sac fry. J. Fish. Res. Board Can. 32(6):809-812.

Button, D. K. 1969. Effect of clay on the availability of dilute organic nutrients to steady-state heterotrophic populations. Limnology and Oceanography 14(1):95-100.

Cairns, J., Jr. 1967. Suspended solid standards for the protection of aquatic organisms. 22nd Purdue Industrial Waste Conference. May 2-4. Purdue University. p. 16-27.

Carlile, B. L., B. L. McNeal, J. A. Kittrick, L. C. Johnson, and H. H. Cheng. 1974. Characterization of suspended sediments in water from selected watersheds as related to control processes, nutrient contents and lake eutrophication. PB-232 167, Natl. Tech. Infor. Serv., Springfield, Va. 22161. 92 p.

Chen, K. Y., S. K. Gupta, A. Z. Sycip, J. C. S. Lu, M. Knezevic, and W. W. Choi. 1976. Research study on the effect of dispersion, settling, and re-sedimentation on the migration of chemical constituents during open-water disposal of dredge materials. Dredged Material Research Program. Contract Report D-76-1, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Miss. 39180. 247 p.

Chidambaram, S., R. K. Meyer, and A. D. Hasler. 1972. Effects of hypophysectomy, pituitary autographs, prolactin, temperature and salinity of the medium on survival and natrimia in the bullhead, *Ictalurus melas*. Comp. Biochem. Physiol. 43A:443-457.

Chittenden, M. E., Jr. 1973. Salinity tolerance of young American shad, *Alosa sapidissima*. Chesapeake Sci. 14:207.

Choi, W. W., and K. Y. Chen. 1976. Associations of chlorinated hydrocarbons with fine particles and humic substances in nearshore surficial sediments. Environ. Sci. & Technol. 10(8):782-786.

Cleave, M. L., D. B. Porcella, and V. D. Adams. 1976. Possible impacts of oil shale development on the Colorado River system. Paper presented before the Pacific Section, American Society of Limnology and Oceanography, June 1976. Missoula, Montana.

Committee on Water Quality Criteria (CWQC). 1973. Water quality criteria 1972. A report of the Committee on Water Quality Criteria, Environmental Studies Board, National Academy of Sciences, National Protection Agency, EPA-R3-73-003, Gov. Printing Office, Washington, D.C. 20402.

Davis, K. B., and B. A. Simco. 1976. Salinity effects on plasma electrolytes of channel catfish, *Ictalurus punctatus*. J. Fish. Res. Board Can. 33:741-746.

Debyle, N. V., and P. E. Packer. 1972. Plant nutrient and soil losses in overland flow from burned forest clearcuts. Water Resources Association Proceedings Series 14:296-307.

Digesti, R. D., and H. J. Weeth. 1976. A defensible maximum for inorganic sulfate in drinking water of cattle. J. Animal Sci. 42(6):1498-1502.

Dills, G., and D. T. Rogers, Jr. 1974. Macro-invertebrate community structure as an indicator of acid mine pollution. Environ. Pollut. 6:239-262.

Doig, M. T., III, and D. F. Martin. 1974. The effect of naturally occurring organic substances on the growth of a red tide organism. Water Res. 8:601-606.

Dornbush, J. N., J. R. Anderson, and L. L. Harms. 1974. Quantification of pollutants in agricultural runoff. U.S. Environmental Protection Agency, Environmental Protection Technology Series, EPA-660/2-74-005, Gov. Printing Office, Washington, D.C. 20402. 150 p.

Eisler, R. 1973. Annotated bibliography on biological effects of metals in aquatic environments. U.S. Environmental Protection Agency, Environmental Protection Technology Series, EPA-R3-007, Gov. Printing Office, Washington, D.C. 20402. 287 p.

Eisler, R., and M. Wapner. 1975. Second annotated bibliography on biological effects of metals in aquatic environments. U.S. Environmental Protection Agency, Environmental Protection Technology Series, EPA-600/3-75-008, Gov. Printing Office, Washington, D.C. 20402. 399 p.

Environmental Protection Agency. 1971. Industry waste study. The Hawaii sugar industry waste study. Environmental Protection Agency, Region IX, San Francisco, Ca., PB-238 931 Natl. Tech. Infor. Serv., Springfield, Va. 22161. 115 p.

Environmental Protection Agency. 1972. Waste water survey, St. Regis Paper Co., Cantonment, Florida. Environmental Protection Agency, Surveillance and Analysis Div., Athens, Ga., PB-228 275, Natl. Tech. Infor. Serv., Springfield, Va. 22161. 52 p.

Environmental Protection Agency. 1973. Methods for identifying and evaluating the nature and extent of nonpoint sources of pollutants. U.S. Environmental Protection Agency, EPA-430/9-73-014. Gov. Printing Office, Washington, D.C. 20402. 261 p.

Environmental Protection Agency. 1975. Interim primary drinking water standards. Federal Register 40(51):11995.

European Inland Fisheries Advisory Commission (EIFAC). Working Party on Water Quality Criteria for European Freshwater Fish. 1965. Water quality criteria for European freshwater fish. Report on finely divided solids and inland fisheries (EIFAC Technical Paper No. 1), Air and Water Pollution 9(3):151-168.

Faucon, A. S., and W. D. Hummon. 1976. Effects of mine acid on the longevity and reproductive rate of the Gastrotricha *Lepidodermella squammata* (Dujardin). Hydrobiologia 50(3):265-269.

Federal Power Commission. 1976. Steam-electric plant air and water quality control data for the year ended December 31, 1973. Summary Report, Federal Power Commission, Washington, D.C. 20426, Gov. Printing Office, Washington, D.C. 20402.

Filip, D. S., and E. J. Middlebrooks. 1976. Eutrophication potential of dairy cattle waste runoff. Water Res. 10:89-93.

Fleming, W. R., J. Nichols, and W. I. W. Potts. 1974. The effect of low-calcium sea water and actinomycin-D on the sodium metabolism of *Fundulus kansae*. J. Experimental Biol. 60:267-273.

Fletcher, J. E., D. L. Sorensen, and D. B. Porcella. (In press.) Erosional transfers of nitrogen in desert ecosystems. In: Nitrogen processes of desert ecosystems. N. E. West and J. J. Skujins, eds. Dowden, Hutchinson and Ross, Inc., Stroudsburg, Pa.

Forshage, A., and N. E. Carter. 1973. Effects of gravel dredging on the Brazos River. Proc. 27th Ann. Conf. S. E. Assoc. Game and Fish Comm. p. 695.

Frere, M. H. 1975. Integrating chemical factors with water and sediment transport from a watershed. J. Environ. Qual. 4(2):12-17.

Fulk, R., D. Gruber, and R. Wullshleger. 1975. Laboratory study of the release of pesticide and PCB materials to the water column during dredging and disposal operations. Dredged Material Research Program, Contract Report D-75-6, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Miss. 39180. 113 p.

Gammon, J. R. 1970. The effect of inorganic sediment on stream biota. Environmental Protection Agency, Water Pollution Control Research Series 18050 DW C12/70. Gov. Printing Office, Washington, D.C. 20402.

Glancy, P. A. 1973. A reconnaissance of streamflow and fluvial sediment transport. Incline Village Area, Lake Tahoe, Nevada. Second Progress Report. 1971. Nevada Division of Water Resources, Water Resources Information Series Report. 37 p.

Goldman, C. R. 1974. Eutrophication of Lake Tahoe emphasizing water quality. Environmental Protection Agency, EPA-660/3-74-034, Gov. Printing Office, Washington, D.C. 20402. 408 p.

Green, L. F., J. F. McCarthy, and C. G. King. 1939. Inhibition of respiration and photosynthesis in *Chlorella pyrenoidosa* by organic compounds that inhibit copper catalysis. J. of Biol. Chem. 128:447-462.

Greenfield, S. S. 1942. Inhibitory effects of inorganic compounds on photosynthesis in *Chlorella*. Am. J. of Bot. 29:121-131.

Gum, R. L. 1974. Identification, weights, and measurements of social goals. In: Water resources planning, social goals and indicators: Methodological development and empirical test. The Technical Committee on the Water Resources Research Centers of the Thirteen Western States, PRWG131-1, Utah Water Research Laboratory, Utah State University, Logan, Utah 84322.

Hagius, C. F., E. J. Middlebrooks, and D. B. Porcella. (In press.) Biostimulatory properties of irrigation return flow. Agricultural Experiment Station, Utah State University. Logan, Ut. 84322.

Hamill, L. 1974. Statistical tests of Leopold's system for quantifying aesthetic factors among rivers. Water Resources Research 10(3):395-401.

Hansen, R. S. 1971. Dredging: problems and remedies. Limnos 4(1):3-12.

Harrison, P. F., J. Demal, and C. Remarle. 1974. Cytophysiological changes in the pituitary gland (pars intermedia) of *Carassius auratus* L. during adaptation to a hyperosmotic environment. Arch. Anat. Microscop. Morphol. Exp. 63:299-306.

Hartung, H. O., and J. L. Tuepker. 1969. Influence of raw water characteristics on meeting requirements for water quality--dissolved materials. In: Influence of raw water characteristics on treatment. Proceedings, Eleventh Sanitary Engineering Conf., J. H. Austin and U. Weise, eds., U. of Ill. Bull. 66(121), Urbana, Ill. 61801. 143 p.

Harville, J. P. 1971. Kaiser refractories environmental studies. COM-71-00107, Natl. Tech. Infor. Serv., Springfield, Va. 22161.

Hem, J. D. 1970. Study and interpretation of the chemical characteristics of natural water. (2nd Edition) USGS Water Supply Paper No. 1473. Gov. Printing Office, Washington, D.C. 20402 363 p.

Hill, D. M. 1972. Stream faunal recovery after manganese strip mine reclamation. No. 72-16289. University Microfilms. Ann Arbor, Michigan 48106. 73 p.

Hollander, E. E., and J. W. Avault, Jr. 1975. Effects of salinity on survival of buffalo fish eggs through yearlings. Progressive Fish Culturist 37(1):47-51.

Horkel, J. D., and W. D. Pearson. 1976. Effects of turbidity on oxygen consumption of Green Sunfish, *Lepomis cyanellus*. Trans. Amer. Fisheries Soc. 105(1):107-113.

Huang, P. M. and C. P. Hwang. 1973. Inorganic and organic phosphorus distribution in domestic and municipal sewage. Water and Sewage Works 120(6)82-83.

Hynes, H. B. N. 1970. The ecology of flowing waters in relation to management. Jour. WPCF 42(3):418-424.

Kahn, S. U. 1974. Adsorption of 2,4-D from aqueous solution by fulvic acid--clay complex. Environ. Sci. & Technol. 8(3):236-238.

Kerekes, J., and J. R. Nursall. 1966. Eutrophication and senescence in a group of Prairie-Parkland Lakes in Alberta, Canada. Verh. Internat. Verein. Limnol. 16(1):65-73.

King, L. G., and R. J. Hanks. 1975. Management practices affecting quality and quantity of irrigation return flow. U.S. Environmental Protection Agency, Environmental Protection Technology Series. EPA-660/2-75-005. Gov. Printing Office, Washington, D.C. 20402. 156 p.

Lal, P., and K. S. Singh. 1973. Effects of qualities of irrigation water and fertilizers on soil properties, yield and nutrient uptake by wheat. Indian J. Agric. Sci. 43(4):392-400.

Landless, P. J. 1976. Acclimation of rainbow trout to sea water. Aquaculture 7:173-179.

Larsen, D. P., K. W. Malueg, D. W. Shults, and R. M. Buie. 1975. Response of eutrophic Shagawa Lake, Minnesota, USA, to point source, phosphorus reduction. Verh. Internat. Verein. Limnol. 19:884-892.

Larson, D. W. 1970. Limnology studies on lakes in the Deschutes National Forest, Oregon. I. Odell Lake. WRRI-4, Oregon St. U., Corvallis, Water Resources Research Institute. 34 p.

Lasker, R., R. H. Tenaza, and L. L. Chamberlain. 1972. The response of Salton Sea fish eggs and larvae to salinity stress. Calif. Fish and Game 58(1): 58-66.

Law, J. P., Jr., and G. V. Skogerboe. 1972. Potential for controlling quality of irrigation return flows. J. Environ. Qual. 1(2):140-145.

Lawrence, C. H. 1975. Estimating indirect cost of urban water use. J. Environ. Eng. Div. ASCE 101(4):517-533.

Leatherland, J. F., M. Hyder, and D. M. Ensor. 1974. Regulation of plasma Na and K concentrations in five African species of *Tilapia* fishes. Comp. Biochem. Physiol. 48A:699-710.

Leduc, G. 1972. Changes in blood chloride and osmolarity in two stocks of salmon parr (*Salmo salar*) during short-term exposure to seawater. Can. J. Zool. 50:1019-1021.

Lee, G. F., M. D. Piwoni, J. M. Lopez, G. M. Mariani, J. S. Richardson, D. H. Homer, and F. Saleh. 1975. Research study for the development of dredged material disposal criteria. Dredged Material Research Program, Contract Report D-75-4, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Miss. 39180. 379 p.

LeGore, R. S., and D. M. DesVoigne. 1973. Absence of acute effects on three-spine sticklebacks (*Gasterosteus aculeatus*) and coho salmon (*Oncorhynchus kisutch*) exposed to resuspended harbor sediment contaminants. J. Fish. Res. Board Can. 30(8):1240-1242.

Leopold, L. B. 1969. Landscape esthetics: How to quantify the sciences of a river valley. Natural History 78(8):36-45.

Liao, P. B. 1970. Pollution potential of salmonid fish hatcheries. Water and Sewage Works 117(12):291-297.

Likens, G. E., F. H. Bormann, N. M. Johnson, D. W. Fisher, and R. S. Pierce. 1970. Effects of forest cutting and herbicide treatment on nutrient budgets in the Hubbard Brook watershed-ecosystem. Ecological Monographs 40(1):23-47.

Lin, S. 1972. Nonpoint rural sources of water pollution. Circular 111. Illinois State Water Survey, Urbana, Ill. 36 p.

Lund, J. W. G. 1969. Phytoplankton. In: Eutrophication: Causes, consequences, correctives. G. A. Rohlich, ed., Natl. Acad. Sci., Washington, D.C. pp. 306-330.

Lund, L. J., H. Kohnke, and M. Paulet. 1972. An interpretation of reservoir sedimentation: II. Clay minerology. J. Environ. Qual. 1(3):303-307.

Lutz, P. L. 1972. Ionic and body compartment responses to increasing salinity in the perch *Perca fluviatilis*. Comp. Biochem. Physiol. 42A:711-717.

Manigold, D. B., and J. A. Schulze. 1969. Pesticides in selected western streams, a progress report. Pest. Monit. J. 3(2):124-135.

Masteller, M. B., W. H. Andrews, L. C. Langord, and G. E. Madsen. 1976. Measurement of streamflow aesthetic values. In: Methodologies for determination of stream resource flow requirements: An assessment. C. B. Stalnaker and J. L. Arnette, eds. Utah State University, Logan, Utah. Available: U. S. Dept. of Interior, Fish and Wildlife Service, Washington, D.C. 20240. p. 167-199.

May, R. C. 1976. Effects of Salton Sea water on the eggs and larvae of *Bairdeilla icistia* (Pisces: Sciaenidae). Calif. Fish and Game 62(2):119-131.

Middlebrooks, E. J. 1974. Review paper: Animal waste management and characterization. Water Research 8:697-712.

Miner, J. R., R. I. Lipper, L. R. Fina, and J. W. Funk. 1966. Cattle feedlot runoff--its nature and variation. Jour. WPCF 38(10):1582-1591.

McFarland, J. W. 1975. Groundwater management and salinity control--case study in Northwest Mexico. Amer. J. Agricultural Economics 57(3):457-462.

McGaha, Y. J., and J. P. Steen. 1974. The effects of variations in turbidity on cycles of planktonic and benthic organisms in flood control reservoirs of northern Mississippi. PB-234 437, Natl. Tech. Infor. Serv., Springfield, Va. 22161; Selected Water Resources Abst. 7(20),W74-10532.

McGauhey, P. H., G. L. Dugan, and D. B. Porcella. 1971. Eutrophication of surface waters--Lake Tahoe. Environmental Protection Agency, Water Pollution Control Research Series 16010 DSW 05/71. Gov. Printing Office, Washington, D.C. 20402. 154 p.

McGauhey, P. H., and E. J. Middlebrooks. 1972a. Management of wastewaters for reclamation and reuse. Water and Sewage Works 119(3):76-82.

McGauhey, P. H., and E. J. Middlebrooks. 1972b. Wastewater management. Water and Sewage Works 119(7):49-53.

McKee, J. E., and H. W. Wolf, eds. 1963. Water quality criteria. 2nd Ed. Publ. No. 3-A. The Resources Agency of California, State Water Quality Control Board. Sacramento, Calif. 548 p.

McKim, J. M., R. L. Anderson, D. A. Benoit, R. L. Spehar, and G. N. Stokes. 1976. Effects of pollution on freshwater fish. Jour. WPCF 48(6):1544-1620.

McKim, J. M., D. A. Benoit, K. E. Bresinger, W. A. Brungs, and R. E. Siefert. 1975. Effects of pollution on freshwater fish. Jour. WPCF 47(6):1711-1768.

McKim, J. M., G. M. Christensen, J. H. Tucker, D. A. Benoit, and M. J. Lewis. 1974. Effects of pollution on freshwater fish. Jour. WPCF 46(6):1540-1591.

McKim, J. M., G. M. Christensen, J. H. Tucker, and M. J. Lewis. 1973. Effects of pollution on freshwater fish. Jour. WPCF 45(6):1370-1407.

Narasimham, C., and V. Parvatheswararao. 1974. Adaptations to osmotic stress in a fresh-water euryhaline teleost, *Tilapia mossambica* X. Role of mucopolysaccharides. Act. Histochem. Bd. 51:37-49.

Oschwald, W. R. 1972. Sediment water interactions. J. Environ. Qual. 1(4): 360-366.

Patalas, K. 1973. The eutrophication of lakes in the Okanagan Valley, British Columbia. In: Proceedings, symposium on the lakes of western Canada, June 1973. Water Resources Center, University of Alberta, Edmonton. p. 336-346; Selected Water Resources Abstracts 8(20),W75-10075.

Patalas, K., and A. Salki. 1973. Crustacean plankton and the eutrophication of lakes in the Okanagan Valley, British Columbia. J. Fish. Res. Baord Can. 30(4):519-542.

Patrick, R. 1972. Aquatic communities as indices of pollution. In: Indicators of environmental quality. W. A. Thomas, ed. Plenum Press, New York. pp. 93-100.

Paulet, M., H. Kohnke, and L. J. Lund. 1972. An interpretation of reservoir sedimentation: I. Effect of watershed characteristics. J. Environ. Qual. 1(2):146-150.

Pearsall, W. H. 1932. Phytoplankton in English lakes. II. The composition of the phytoplankton in relation to dissolved substances. J. of Ecology 20(2):241-262.

Perry, W. G. 1976. Black and bigmouth buffalo spawn in brackish water ponds. Progressive Fish Culturist 38(2):81.

Peterka, J. J. 1972. Effects of saline waters upon survival of fish eggs and larvae and upon the ecology of the fathead minnow in North Dakota. PB-223 017, Natl. Tech. Infor. Serv., Springfield, Va. 22161.

Pfister, R. M., P. R. Dugan, and J. I. Frea. 1969. Microparticulates: Isolation from water and identification of associated chlorinated pesticides. Science 166:878-879.

Pierce, R. H., Jr., C. E. Olney, and G. T. Felbeck, Jr. 1974. PP'-DDT adsorption to suspended particulate matter in sea water. Geochimica Et Cosmochimica Acta 38:1061-1073.

Poirrier, M. A., B. R. Bordelon, and J. L. Laseter. 1972. Adsorption and concentration of dissolved carbon-14 DDT by coloring colloids in surface waters. Environ. Sci. and Tech. 6(12):1033-1035.

Provasoli, L. 1969. Algal nutrition and eutrophication. In: Eutrophication: Causes, consequences, correctives. G. A. Rohlich, ed. Natl. Acad. Sci., Washington, D.C. pp. 574-593.

Provasoli, L., J. J. A. McLaughlin, and I. J. Pintner. 1954. Relative and limiting concentrations of major mineral constituents for the growth of algae flagellates. Trans. New York Acad. Sci. 16(8):412-417.

Rao, G. M. M. 1971. Influence of activity and salinity on the weight-dependent oxygen consumption of the rainbow trout *Salmo gairdneri*. Marine Biol. 8(3): 205-212.

Rao, T. R. 1974. Influence of salinity on the eggs and larvae of the California killifish *Fundulus parvipinnis*. Marine Biol. 24:155-162.

Reid, G. K. 1961. Ecology of inland waters and estuaries. Reinhold Publishing Corp., New York. 375 p.

Reimold, R. J., and W. H. Queen (eds.). 1972. Ecology of halophytes. Symposium, Minneapolis, Minnesota. U.S.A. August 1972. Academic Press Inc., New York, N.Y.

Richards, L. A. 1954. Diagnosis and improvement of saline and alkaline soils. USDA Handbook No. 60. Gov. Printing Office, Washington, D.C. 20402.

Ritchie, J. C. 1972. Sediment, fish, and fish habitat. J. Soil and Water Conserv. 27:124.

Rizwanul, H., D. W. Schmedding, and V. H. Freed. 1974. Aqueous solubility, adsorption, and vapor behavior of polychlorinated biphenyl aroclor 1254. Environ. Sci. & Technol. 8(2):139-142.

Robeck, G. G. 1969. Influence of raw water color and turbidity on treatment and effluent quality. In: Influence of raw water characteristics on treatment. Proceedings, Eleventh Sanitary Eng. Conf., J. H. Austin and U. Weise, eds., U. of Ill. Bull. 66(121), Urbana, Illinois 61801. p. 143.

Rosenberg, D. M., and A. P. Wiens. 1975. Experimental sediment addition studies on the Harris River, N.W.T., Canada: The effect on macro-invertebrate drift. Verh. Internat. Verein. Limnol. 19:1568-1574.

Ruttner, F. 1952. Fundamentals of limnology. University of Toronto Press, Toronto. 295 p.

Sartor, J. D., G. B. Body, and F. J. Agardy. 1974. Water pollution aspects of street surface contaminants. Jour. WPCF 46(3):458-467.

Schiewer, V. U. 1974. Salt tolerance and the influence of increasing NaCl concentrations on the contents of nitrogen, carbohydrates, pigments and the production of extracellular carbohydrates in some freshwater bluegreen algae. Arch. Hydrobiol./Suppl B. 46(2):171-184.

Schmidbauer, A., and A. Ried. 1967. Einfluss hyperton ischer medien auf den Stoffwechsel synchron kultwierter Chlorella. Arch. Mikrobiol. 58:275-295.

Seenayya, G. 1973. Ecological studies in the plankton of certain freshwater ponds of Hyderabad-India. III. Zooplankton and bacteria. Hydrobiologia 41(4):529-540.

Shakuntala, K. 1975. Effects of temperature-salinity combinations on the digestion rates of *Gambusia affins*. Proc. Indian Acad. Sci. 81(6):249-253.

Shirgur, G. A., and H. G. Kewalramani. 1973. Observations on salinity and temperature tolerance of some of the fresh-water insects. J. Biol. Sci. 16:42-52.

Singley, J. A., and W. Chavin. 1975. The adrenocortical-hypohyseal response to saline stress in the goldfish *Carassius auratus* L. Comp. Biochem. Physiol. 51:749-756.

Skau, C. M., and J. C. Brown. 1974. Nutrients and suspended sediments from forested watersheds in the east-central Sierra Nevada. PB-238 363, Natl. Tech. Infor. Serv., Springfield, Va. 22161.

Slotta, L. S., and K. J. Williamson. 1974. Monitoring dredge spoils. Proceedings of Sem. on Meth. for Monitoring the Marine Env. (Seattle) Env. Monitoring Series EPA--600/4-74-004, U.S. Env. Protection Agency. Gov. Printing Office, Washington, D.C. 20402. p. 303-613.

Smith, L. L., Jr., R. H. Krainer, and J. C. McLeod. 1965. Effects of pulpwood fibers on fathead minnows and walleye fingerlings. Jour. WPCF 37(1):130-140.

Snyder, G. G., H. F. Haupt, and G. H. Belt, Jr. 1975. Clearcutting and burning slash alter quality of stream water in Northern Idaho. Research Paper Int.-168, U.S. Dept. of Agricul., Forest Service. 26 p.

Sorensen, D. L., T. C. Hughes, C. E. Israelsen, A. L. Huber, E. K. Israelsen, M. V. Mandavia, and L. Baker. 1976. Inventory related to water quality objectives. Bear River Basin Type IV study Idaho-Utah-Wyoming. United States Department of Agriculture, Soil Conservation Service, Salt Lake City, Utah.

Southerland, E. V. 1974. Agricultural and forest land runoff in upper South River near Waynesboro, Virginia. PB-239 967, Natl. Tech. Infor. Serv., Springfield, Va. 22161. 148 p.

Specht, D. T. 1975. Seasonal variation of algal biomass production potential and nutrient limitation in Yaquine Bay, Oregon. In: Biostimulation and nutrient assessment. E. J. Middlebrooks, D. H. Falkenborg, and T. E. Maloney, eds., Ann Arbor Science, Ann Arbor, Michigan. p. 149-174.

Spoon, D. M. 1975. Survey, ecology, and systematics of the upper Potomic estuary biota: Aufwuchs microfauna, Phase I, Proj. No. B-002-DC. Water Resources Research Center, Washington Technical Institute, Washington, D.C. 20008. 125 p.

Spoon, D. M. 1976. Survey and ecology of aufwuch protozoa and micrometazoa of the Potomic estuary 1971 and 1974. J. Protozool. 23(2):25A. (Abstract).

Stalnaker, C. B., and J. L. Arnette (Editors). 1976. Methodologies for the determination of stream resource flow requirements: An assessment. Utah State University, Logan, Utah. Available: U.S. Dept. of Interior, Fish and Wildlife Service, Washington, D.C. 20240. 199 p.

Stickney, R. R., and B. A. Simco. 1971. Salinity tolerance of catfish hybrids. Trans. Amer. Fish Soc. 100(4):790-792.

Subramanyam, O. V. 1974. Effect of salinity acclimation on the succinic dehydrogenase activity in a freshwater fish, *Heteropneustes fossilis* (Teleostei: Siluroidea). Proc. Indian Acad. Sci. 80:26-30.

Sykora, J. L., E. J. Smith, and M. Synak. 1972. Effect of lime neutralized iron hydroxide suspensions on juvenile brook trout (*Salvelinus fontinalis*, Mitchell). Water Res. 6(8):935-950.

Swale, E. M. F. 1964. A study of the phytoplankton of a calcareous river. J. Ecology 52:433-446.

Topping, M. S. 1975. Effect of environmental factors on standing crop of plankton in British Columbia lakes. Verh. Internat. Verein. Limnol. 19:524-529.

Turner, J. L. 1976. Striped bass spawning in the Sacramento and San Joaquin Rivers in central California from 1963-1972. Calif. Fish and Game 62(2): 106-118.

Turner, J. L., and T. C. Farley. 1971. Effects of temperature, salinity, and dissolved oxygen on the survival of striped bass eggs and larvae. Calif. Fish and Game 57(4):268-273.

U.S. Department of Commerce, Bureau of the Census. 1975. Census of manufacturers, 1972. Special Report Series: Water use in manufacturing, MC72(SR)-4. Gov. Printing Office, Washington, D.C. 20402. 198 p.

USU Foundation. 1969. Characteristics and pollution problems of irrigation return flow. U.S. Department of the Interior, Federal Water Pollution Control Administration, Robert S. Kerr Water Research Center, Ada, Oklahoma 74820. 237 p.

UWRL. 1976. Erosion control during highway construction. Volume II, Manual of erosion control principles and practices. NCHRP Project 16-3. Utah Water Research Laboratory, Utah State University, Logan, Utah. Available: Program Director, National Highway Research Program, Transportation Research Board, 2101 Constitution Ave., N. W., Washington, D.C. 20418. 200 p. and 8 maps.

Umminger, B. L. 1971. Osmoreularity role of serum glucose in freshwater-adapted killifish (*Fundulus heteroclitus*) at temperatures near freezing. Comp. Biochem. Physiol. 38A:141-145.

Utah State University. 1975. Colorado River regional assessment study. I. Executive summary, basin profile and report digest. PRWG165-1, Utah State University, Utah Water Research Lab., Logan, Utah 84322.

Utah State University. 1975. Colorado River regional assessment study. IV. Bibliography and appendices. PRWG165-4, Utah State University, Utah Water Research Lab., Logan, Utah 84322.

Wallen, I. E. 1951. The direct effect of turbidity on fishes. Oklahoma Ag. and Mech. College Bull. 48(2):1-27.

Warren, C. E. 1971. Biology and water pollution control. W. B. Saunders Company, Philadelphia. 434 p.

Wershaw, R. L., P. J. Burcar, and M. C. Goldberg. 1969. Interactions of pesticides with natural organic material. Environ. Sci. & Technol. 3(3): 271-273.

Wetzel, R. G. 1973-1974. Dissolved organic matter and lake metabolism. COD 1599-79, Natl. Tech. Infor. Serv., Springfield, Va. 22161. 31 p.

Wetzel, R. G., and D. L. McGregor. 1968. Axenic culture and nutritional studies of aquatic macrophytes. Am. Midland Natur. 80:52-63.

Whipple, W., J. V. Hunter, and S. L. Yu. 1974. Unrecorded pollution from urban runoff. Jour. WPCF 46(5):873-885.

Wichard, W., and H. Komnick. 1974. Fine structure and function of the rectal chloride epithelia of damselfly larvae. J. Insect Physiol. 20:1611-1621.

Williams, R., and M. F. Harcup. 1974. The fish populations of an industrial river in South Wales. J. of Fish Biol. 6(4):395-414.

Wolman, M. G. 1971. The nation's rivers. Science 174(4012):905-918.

Zafar, A. R. 1967. On the ecology of algae in certain fish ponds of Hyderabad, India: III. The periodicity. Hydrobiologia 30(1):96-112.

Zeitoun, I. H., J. E. Halver, D. E. Ullrey, and P. I. Tack. 1973. Influence of salinity on protein requirements of rainbow trout (*Salmo gairdneri*) fingerlings. J. Fish. Res. Board Can. 30(12):1867-1973.

Zeitoun, I. H., D. E. Ullrey, and P. I. Tack. 1974a. Effects of water salinity and dietary protein levels on total serum protein and hematocrit of rainbow trout (*Salmo gairdneri*) fingerlings. J. Fish. Res. Board Can. 31(6):1133-1134.

Zeitoun, I. H., D. E. Ullrey, J. E. Halver, P. I. Tack, and W. T. Magee. 1974b. Influence of salinity on protein requirements of coho (*Oncorhynchus kisutch*) smolts. J. Fish. Res. Board Can. 31(6):1145.

Zitko, V. 1974. Uptake of chlorinated paraffins and PCB from suspended solids and food by juvenile Atlantic salmon. Bull. of Environmental Contamination and Toxicology 12(4):406-412.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/3-77-042	2.	3. RECIPIENT'S ACCESSION NO.
4. TITLE AND SUBTITLE SUSPENDED AND DISSOLVED SOLIDS EFFECTS ON FRESHWATER BIOTA: A REVIEW		5. REPORT DATE April 1977 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) D.L. SORENSEN, M.M. McCARTHY, E.J. MIDDLEBROOKS, AND D.B. PORCELLA		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS UTAH STATE UNIVERSITY FOUNDATION, AND THE UTAH WATER RESEARCH LABORATORY, COLLEGE OF ENGINEERING, UTAH STATE UNIVERSITY, LOGAN, UTAH 84322		10. PROGRAM ELEMENT NO. 1BA608 11. CONTRACT/GRANT NO. P.O. No. CC6991630-J
12. SPONSORING AGENCY NAME AND ADDRESS U.S. ENVIRONMENTAL PROTECTION AGENCY - CORVALLIS, OR CORVALLIS ENVIRONMENTAL RESEARCH LABORATORY 200 SOUTHWEST 35th STREET CORVALLIS, OREGON 97330		13. TYPE OF REPORT AND PERIOD COVERED FINAL, JULY-DECEMBER, 1976 14. SPONSORING AGENCY CODE EAP/600/02
15. SUPPLEMENTARY NOTES		

16. ABSTRACT

It is widely recognized that suspended and dissolved solids in lakes, rivers, streams, and reservoirs affect water quality. In this report the research needs appropriate to setting freshwater quality criteria or standards for suspended solids (not including bedload) and dissolved solids are defined by determining the state of our knowledge from a critical review of the recent literature in this field. Although some 185 journal articles, government reports, and other references were cited herein, there is a dearth of quantitative information on the response of freshwater buita, especially at the community level, to suspended and dissolved solids.

The major research need was defined as the development and/or application of concepts of community response to suspended and dissolved solids concentrations and loads. These concepts need to be applied especially to the photosynthetic, the microfauna, and macrofauna levels. Fish studies are of lower priority since more and better research has been reported for these organisms.

In addition, the role of suspended solids in transporting toxic substances (organics, heavy metals), aesthetic evaluation of suspended solids in aquatic ecosystems, and dissolved solids in drinking water, and economic aspects of dissolved solids in municipal-industrial water were defined as research needs.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
review, suspended solids, suspended sediment, turbidity, residue, dissolved solids, salinity, TDS, conductivity, freshwater, fish, invertebrates, zooplankton, algae, water supply, livestock, aesthetics, nutrients, chlorinated organics, erosion, irrigation, standards, research needs		0203 2006 0502 0603 0704 0801 0808 1407

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO. OF PAGES 73
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (9-73)

☆ U. S. GOVERNMENT PRINTING OFFICE: 1977—797-589/99 REGION 10

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Technical Information Staff
Cincinnati, Ohio 45268

OFFICIAL BUSINESS
PENALTY FOR PRIVATE USE, $300
AN EQUAL OPPORTUNITY EMPLOYER

POSTAGE AND FEES PAID
U.S. ENVIRONMENTAL PROTECTION AGENCY
EPA-335

U.S.MAIL

Special Fourth-Class Rate
Book

If your address is incorrect, please change on the above label; tear off; and return to the above address.
If you do not desire to continue receiving this technical report series, CHECK HERE ☐; tear off label, and return it to the above address.

UMA | AECOM

CAPP Freshwater Salinity Working Group and the Salt Technical Advisory Sub-committee of the British Columbia Upstream Petroleum Committee

A Review of the Toxicological Literature for Salt – 2002 to 2007

Prepared by:

Doug A. Bright, Ph.D., RPBio. and Michelle Meier, B.Sc.
UMA Engineering Ltd.

200-415 Gorge Rd. E
Victoria, BC, V8T 2W1,
Tel. 250-475-6355;

Project Number G016 002 00

September 2007

EXECUTIVE SUMMARY

As a follow up to the Derivation of Soil Quality Matrix Soil Standards for Salt under the British Columbia Contaminated Sites Regulation Draft Document, prepared by Doug Bright and Jan Addison (2002), a literature review was conducted to assess any new scientific research into the toxicity potential of salt ions to aquatic life and plants. In particular, there is an interest in whether recent and emerging scientific knowledge would alter conclusions in Bright and Addison (2002) regarding thresholds of toxicity for sodium or chloride in soil and water. The literature review, conducted by UMA Engineering Ltd. (UMA) using online searchable databases of scientific journals, was limited to new research published since 2002.

Seventeen relevant research papers were identified, although very few of these were directly related to sodium chloride (NaCl) toxicity to terrestrial and freshwater life. The purpose of this report is to provide a summary of recent research examining salt toxicity to wildlife and plants and to evaluate this new data in the context of the draft soil matrix standards for salts and the BC Working and Approved Water Quality Guidelines for the protection of aquatic life.

Table S-1 summarizes the toxicity data applicable to plant and animal species that might be surrogates for those found in BC, and compares toxicity values to the draft soil matrix salinity standards (Bright *et al*, 2002) and the provincial Working and Approved Water Quality Guidelines (MoE, 2006). Exposures involving simultaneous exposure to another substance are not summarized in Table 1. Similarly, studies involving hydroponic or soil solution exposures are also not included Table 1, since the basis of expression of exposure concentration is not conducive to comparison with BC draft soil matrix standards for salt ions.

While limited new data were located for freshwater organisms and plants, no new data were found for soil invertebrates.

Table S-1: Summary of Toxicity Test Results for BC Species				
Study	**Toxicity Test**	**Organism**	**Result**	**Guideline**
Davies and Hall (2006)	48 hour acute Cl^- toxicity test in water with varying Ca:Mg ratios.	*Daphnia magna*	LC50 results: 3,136 mg/L Cl^- (0.7 Ca:Mg); 3,222 mg/L Cl^- (1.8 Ca:Mg); 3,137 mg/L Cl^- (7.0 Ca:Mg);	BC Approved Water Quality Guideline for chloride: 150 mg/L
Diamond *et al* (2005)	Pulsed exposure of NaCl in water at varying concentrations and durations for 7 days	Fathead minnows (*Pimephales pomelas*)	Decreased survival with 4 g/L NaCL for 96 hours, 8 g/L for 24 hours and 12 g/L for 3 hours	BC Approved Water Quality Guideline for chloride: 150 mg/L
Sanzo and Hecnar (2005)	96 hour acute NaCl toxicity test	Wood frog tadpoles (*Rana sylvatica*)	LC50 results: 2,636 mg/L by Spearman-Kaber and 5,109 mg/L by probit analysis	BC Approved Water Quality Guideline for chloride: 150 mg/L
Sanzo and Hecnar (2005)	90 day chronic NaCl toxicity test using concentrations of 0.0, 0.39, 77.8, and 1030 mg/L NaCl	Wood frog tadpoles (*Rana sylvatica*)	Lower survivorship, decreased time to metamorphosis at 1030 mg/L NaCl, as well as reduced weight and activity, and increased physical abnormalities with increasing salt concentration.	BC Approved Water Quality Guideline for chloride: 150 mg/L

Based on a review of the most recent published scientific information, it is concluded that toxicological data for salt which became available post-2002 would not substantively change the draft matrix soil standards derived in 2002 for either sodium (Na^+) or chloride (Cl^-).

TABLE OF CONTENTS

1. Introduction

As a follow up to the Derivation of Soil Quality Matrix Soil Standards for Salt under the British Columbia Contaminated Sites Regulation Draft Document, prepared by Doug Bright and Jan Addison (2002), a literature review was conducted to assess any new scientific research into the toxicity potential of salt ions to aquatic life and plants. In particular, there is an interest in whether recent and emerging scientific knowledge would alter conclusions in Bright and Addison (2002) regarding thresholds of toxicity for sodium or chloride in soil and water. The purpose of this report is to provide a summary of scientific research published between 2002 and August 2007 regarding the ecotoxicity of chloride or sodium ion.

The literature review was conducted by UMA Engineering Ltd. (UMA) and utilized online searchable databases of scientific journals. The literature review was limited to new research published since 2002. Online databases searched included –

Springerlink,	ScienceDirect,	Wiley Interscience,
Royal Society of Chemistry,	Oxford University Press,	JSTOR,
American Chemical Society,	SETAC online journals	

A summary is provided below of recent knowledge on –

- Salt Toxicity to Aquatic Life (Section 2), and
- Salt Toxicity to Plants (Section 3).

No new studies on soil invertebrates and salt exposures were found.

Section 4 provides major conclusions. Sections 2 and 3 include a review of studies where the test organism(s) were exposed to multiple substances including salt ions, as well as hydroponic exposures of plants. Such studies, however were deemed to be not relevant for assessing salt toxicity thresholds.

2. Salt Toxicity to Freshwater Life

Recent research on salt toxicity to wildlife is limited. Few studies were found that pertain directly to NaCl toxicity. Several studies found pertained to the chemical toxicity of metals, organic and inorganic pollutants in saline solutions and whether or not salts enhanced or inhibited a toxicological response in test organisms.

Dethloff *et al* (2007) examined the chronic toxic effects of silver on the early life stages of rainbow trout (*Oncorhynchus mykiss*) in the presence and absence of 49 mg/L NaCl and assessed possible protective effects of sodium chloride to silver toxicity. Chronic toxicity did not appear to be greatly modified by the presence of sodium chloride in this experiment although a reduction in silver uptake was observed based on whole body analyses.

A similar study was conducted by **Naddy *et al* (2007)** that looked at chronic exposure of an early life stage and short term chronic effects of silver toxicity on fathead minnows (*Pimephales pomelas*) in water amended with or without 60 mg/L NaCl. The researchers found that chronic toxicity of silver was mitigated to some extent by NaCl addition based on increases in the maximum acceptable tolerable concentration (MATC) and inhibition concentration of 20% of the population (IC_{20}) compared to those found in unamended waters. Sodium chloride also appeared to provide a level of protection from silver accumulation in the early life stage study but not in the short term chronic study. The results of this research suggest a protective chloride and/or sodium effect in short-term and chronic exposures from ionic Ag^+ using fathead minnows.

Protective effects of chloride from sulfate toxicity were assessed by **Soucek and Kennedy (2005)** in acute toxicity tests of sulfate to *Ceriodaphnia dubia*, *Chironomus tentans*, *Hyalella azteca*, and *Sphaerium simile*. The toxicity tests were used to determine the LC_{50} concentrations for sulphate in solutions of varying water compositions including high TDS solutions. Protective effects of chloride on sulfate toxicity to *H. azteca* was assessed by exposing *H. azteca* to 2800 mg/L sulphate in six different concentrations of chloride for 96 hours. The results showed that sulfate toxicity to *H. azteca* decreased with increased levels of chloride. At the lowest measured chloride concentration of 5 mg/L, only 20% of the test organisms exposed to 2846 mg/L sulfate were alive after 96 hours. At 13 mg/L Cl^- survival increased nominally, but not significantly; however significant increases in survival were observed at and above 18 mg/L Cl^-. Survival was 85% and 100% in the 36 and 67 mg/L Cl^- treatments respectively. The results of the *H. azteca* experiment support the hypothesis that chloride has a protective effect against sulphate toxicity, because incremental increases in chloride were associated with incremental increases in survival.

In another experiment, **Soucek (2007)** examined the influence of both chloride and water hardness on acute toxicity of sodium sulfate to *Hyalella azteca* and *Ceriodaphnia dubia*. Results from the study indicated that chloride had varying effects on sodium sulfate toxicity to *C. dubia* and *H. azteca* over the range of 5 to 500 mg/L Cl^-. For *H. azteca,* increasing chloride concentration from 5 to 25 mg/L resulted in increased sulfate LC_{50}s. For *C. dubia* the slope was not significantly different from zero over this chloride concentration range. In addition LC_{50}s for *C. dubia* were higher than those for *H. azteca* for each chloride concentration over this range. Although a positive relationship between chloride concentration and sulphate LC_{50} was observed for *H. azteca* over the range of 5 to 25 mg/L Cl^-, a significant negative trend was observed over the range of 25 to 500 mg/L Cl^-. An even stronger negative relationship was observed for *C. dubia* over the same chloride range.

These studies did not asses the toxic effects of chloride directly on test organisms; therefore, a comparison to the BC Approved Water Quality Guidelines (BC AWQG) for chloride could not be made.

Only one study was found that investigated chloride toxicity to *Daphnia magna* in exposure waters of varying Ca:Mg ratios and hardness.

Davies and Hall (2006) conducted acute chloride toxicity tests using reformulated waters with Ca:Mg ratios of 0.7, 1.8, and 7.0. *D. magna* was exposed to saline waters (NaCl) at 100 mg/L hardness for all Ca:Mg ratios. Mortality was determined at the end of the 48 hour exposure period. The mean median lethal concentration for *D. magna* in NaCl toxicity tests at 100 mg/L hardness expressed as Cl^- anion concentration was 3,136 mg/L (0.7 Ca:Mg), 3,222 mg/L (1.8 Ca:Mg), and 3,137 mg/L (7.0 Ca:Mg). No significant difference was found between exposure waters at different Ca:Mg ratios and chloride toxicity. The chloride concentrations found to elicit mortality in this study were far higher then the BC AWQG for chloride, which is 150 mg/L chloride. This indicates that the BC water quality guideline for chloride would be protective of chloride toxicity to freshwater aquatic invertebrates.

In the above-described studies by Dethloff *et al* (2007) and Naddy *et al* (2007), protective effects of sodium chloride to silver toxicity on two freshwater fish species was observed. Similarly a protective effect of chloride on sulfate toxicity to *H. azteca* was also found by Soucek and Kennedy. However, a study conducted by Barwardi (2007) found sodium chloride to enhance the toxic effects of the agricultural pesticide fethion on three freshwater fish species.

Bawardi *et al* (2007) examined the impacts of hypersaline water on the biotransformation and toxicity of fethion, an agricultural pesticide, on rainbow trout (*Oncorhynchus mykiss*), striped bass (*Morone saxatilitis x Morone chrysops*) and tilapia (*Oreochromis mossambicus*). Results from the

96 hour toxicity test indicated that rainbow trout exposed to fenthion in a hypersaline environment experience significantly greater toxicity (LC_{50} of 0.18 mg/L fenthion) than those fish in freshwater treatments with no additional NaCl (LC_{50} 1.12 mg/L fenthion). The LC_{50} for freshwater treated tilapia was 6.59 mg/L fenthion, compared to 4.56 mg/L fenthion in hypersaline conditions. Although a trend toward salinity-enhanced toxicity was observed, a significant difference between the two treatments was not observed in experiments with rainbow trout and tilapia. In contrast, toxicity testing with striped bass indicated a significant difference between freshwater and hypersaline environments, with LC_{50} values being 13.2 mg/L fenthion and 2.8 mg/L fenthion, respectively. A comparison of the three freshwater fish species indicated that trout were approximately 10 times more sensitive to fenthion toxicity than striped bass or tilapia in freshwater treatments. In hypersaline conditions, trout were 36 and 16 times more sensitive than tilapia or striped bass respectively.

Although these studies are not directly related to NaCl toxicity on aquatic life they do indicate that hypersaline solutions may enhance or inhibit the chemical toxicity of pollutants within the aqueous environment on aquatic organisms.

Two studies were found that included toxicity tests of NaCl on fish species.

Arezon *et al* (2003) evaluated the feasibility of use of a Brazilian fish, *Cynopoecilus melanotaenia,* as a test organism in toxicity tests. Three reference substances were used in a 96 hour acute toxicity test, one of which was sodium chloride (NaCl). Fry of *C. melanotaenia* were placed in five concentrations of NaCl plus one control (0.6, 0.9, 1.4, 1.7 and 2.0 g/L NaCl). Results of the 96 hour toxicity test for sodium chloride showed a mean EC_{50} of 1.7 g/L NaCl. Although this fish species is not found in British Columbia,a comparison of the EC_{50} result for sodium chloride in this study with the BC AWQG for salinity in freshwater environments shows that the BC guideline of 150 mg/L (0.15 g/L) NaCl would provide protection from NaCl toxicity for this fish species.

The second, more relevant study of NaCl toxicity to fish was conducted by **Diamond *et al* (2005)** who examined the toxic effects of pulsed or fluctuating contaminant exposures to fathead minnows (*Pimephales pomelas*) using concentrations of NaCl. Six day old fathead minnows were exposed to pulses of NaCl at varying concentrations and durations for 7 days (4 g/L for 96 hours, 6 g/L for 24 hours, 8 g/L for 24 hours, and 12 g/L for 3 hours). Results showed significant decreases in survival with the 4 g/L 96 hour, 8 g/L 24 hour and 12 g/L 3 hour pulses compared to the control. Fish lethality generally occurred within 24 hours of the pulse, however, continued mortality over time was observed in the highest magnitude pulse (12 g/L) when exposure duration was relatively short (3 hours). The lack of change in survival after 72 hours of testing in most treatments indicated that mortality effects of NaCl had stabilized.

Gomez-Mestre and Tejedo (2003) conducted several experiments to determine the effect of salinity on embryonic and early larval stages of *Bufo calamita*, a species of frog that breeds in both brackish and freshwater environments in Spain. Through a combination of field transplant and common garden experiments this study showed that water salinity decreased survival probability of individuals in all populations, prolonged their larval period and reduced their mass at metamorphosis. Acute toxicity experiments on embryonic and early larval phases found that for three freshwater populations of *B. calamita* mortality within the first 48 hours remained low until a concentration of 10 g/L total dissolved solids (TDS) was reached and increased steeply afterward. Embryos from the brackish populations of *B. calamita* suffered no mortality at all during the first 48 hours of the experiment at all salinity concentrations (2, 4, 6, 8, 10, 12 g/L total dissolved solids). Differences in tolerance among populations were highly significant during the first 48 hours of exposure. After 72 hours, differences among populations had disappeared. LC_{50} values decreased as time went on, suggesting that mortality was not just restricted to the impact of the initial exposure to the osmotic stress but that water salinity had a chronic effect on continuous exposure. Embryos from the brackish water population showed an initial higher tolerance to osmotic stress, but after three days of exposure the acute levels of salinity had the same effect on it as on the freshwater populations and all ended up with LC_{50} values ranging from 8 to 10 g/L. Higher osmotolerance of brackish water populations was further supported by the results of the embryonic common garden experiment. Survival of the embryos decreased at the highest salinity on average 62% in the freshwater populations but only 20% in the brackish populations. In BC, there are no approved guidelines for TDS for the protection of freshwater aquatic life; however, there exists a working water quality guidelines for TDS for livestock watering (1 g/L for sensitive species, and 3.0 g/L for other species), and for irrigation (0.5 to 3.5 g/L max, crop and soil dependent). A concentration of 500 mg/L is the BC AWQG for TDS in drinking water.

Sanzo and Hecnar (2005) examined the effects of roads salts (NaCl) on wood frog (*Rana sylvatica*) tadpoles exposed for 96 hours. Tests revealed 96 hour LC_{50} values of 2636 mg/L by the Spearman-Kaber method and 5109 mg/L by probit anaylsis. Linear regression revealed a significant decrease in tadpole weight at 96 hours as NaCl concentrations increased. Physical and behavioral effects in all salt exposure levels were observed with the most pronounced effects seen at higher NaCl concentrations. A 90 day chronic experiment revealed significantly lower survivorship, decreased time to metamorphosis, reduced weight and activity, and increased physical abnormalities with increasing salt concentration (0.00, 0.39, 77.9 and 1030 mg/L). Time to metamorphosis was significantly different from the control at an exposure concentration of 1030 mg/L. Similarly, number of successfully metamorphosed tadpoles per treatment group was only significantly lower than the control in the 1030 mg/L treatment The BC WWQG for salinity is set at 150 mg/L chloride, which is equivalent to 247 mg/L NaCl assuming both anion and cation is present on an equimolar basis. BC AWQG for chloride in freshwater environments would provide protection from NaCl toxicity for this amphibian species.

Valenti *et al* (2006) conducted a study on the toxicity of chlorine as hypochlorite to freshwater mussels. This study, therefore, is not relevant for assessing toxicity of chloride ion. The objective of the study was to assess the level of risk that chlorine toxicity poses to early life stages of unionids. A series of experiments were conducted with glochidia from various species of freshwater mussels to determine their tolerance of total residual chlorine (TRC). Chronic tests were conducted using juvenile mussels for 21 days. Bioassays with 3, 6 and 12 month old juveniles of *Villosa iris* were conducted to examine the relationship between age and toxicity. In addition a 21 day test with 2 month old juveniles of *Epioblasma capsaeformis*, a United States federally endangered species was conducted to compare sensitivities between species. For the acute toxicity test glochidia were exposed to varying concentrations of calcium hypochlorite (high test hypochlorite, (HTH) in moderately hard reconstituted water as the TRC toxicant. Survivorship was assessed every 24 hours until 72 hours of exposure. Juvenile mussels were exposed to HTH at varying concentrations for 21 days in the chronic toxicity tests. After 21 days the juveniles were removed from test chambers and assess for growth and survivorship. The results of the acute glochidia tests showed that the three endangered species *E. brevidens, E. capsaeformis* and *Alasmidonta. heterodon* were slightly more sensistive to chlorine than *Lampsilis fasciola* and far more sensitive than *V. iris* after 24 hours of exposure. At 250 µg TRC/L average survivorship for the more sensitive species (<20%) was nearly half that of the respective value for *L. fasciola* (35%) and less than a third for *V. iris* (66%). In concentrations of 30 µg TRC/L and lower, survivorship remained greater than 90% for all species after 24 hour except *E. brevidens* (79-87%). All exposed glochidia died at 500 µg TRC/L. After 48 hours of exposure, survivorship in chlorinated treatments differed only slightly for *V. iris* (mean LC50=260 µg/L) and *A. heterodon* (mean LC_{50}=95 µg/L), although it decreased substantially for *L. fasciola* (mean LC_{50}=80 µg/L). Significant declines were observed in experiments with three and six month old *V. iris* juveniles in the 21 day chronic toxicity tests. Adverse effects were observed at lower concentrations in experiments with three month old juveniles as survivorship declined to 50% at 30 µg TRC/L. Survivorship for six month old juveniles remained =90% in concentrations as high as 120 µg/L and was significantly lower than the control only at concentrations =250 µg/L TRC/L. No concentration in the tests caused significant declines in survivorship for 12 month old juveniles and survivorship remained 80% even at 500 µg TRC/L. In terms of growth all three age classes grew significantly less at concentrations =60 µg TRC/L then those in controls. Average growth was reduced relative to controls by 37 to 80% in exposures with TRC concentration of 30 to 120 µg/L and by 90% in exposures of 250 µg/L and greater. The lowest observed adverse effect concentrations for three-month old *V. iris* was determined to be 30 µg/L and 60 µg/L for 6 and 12 month old *V. iris* juveniles. Two month old *E. capsaeformis* juveniles were more sensitive than any age class of *V. iris*. Growth was significantly reduced at concentrations of 20 µg TRC/L and higher, as exposed individuals grew less than 20% relative to those in the control. Observed mortalities was considerably high in the tests, as 50% or more of the individuals died at concentrations of 30 µg/L and higher. The lowest observed adverse effect concentration for *E. capsaeformis* approached the US EPA 4-day maximum freshwater water quality criteria of 11 µg/L TRC. All individuals in the 120 µg/L exposure died after 21 days of exposure, whereas those in the control and 5 µg/L had average survivorship of 80 and 100%, respectively. The results of this study show that glochidia are more tolerant of TRC than many aquatic species. In particular, researchers have reported toxicological endpoints for cladocerans that were much lower than those found in this study. This study also showed that juvenile mussels may be able to survive high dose acute exposures; the impact of long-term exposure to low doses may result in sublethal impairment that could lower their chances of surviving the multi-year juveniles stage and being recruited to the reproducing population. Since this study looked at toxicity of total residual chlorine in water no direct comparison can be made to the chloride concentration set to protect freshwater aquatic life in British Columbia.

3. Salt Toxicity to Plants

Research found on toxicity of salt to plants focused mainly on agricultural crops (soybeans, barley, *etc*) and its effect on nutrient uptake, root and shoot growth. Two studies were found to pertain to forest tree species and NaCl toxicity. A significant limitation of many of these studies relative to define acceptable soil-based sodium or chloride thresholds is that the plants were exposed hydroponically (Cramer, 2002; Bayuelo-Jimeniz *et al.,* 2003; Luo *et al*, 2004) or the concentration was expressed as a concentration in irrigation water (Franklin et al., 2002; Wilson et al., 2006; Apostol *et al,* 2002).

Cramer (2002) evaluated the inhibitory effect of salinity on leaf extension of three different grass species: *Hordeum jubatum* L.(Foxtail Barley), *Hordeum vulgare* L. (Common Barley) and *Zea mays* L (Sweet Corn). Plants were exposed to NaCl added to a hydroponic solution (0, 40, 80 and 120 mM NaCl) and changes in leaf elongation rate (LER) were measured over time with a displacement transducer. Leaf elongation of plants was reduced immediately by the addition of salinity to the nutrient solution. Initially, LER declined rapidly in response to salinity, but then entered a recovery phase, reaching a steady state rate after 5 hours for all species and salt treatments. The steady-state response was lower than the controls and was proportional to the level of salinity. At lower salt concentrations (40 and 80 mM), distinctions between species was small. However, clear distinctions were found between species responses at 120 mM NaCl. In general *H. jubatum* was more tolerant than *Z. mays*, which was more salt tolerant than *H. vulgare* to these short-term salinity stresses. In contrast, barley was more salt tolerant then maize over the long term. The mechanism of inhibition of LER by salinity as tested by the applied tension technique varied with the species examined affecting either the apparent yield threshold, the hydraulic conductance of the whole plant or both.

Wilson *et al* (2006) studied the physiological processes involved in cowpea differential growth response of four major USA cowpea cultivars to increasing salinity. The effect of salinity on leaf gas exchange of net photosynthetic rate per unit leaf mass (Pnm), and per unit leaf area (Pna) and stomatal conductance (gs) were examined. Seven salinities ranging from 2.6 to 20.5 dSm-1 were constructed using NaCl, $CaCl_2$ and $MgSO_4$ as the salinization salts. A highly significant salt effect on Pna, Pnm, gs and specific leaf weight (SLW) was found for all four cowpea cultivars. The salinity level resulting in a maximum leaf gas exchange (C_{max}) was determined to be 6.1 dSm^{-1} compared to 6.0 dSm^{-1} for the vegetative stage and the flowering stage respectively, and C_{50} values (salinity level resulting in a 50% reduction of the maximum leaf exchange) was 17.5 dSm^{-1} versus 17.8 dSm^{-1} for the vegetative stage and the flowering stage respectively.

Bayuelo-Jimeniz *et al* (2003) studied the effects of salinity on four wild (*Phaseolus angustissimus, P. filiformis, P. microcarpus, and P. vulgaris*) and two cultivated (*P. acutifolius and P. vulgaris* L.) *Phaseolus* species. Relative growth rate (RGR), unit leaf rate (ULR), leaf area ratio (LAR), specific leaf area (SLA) leaf weight ratio (LWR) and rate of ion uptake were calculated for the period between 10 and 20 days after planting. Salinity stress had a significant effect on root, shoot and total dry weight and root:shoot ratio, and differences among species for all characters were highly significant. In both cultivated and wild accessions, salinity inhibited shoot growth more than root growth. Shoot dry weight was significantly reduced in all taxa at all salinity levels, whereas root dry weight was significantly reduced at 40 mM NaCl but no further reduction was observed at 80 mM NaCl. At day 20 shoot and root dry weights of *P. filiformis* were reduced to 50 and 25% of control plants by 80 mM NaCl. For all other species, shoot and root dry weights were reduced by 60 -75% and 29-56% respectively of the control values in the 80 mM NaCl treatment. Salinity increased the root:shoot ratio, but these ratios significantly decreased in *P. acutifolius* after 20 days of growth. The effect of salt stress on the number of leaves and leaf area were similar to that on shoot growth in all accessions. Number of leaves at day 20 significantly decreased with increasing salinity and duration of salt stress, resulting in decreased leaf area. After 20 days of salt stress total leaf area was reduced by 44-66% in accessions grown in 40 mM NaCl and 77-91% in those grown in 80 mM NaCl. Salinity stress decreased RGR. Differences in RGR between unsalinized plants and plants treated with 40 mM NaCl were evident after 20 days. In all species RGR decreased with increasing salinity and with the period of exposure. ULR decreased over time in all species, particularly in salinized plants. Salinity reduced LAR in all species. The lowest LAR was found in one of the slowest growing species, *P. angustissimus*. Salinity significantly affected leaf water, osmotic, and turgor potentials as well as stomatal conductance and CO_2 assimilation rate. Tissue concentrations of Cl^- and Na^+ ions increased significantly in response to salt treatments. In all taxa the concentration of Na^+ increased almost in parallel in stems and roots with increasing salinity, whereas the concentration of Cl^- increased more in stems and leaves than in roots. Uptake rate of K^+, Ca^{2+}, and Mg^{2+} were significantly affected by increasing NaCl. On day 20 salinity had significantly increased the absorption rates of Cl and Na.

Luo *et al* (2004) conducted experiments to determine ion-specific stress effects of Na^+ and Cl^- on seedlings of cultivated (*Glycine max* L. Merr) and wild soybean (*Glycine soja* Sieb & Zucc.). Results showed that under NaCl stress Cl^- was more toxic than Na^+ to seedlings of *G. max*. A positive correlated was found with the content of Cl^- in the leaves and ionic stress resulting in injury in *G. max*. A negative correlation was found between Cl^- content in roots and plant injury. Seedlings of *G. max* cultivars (salt-tolerant Nannong 1138-2 and salt sensitive Zhongzihuangdou-yi) and two *G. soja* populations were exposed to 150 mM Na^+, Cl^- and NaCl respectively. *G. max* Nannong 1138-2 and Zhongzihuangdou-yi were damaged much more heavily in the solution of Cl^- than in that of Na^+. The leaves were found to be more sensitive to Cl^- than to Na^+, and salt tolerance of these two *G. max* cultivars was mainly due to successful withholding of Cl^- in the roots and stems to decrease its content in the leaves. A reverse response was observed to isoosmotic stress of 150 mM Na^+ and Cl^- was shown in *G. soja* populations, their leaves were not as susceptible to toxicity of Cl^- as that of Na^+. In this case salt tolerance was mainly due to successful withholding of Na^+ in the roots and stems to decrease its content in the leaves. The results of this experiment indicate that *G. soja* have advantages over *G. max* in those traits associated with the mechanism of Cl^- tolerance, such as its withholding in roots and vauoles of leaves.

Franklin *et al* (2002) compared the effects of NaCl and Na_2SO_4 on the nutrient status of jack pine (*Pinus banksiana*). Twenty-eight week old germinated seedlings of *P. banksiana* were exposed to 60 mM NaCl and 60 mM Na_2SO_4 and maintained for 10 weeks. Salt treatments decreased shoot dry weights and shoot elongation rates. Shoot dry weight was reduced by 36% in 60 mM Na_2SO_4 treated seedlings and by 40% in 60 mM NaCl treated seedlings. The percentage of seedlings exhibiting terminal bud flush decreased slightly from 98.4% in the control seedlings to 91.9% and 90.2% in Na_2SO_4 and NaCl treatments, respectively. Growth and injury of seedlings were found to be more affected by sodium chloride than sodium sulfate. While a small but significant amount of needle necrosis (8%) occurred in Na_2SO_4 treated seedlings, NaCl treatment resulted in a significantly greater necrosis of approximately 21% of the needle dry weight. Chlorophyll *a* and total carotenoid content were reduced in NaCl treated plants. Sodium chloride treated plants exhibited a delay in flushing of the terminal buds, reduced carotenoid content, extensive needle necrosis, and elevated levels of K, Mg, Mn, N and P in the shoot. Na_2SO_4 treated seedlings resulted in reduced shoot calcium and potassium concentrations, while those of nitrogen and phosphorus increased. Needle necrosis was correlated with tissue Na^+ only in sodium chloride treated plants, and no relationship was found between growth or necrosis, and tissue levels of Cl^- or nutrition elements. This study showed that greater toxicity of sodium chloride in jack pines was not due to nutrient deficiency.

Apostol *et al* (2002) examined the response of 6 month old jack pine seedlings to boron and salinity (NaCl and Na_2SO_4) treatments. During 4 weeks of exposure 60 mM NaCl and 60 mM Na_2SO_4 significantly decreased survival, new shoot length, number of new roots, shoot to root dry weight ratio and transpiration rates. When applied in absence of the salts Boron had little effect on the measured variables. However, when applied together with salts, Boron decreased seedling survival, increased needle injury and altered tissue elemental concentrations in jack pine seedlings. In 2 mM Boron treatment Boron concentration was higher in shoots than in the roots. However, when 2 mM Boron was present in NaCl and Na_2SO_4 treatments, shoot Boron concentration declined and greater proportion of Boron accumulated mostly in the roots. Based on the electrolyte leakage and needle necrosis data, Cl^- appears to be the major factor contributing to seedling injury and Boron aggravates the injurious effects of NaCl. The authors suggest that Cl^- may contribute to Na and B toxicity in jack pine by altering cell membrane permeability leading to increased Na concentration in the shoots.

4. Conclusions

Very limited research has been conducted since 2002 on the toxicity of sodium chloride to plants and wildlife. Seventeen relevant research papers were identified, although very few of these were directly related to sodium chloride (NaCl) toxicity to terrestrial and freshwater life.

Table 1 summarizes the toxicity data applicable to plant and animal species that might be surrogates for those found in BC, and compares toxicity values to the draft soil matrix salinity standards (Bright *et al*, 2002) and the provincial Working and Approved Water Quality Guidelines (MoE, 2006). Studies involving simultaneous exposure to one or more additional substance are not summarized in Table 1, since these are not deemed to provide comparable data for assessing salt toxicity in isolation.

Table 1: Summary of Toxicity Test Results for BC Species

Study	Toxicity Test	Organism	Result	Guideline
Davies and Hall (2006)	48 hour acute Cl^- toxicity test in water with varying Ca:Mg ratios.	*Daphnia magna*	LC50 results: 3,136 mg/L Cl^- (0.7 Ca:Mg); 3,222 mg/L Cl^- (1.8 Ca:Mg); 3,137 mg/L Cl^- (7.0 Ca:Mg);	BC Approved Water Quality Guideline for chloride: 150 mg/L
Diamond *et al* (2005)	Pulsed exposure of NaCl in water at varying concentrations and durations for 7 days	Fathead minnows (*Pimephales pomelas*)	Decreased survival with 4 g/L NaCL for 96 hours, 8 g/L for 24 hours and 12 g/L for 3 hours	BC Approved Water Quality Guideline for chloride: 150 mg/L
Sanzo and Hecnar (2005)	96 hour acute NaCl toxicity test	Wood frog tadpoles (*Rana sylvatica*)	LC50 results: 2,636 mg/L by Spearman-Kaber and 5,109 mg/L by probit analysis	BC Approved Water Quality Guideline for chloride: 150 mg/L
Sanzo and Hecnar (2005)	90 day chronic NaCl toxicity test using concentrations of 0.0, 0.39, 77.8, and 1030 mg/L NaCl	Wood frog tadpoles (*Rana sylvatica*)	Lower survivorship, decreased time to metamorphosis at 1030 mg/L NaCl, as well as reduced weight and activity, and increased physical abnormalities with	BC Approved Water Quality Guideline for chloride: 150 mg/L

			increasing salt concentration.	

Similarly, experiments involving soil solution or hydroponic exposures are excluded from the Table 1 summary, since the data are of limited relevance in the context of this exercise. Additional research not directly related to BC species or sodium chloride toxicity was included in this review to provide complimentary information for risk assessors on salt interactions with other toxiciants, and on altered bioavailability of environmental contaminants to terrestrial and aquatic life.

Based on a review of the most recent published scientific information, it is concluded that toxicological data for salt which became available post-2002 would not substantively change the draft matrix soil standards derived in 2002 for either sodium (Na+) or chloride (Cl-).

5. References

Apostol, K.G., Zwiazek, J.J., MacKinnon, M.D. (2002). NaCl and Na_2SO_4 alter responses of jack pine (*Pinus banksiana*) seedlings to boron. Plant and Soil 240 pp 321-329

Arenzon, A., Pinto, R.F., Colombo, P., Raya-Rodriguez, M.T. (2003). Assessment of the freshwater annual fish *Cynopoecilus melanotaenia* as a toxicity test organism using three reference substances. Environmental Toxicology and Chemistry Vol 22 No. 9 pp 2188-2190.

Bayuelo-Jimenez, J.S., Debouck, D.G., Lynch, J.P. (2003). Growth, gas exchange, water relations, and ion composition of *Phaseolus* species grown under saline conditions. Field Crops Research 80 pp 207-222.

Bawardi, O., Rimoldi, J., Schlenk, D. (2007). Impacts of hypersaline water on the biotransformation and toxicity of fenthion on rainbow trout (*Oncorhynchus mykiss*), striped bass (*Morone saxatilis* X *Morone chrysops*) and tilapia (*Oreochromis mossambicus*). Pesticide Biochemistry and Physiology 88 pp 321-327.

Cramer, G.R. (2003) Differential effects of salinity on leaf elongation kinetics of three grass species. Plant and Soil Volume 253 Number 1 pp 233-244

Davies, T.D., Hall, K.J. (2007). Importance of calcium in modifying the acute toxicity of sodium sulphate to *Hyalella azteca* and *Daphnia magna*. Environmental Toxicology and Chemistry Vol 26 No 6 pp 1243-1247.

Dethloff, G.M., Naddy, R.B., Gorsuch, J.W. (2007). Effects of sodium chloride on chronic silver toxicity to early life stages of rainbow trout. Environmental Toxicology and Chemistry Vol. 26, No. 8. pp. 1717-1725

Diamond, J., Bowersox, M., Latimer, H., Barbour, C., Bearr, J., Butcher, J. (2005). Effects of pulsed contaminant exposures on early life stages of fathead minnow. Archives of Envionmental Contamination and Toxicology 49 pp 511-519

Franklin, J.A, Zwiazek, J.J., Renault, S., Croser, C. (2002). Growth and elemental composition of jack pine (*Pinus banksiana*) seedlings treated with sodium chloride and sodium sulfate. Trees 16 pp 325-330.

Gomez-Mestre, I., Tejedo, M. (2003). Local adaptation of an anuran amphibian to osmotically stressful environments. Evolution Vol. 57 No. 8 pp 1889-1899.

Luo, Q., Yu, B., Liu, Y. (2005). Differential sensitivity to chloride and sodium ions in seedlings of Glycine max and G. soja under NaCl stress. Journal of Plant Physiology 162 pp 1003-1012.

MoE of Environment, A Compendium of Working Water Quality Guidelines for British Columbia. Updated August 2006. Retrieved from http://www.env.gov.bc.ca/wat/wq/BCguidelines/working.html. Accessed July 18th 2007.

MoE of Environment, British Columbia Approved Water Quality Guidelines 2006 Edition. Updated August 2006. Retrieved from http://www.env.gov.bc.ca/wat/wq/BCguidelines/approv_wq_guide/approved.html. Accessed July 18th, 2007.

Naddy, R.B, Rehner, A.B., McNerney, G.R., Gorsuch, J.W., Kramer, J.R., Wood, C.M., Paquin, P.R., Stubblefield, W.A. (2007) Comparison of short-term chronic and chronic silver toxicity to fathead minnows in unamended and sodium chloride amended waters. Environmental Toxicology and Chemistry Vol. 26 No. 9

Sanzo, D., Hecnar, S.J. Effects of road de-icing salt (NaCl) on larval wood frogs (*Rana sylvatica*). (2006). Environmental Pollution 140 pp 247-256.

Soucek, D.J., Kennedy, A.J. (2005). Effects of hardness, chloride, and acclimation on the acute toxicity of sulfate to freshwater invertebrates. Environmental Toxicology and Chemistry Vol. 24 No. 5 pp 1204-1210

Soucek, D.J. (2007). Comparison of hardness and chloride regulated acute effects of sodium sulfate on two freshwater crustaceans. Environmental Toxicology and Chemistry Vol 26 No. 4 pp 773-779.

Valenti, T.W., Cherry, D.S., Currie, R.J., Neves, R.J., Jones, J.W., Mair, R., Kane, C.M. (2006). Chlorine toxicity to early life stages of freshwater mussels (Bivalvia: Unionidae). Environmental Toxicology and Chemistry Vol. 25, No. 9 pp 2512-2518.

Wilson, C., Liu, X., Lesch, S.M., Suarez, D.L. (2006). Growth response of major USA cowpea cultivars. Effect of salinity on leaf gas exchange. Plant Science 170 pp 1095-1101.

Environ Monit Assess (2013) 185:9299–9320
DOI 10.1007/s10661-013-3253-9

Application of a benthic observed/expected-type model for assessing Central Appalachian streams influenced by regional stressors in West Virginia and Kentucky

Gregory J. Pond · Sheila H. North

Received: 11 January 2013 / Accepted: 11 May 2013 / Published online: 4 June 2013

Abstract Stream bioassessments rely on taxonomic composition at sites compared with natural, reference conditions. We developed and tested an observed/expected (*O*/*E*) predictive model of taxonomic completeness and an index of compositional dissimilarity (BC index) for Central Appalachian streams using combined macroinvertebrate datasets from riffle habitats in West Virginia (WV) and Kentucky (KY). A total of 102 reference sites were used to calibrate the *O*/*E* model, which was then applied to assess over 1,200 sites sampled over a 10-year period. Using an all subsets discriminant function analysis (DFA) procedure, we tested combinations of 14 predictor variables that produced DF and *O*/*E* models of varying performance. We selected the most precise model using a probability of capture at >0.5 ($O/E_{0.5}$, SD=0.159); this model was constructed with only three simple predictor variables—Julian day, latitude, and whether a site was in ecoregion 69a. We evaluated *O*/*E* and BC indices between reference and test sites and compared their response to regional stressors, including coal mining, residential development, and acid deposition. The Central Appalachian *O*/*E* and BC indices both showed excellent discriminatory power and were significantly correlated to a variety of regional stressors; in some instances, the BC index was slightly more sensitive and responsive than the $O/E_{0.5}$ model. These indices can be used to supplement existing bioassessment tools crucial to detecting and diagnosing stream impacts in the Central Appalachian region of WV and KY.

Keyword Predictive model · *O*/*E* · Macroinvertebrates · Bioassessment · Mining · Acid deposition · Residential development

G. J. Pond (✉)
Office of Monitoring and Assessment,
U.S. Environmental Protection Agency, Region III,
Wheeling, WV, USA
e-mail: pond.greg@epa.gov

S. H. North
Office of Research and Development, Dynamac
Corporation, c/o US Environmental Protection Agency,
Cincinnati, OH, USA

Introduction

Macroinvertebrates are the most commonly used taxonomic group for assessing stream conditions worldwide (Rosenberg and Resh 1993). In the USA, macroinvertebrate bioassessment tools vary across state and federal agencies, but additive multimetric indices (MMIs) and observed/expected (*O*/*E*) models are the primary methods employed (e.g., Barbour et al. 1999; Hawkins 2006). MMI and *O*/*E* models are constructed differently yet both aim to detect deviation of macroinvertebrate community structure from reference conditions. MMIs use summary attributes of benthic taxa lists (e.g., % Ephemeroptera, Number of Intolerant taxa), while *O*/*E* indices can be described as a measure of taxonomic completeness, measuring the proportion of expected taxa observed at a particular site (Hawkins 2006). The most widely applied macroinvertebrate *O*/*E* models follow the UK's River Invertebrate Prediction and Classification System (RIVPACS; Wright et al. 2000; Clarke et al. 2003), which makes site-specific predictions of taxa that occur under unimpaired reference conditions. Environmental protection agencies in Kentucky (KY) and West Virginia (WV) have MMIs available for statewide assessments; these MMIs were typically developed to account for natural variation by adopting ecoregion, seasonal, or stream size classes (Pond et al. 2003; Pond et al. 2012). Rather than classifying assemblages into discrete groups, *O*/*E* models predict the probability of capturing each taxon along continuous natural gradients; the sum of these capture probability is the expected taxa richness which is compared with that observed at the site. Several recent studies have also applied continuous-gradient modeling to MMIs in regions with naturally high environmental variation (e.g., Pont et al. 2009; Hawkins et al. 2010).

Streams in the Central Appalachian Mountains harbor diverse and sensitive macroinvertebrate assemblages, but many have been degraded by land uses such as mineral extraction and residential development, and other stressors such as acid precipitation. In KY and WV, biological impacts stemming from mountaintop coal mining have been well studied over the last several years (Hartman et al. 2005; Pond et al. 2008; Fritz et al. 2010; Palmer et al. 2010; Merriam et al. 2011; Lindberg et al. 2011; Bernhardt et al. 2012); these studies have reported impacts to benthic assemblages downstream of mining operations. Because of topographical constraints in this ecoregion, most residential developments and associated infrastructure (e.g., roads, utility lines, etc.) occur along streams, which also leads to impacts to benthic assemblages (e.g., Pond 2010; Merriam et al. 2011; Pond 2012); previous studies have generally used combinations of metrics, individual taxa groups, and MMIs to detect impacts. Regional acid precipitation patterns are well-documented in the eastern USA and throughout Europe (e.g., Herlihy et al. 1993). Although atmospheric deposition of acids from coal-fired power generation has led to widespread freshwater acidification in the New England region of the USA (Wigington et al. 1996), the impacts are sporadic in the Central Appalachians and effects are most pronounced in poorly buffered (low-base bedrock geology) and small, higher elevation catchments (Herlihy et al. 1993).

Recently, several large landscape-scale *O*/*E* models have been developed at the state (e.g., Hargett et al. 2007; Hubler 2008), regional (Hawkins 2006; Ode et al. 2008), and national levels (Yuan et al. 2008). Previous efforts to develop predictive *O*/*E* indices in parts of the Appalachians (Hawkins 2006; Carlisle et al. 2008) produced relatively precise and robust models. However, in those studies, sampling methods or catchment sizes differed considerably from methods currently employed by KY and WV environmental agencies and since *O*/*E* models are sensitive to sampling methods, we chose to develop more specific models that conform to these state assessment methodologies and our region of interest. Our objective was to develop an *O*/*E* index for use as a supplemental, robust biological tool for detecting adverse impacts to aquatic resources in the Central Appalachians. Here, we apply an *O*/*E* model to a single ecoregion using state sampling and processing methods. Moreover, by combining genus-level taxonomic data from both KY and WV, we sought to develop and apply a geographically seamless assessment tool to aid in detecting degradation from mountaintop mining, residential development, and acid deposition. We evaluated *O*/*E* sensitivity and responsiveness using independent datasets.

Methods

Study area

The Central Appalachian Mountains ecoregion (i.e., ecoregion 69 described in Woods et al. 1996 and Woods et al. 2002) is composed of a highly dissected plateau with high local relief spanning portions of Tennessee, KY, Virginia, WV, and Pennsylvania (Fig. 1). The ecoregion is largely underlain by sedimentary sandstones and conglomerates, shale, and Pennsylvanian-age coal and supports diverse, mixed mesophytic, and central hardwood forest. Regional land uses are varied and generally include coal mining, oil and gas extraction, silviculture, residential development, and minor agriculture. Because of the dissected nature of the landscape, drainage density is high; streams in the region range in temperature from cold to warm, are of moderate to high gradient, and have cobble-boulder substrates. Our study focused on the southern portion of this ecoregion, in KY and WV, where mountaintop mining is most prevalent.

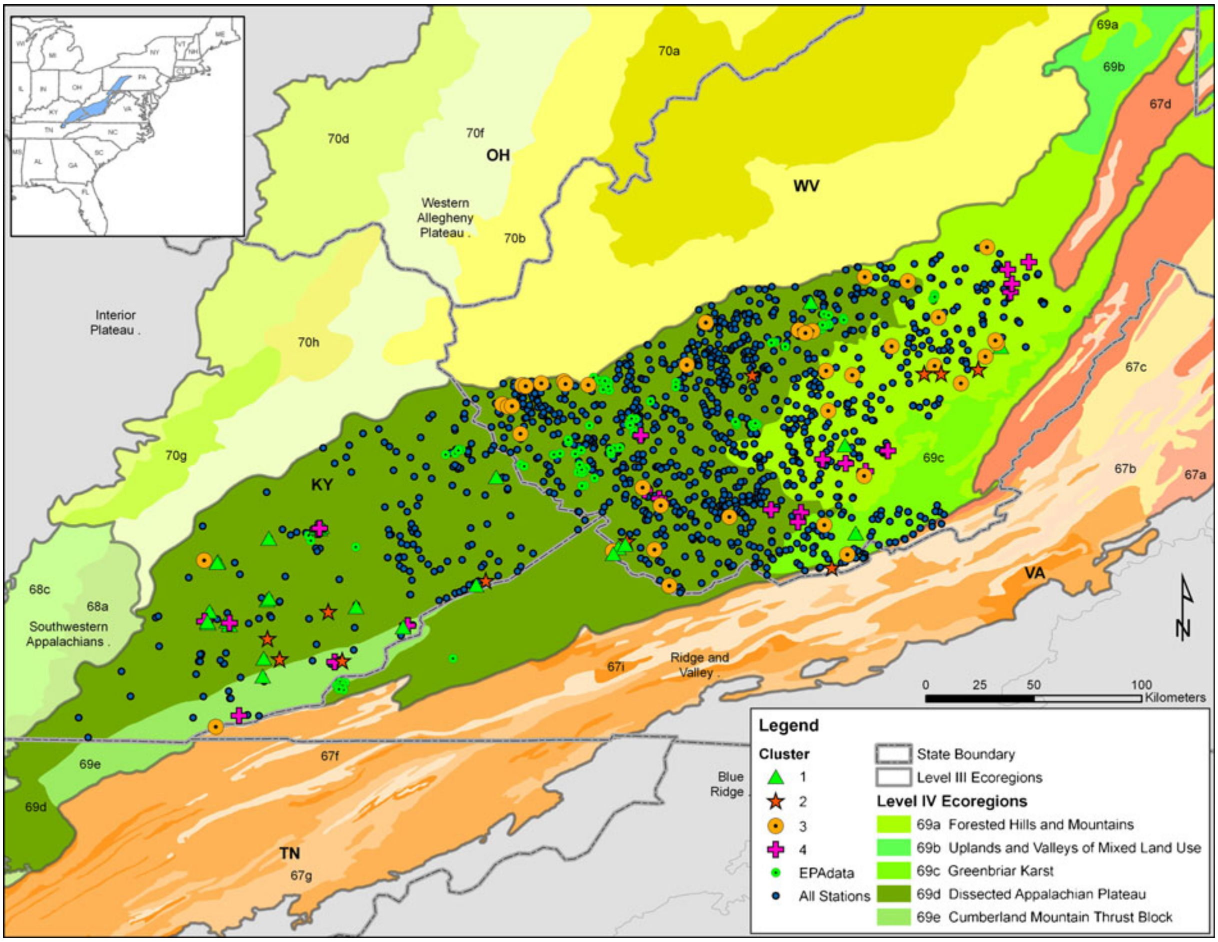


Fig. 1 Map of study area showing distribution of all sites in KY and WV. Sites symbolized by cluster number are calibration reference sites (see text). Independent sites ("EPA sites") in WV are from Pond et al. (2008), and independent sites in KY are from Pond (2010, 2012). Names of the subecoregions for adjacent ecoregion 67 and 70 can be found in Woods et al. (1996, 2002)

Development dataset

Our region of interest specifically centered around previous work done through a programmatic environmental impact statement on mountaintop removal/valley filling (US Environmental Protection Agency 2005). We limited our dataset to samples collected in areas of ecoregion 69 that were within the primary vicinity of mountaintop coal mining in WV and KY (Fig. 1); this area is generally demarcated in the north by the Elk River drainage divide (WV's "southern coalfield") and in the south by the KY border with Tennessee and Virginia (KY's "eastern coalfield"). Thus, our study domain covers parts of subecoregions 69a, 69c, 69d, and 69e conforming to areas with surface coal mining. We queried WV and KY Department of Environmental Protection (DEP) state databases for benthic samples collected from this portion of ecoregion 69. The samples identified were from flowing riffle habitat (e.g., primarily perennial streams but seasonal intermittency can occur in smaller streams) between January and October (1998–2009) that had genus-level taxonomy. However, few reference samples existed with January collection dates, so we only used sampling events spanning February to early October. Prior to defining data for possible inclusion, we examined frequency histograms of all sample dates across the February to October seasonal gradient and detected no seasonal bias. Reference sites in KY and WV passed a series of screening criteria (Kentucky Department for Environmental Protection KYDEP 2009; West Virginia Department of Environmental Protection WVDEP 2011) based on water chemistry, habitat, general land use, and lack of point source discharges. We compared abiotic data from both state reference site lists to ensure comparability. Reference sites in both states ranged from "minimally impacted" to "least disturbed" (after Stoddard et al. 2006). We retained sites that were <100 km^2 in size, because very few reference sites existed above this catchment size. For model calibration, we also excluded duplicate samples and samples collected at the same site within a 5-year period; the resulting dataset included 1,410 sites. We used 102 reference sites (80 %) for calibration (CAL) purposes (65 from WV and 37 from KY) and set aside 22 reference sites (20 %) for model validation (VAL); however, it is often recommended that a minimum of 30–50 validation sites be evaluated (Van Sickle, personal communication). However, we tried to balance the need to have enough CAL sites to build the model while retaining some VAL sites for testing. The remaining 1286 sites were not in reference condition and deemed "TEST" sites.

Macroinvertebrate sampling and processing

Both KY and WV sample riffle habitats similarly (i.e., composite of four 0.25-m^2 kicknet samples); however, KYDEP performs full laboratory sorts of samples, while WVDEP randomly subsamples to 200 (±20 %) organisms in gridded sorting pans. We made the datasets comparable by computer subsampling the KYDEP riffle data to 200 organisms in R (R Development Core Team 2006); samples with <200 organisms were retained. Prior to analyses, we ensured taxonomic similarity by seeking common operational taxonomic units (OTUs) among sites in both datasets; this ensured that sites with identifications at different taxonomic levels would not lead to ambiguous taxa and double counting. This required close examination and scrutiny of site taxa lists to assign unambiguous OTUs that maximized biological information while providing consistent taxonomy across sites.

We assessed additional independent datasets from US Environmental Protection Agency (EPA) sampling efforts at sites downstream of mountaintop mining in WV (Pond et al. 2008; n=37) and sites affected by mining and residential influence in KY (i.e., raw taxonomic data were extracted from the KYDEP database for sites used in Pond 2010, 2012; n=49). These benthic samples were collected and identified with identical techniques as WVDEP and KYDEP and then modified to match the defined OTUs. At a minimum, all sites had habitat scores (Rapid Bioassessment Protocol; Barbour et al. 1999), pH, specific conductance, temperature, and dissolved oxygen measurements.

Land cover data

For the CAL, VAL, TEST, and independent sites, we used geographic information system (GIS) to characterize catchment land cover using the 2001 and 2006 National Land Cover Dataset coverage (NLCD; Fry et al. 2011). Catchments were delineated from a 10-m digital elevation model (DEM) using terrain processing routines in ArcHydro Tools 9 (v 1.3; ESRI, Redlands, CA). For each NLCD, the barren and grassland categories were converted into a single polygon dataset (since grassland land cover frequently indicates reclaimed mine land in this ecoregion). Mining permit point locations were downloaded for WV (WVDEP Division of Mining and Reclamation Data; http://gis.dep.wv.gov/data/omr.html) and KY (KY Department of Natural Resources, Division of Mine Permits Data: http://minepermits.ky.gov/pages/spatialdata.aspx). Polygons in the barren-grassland dataset that were located within 500 m of WV and KY surface/underground mining permit points were considered to represent mining areas. For the few watersheds that extended into Virginia and Tennessee, as well as all remaining polygons at >85,000 m^2 in size, mining areas were identified visually using satellite imagery and topographic maps. Polygons within urban areas (which also can have grassland and barren land cover) were not considered mining land cover. For the independent sites, land disturbances were often more recent (i.e., later than 2006); therefore, we derived percent mining land cover estimates for these sites by measuring areas of polygons (from digitized mine maps) located upstream of sample sites and then used up-to-date aerial photography to confirm disturbance extent. For the independent KY dataset, housing density (number of homes per square kilometer) was acquired by counting the number of houses upstream of sample points using aerial photography and 1:24,000 topographic maps.

Model development and testing

Our O/E-type predictive models followed general methods reported by Hawkins et al. (2000) and Van Sickle et al. (2006). We adapted O/E modeling code using R statistical software (R Development Core Team 2006 developed by John Van Sickle (EPA; available at: http://www.epa.gov/wed/pages/models/rivpacs/rivpacs.htm) to our Central Appalachian dataset. Based on previous work (Hawkins 2006; Carlisle et al. 2008), we explored candidate predictor variables (Table 1) from a list of natural abiotic factors (i.e., variables not altered by human disturbance), including site data (Julian day of sample event) and several landscape variables extracted from GIS (site latitude and longitude, elevation, catchment area, and stream slope). Stream segment slope was estimated for each site with GIS using individual National Hydrography Dataset stream segments (available at http://nhd.usgs.gov/) and DEMs (available at http://seamless.usgs.gov/ned13.php); thus, actual site slope was not exact. Mean annual precipitation and minimum and maximum temperature data were interpolated (PRISM Climate Group; available at http://prism.oregonstate.edu/; soil variables (%clay and %sand) and groups B (moderate infiltration rate) and C (slow infiltration rate) hydrologic soil types were also interpolated (Soil Survey Geographic database; available at http://soildatamart.nrcs.usda.gov). Sites were coded (0 or 1) using biogeographic dummy variables (EPA level IV subecoregion (i.e., 69a, 69d, 69e, and 69c) after Woods et al. (1996, 2002). Although the Central Appalachian ecoregion has areas of differing dominant geology (e.g., sandstone and conglomerate, shale, etc.), we found discrepancies in State Soil Geographic data related purely to WV and KY political boundaries and thus, did not include geological predictors. Prior to analyses, we transformed predictor variables (e.g., logarithm, square root, and Box–Cox), where necessary, to approximate statistical normality for each variable.

After removal of rare taxa found at <5 % of the reference sites, CAL reference site assemblages were clustered with flexible β (β=−0.3) using a Bray–Curtis dissimilarity matrix applied to $\log_{10}$ transformed taxon abundances; these rare taxa were added back into the final dataset for calculation of the *O*/*E* indices. The grouping structure identified with cluster analysis was then used with Van Sickle et al.'s (2006) "all subsets" discriminant function analysis (DFA) modeling procedure to select abiotic explanatory variables and predict group membership probabilities of nonreference sites; the DFA produced many different models composed of all possible combinations of explanatory predictor variables. Each model's performance was evaluated by considering its resulting CAL *O*/*E* precision (SD) and bias

Table 1 Minimum (min), mean, and maximum (max) values for all predictor variables among calibration (CAL), validation (VAL), and TEST sites

	CAL			VAL			TEST		
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
Longitude	−83.6205	−81.9506	−80.1844	−83.6547	−82.0463	−80.4606	−84.1435	−81.5847	−80.0258
Latitude	36.6491	37.6705	38.64736	36.6981	37.63719	38.43035	36.6879	37.8707	38.64383
Julian day	59	152	259	96	153	235	71	187	273
Precipitation (mm)	952.9	1,245.7	1,752.0	981.5	1,249.9	1,523.6	922.7	1,195.8	1,595.3
Segment Slope (%)	0.1	4.4	23.0	0.9	4.7	14.5	0.1	2.8	18.9
Annual avg. air temp. (min)	2.1	5.2	7.1	3.3	5.3	6.3	1.3	5.3	6.5
Annual avg. air temp. (max)	13.9	17.9	19.5	15.3	17.9	19.6	13.4	18.1	20.1
Site elevation (m)	192.0	461.8	1,076.9	205.7	472.8	809.9	175.9	401.9	1,156.1
Catchment area (km^2)	2.9	12.3	74.3	3.0	9.9	71.7	2.7	21.2	79.2
Hydrologic group B soils (%)	0.0	43.4	91.0	3.0	40.7	88.0	0.0	34.4	94.0
Hydrologic group C soils (%)	0.0	46.1	83.0	12.0	47.8	83.0	0.0	48.1	93.0
Soil clay (%)	2.6	3.1	4.0	2.9	3.1	3.3	2.6	3.1	4.0
Soil sand (%)	13.9	24.7	4.2	14.7	24.7	31.3	12.6	27.4	40.2
Soil permeability (cm/h)	0.4	1.3	3.1	0.9	1.5	2.5	0.3	1.6	3.1

Four biogeographic (EPA level IV subecoregion) dummy variables (coded as 0 or 1) were also analyzed (see text) but are not included in the table (deviation from 1.0) compared with null models (which assume no dependence on abiotic gradients for probability of capture (P_c)). We also considered other DFA statistics, including Wilks λ and F statistic, re-substitution and cross-validation accuracy, and root mean square error (RMSE) of O/E, for reference CAL and VAL datasets. However, Van Sickle et al. (2006) stated that O/E precision and bias should be considered first; DFA statistics are, thus, reported for informational purposes only.

The top performing DFA model (based on O/E precision and bias) was selected and applied to all taxonomic data for O/E scoring. Specifically, the DFs were used to estimate the probability that a new test site belonged to a specific cluster based on abiotic predictor variables. These group membership probabilities were then used to weight the frequency of occurrence of each taxon within each reference cluster to predict the average P_c for that taxon at the new test site. To predict the expected taxa at a new site, we summed the probabilities of capture, where P_c was >0.5 (e.g., Hawkins et al. 2000; Van Sickle et al. 2007); this identified "common taxa." Thus, E represented the number of common taxa expected with $P_c>0.5$ at a site based on the environmental predictor variables specific to that site. This use of $P_c>0.5$ has shown to result in the most sensitive form of O/E ($O/E_{0.5}$) for assessment purposes, relying on the stronger signal of common taxa to detect impacts (Hawkins et al. 2000; Ostermiller and Hawkins 2004; Van Sickle et al. 2007); hence, excluding low-probability taxa not only improves model precision, but the high-probability taxa often respond more strongly to ecosystem stress (Hawkins et al. 2000).

We also evaluated the BC index (index of compositional dissimilarity using the Bray–Curtis equation; Van Sickle 2008), which measures the average disagreement, across taxa, between each taxon's observed occurrence and its predicted capture probability. Low BC values indicate that a test site has a taxonomic composition similar to its reference expectation. The BC index retains information on differences in low and high probability taxa, whereas O/E models fail to depict taxon-specific disparities because lower probability taxa can cancel out high probability taxa. Finally, calculations in R also flagged outlier TEST sites (statistically not within the model's experience). Outliers were determined from χ^2 tests ($p=0.01$) of predictor variables in relation to calibration reference site centroids. Outliers were not used in subsequent sensitivity and response analyses.

Other performance measures (sensitivity and responsiveness) of O/E and BC were examined with VAL and TEST sites, and separately with the independent WV ($n=37$) and KY ($n=49$) data from sites downstream of mountaintop mining and residential development. Since both KYDEP and WVDEP use the 5th percentile of reference site MMI scores as a threshold of impairment, we applied this same percentile for $O/E_{0.5}$ and BC (95th percentile) to calculate percent discrimination efficiencies (%DE) as simply the number of test sites scoring below the reference site 5th percentile, divided by total number of test sites and multiplied by 100. Kruskal–Wallis and Mann–Whitney U statistics were used to test for significant ($\alpha=0.05$) differences among reference, mined, unmined, and residential land use categories, respectively. Regression analysis was conducted to examine response of the $O/E_{0.5}$ and BC indices to common Central Appalachian mining stressors (habitat quality, specific conductance, % mining land use, and housing density). We also evaluated a subset of TEST sites that were influenced entirely by acid deposition; here, we selected 23 TEST sites that had pH<6.0, >90 % forest land cover, optimal instream habitat (total RBP score, >150), and very low specific conductance (<40 μS/cm). Relationships between pH and $O/E_{0.5}$ and BC values were analyzed with simple linear regression.

Finally, $O/E_{0.5}$ and BC values were compared with state MMI scores for the independent WV and KY datasets. For the KY sites, we calculated the KY Macroinvertebrate Bioassessment Index (MBI; after Pond et al. 2003); the WV sites were scored using the Genus-level Index of Most Probable Stream Status (GLIMPSS; after Pond et al. 2012). Comparisons of MMIs and the O/E-type indices focused on percent agreement of sites considered either impaired or unimpaired based on the 5th percentile (95th percentile for BC) of reference distributions. The MBI and GLIMPSS impairment thresholds (5th percentile of reference) were calculated from a different, but partially overlapping, set of reference sites specific to each state's MMI classification strata (see Pond et al. 2003, 2012; ecoregion 69 reference sites are shared between MMI and O/E). For example, WV's "mountain" bioregion incorporates reference sites from a two-ecoregion area (67 and 69), while the KY "mountain" bioregion uses reference sites from a three-ecoregion area (68, 69, and 70). Nevertheless, our goal here was to simply evaluate the correlation between MMIs and O/E-type models and assess how each state's MMI would rate a set of independent sites as compared with our *O*/*E*-type model.

Results

Macroinvertebrates

A total of 471 taxa were included from the entire dataset. Eighty-two of these taxa were ambiguous (e.g., coarser resolution or revised genus-level taxonomy based on updated systematics) and after careful inspection, were further modified by hierarchical lumping, name changes, or omission. The total number of retained OTUs (mostly genus level) from all sites was 389. The 102 CAL reference sites contained 199 invertebrate OTUs from 61 families (Appendix 1), but rare taxa occurring at <5 % of CAL reference sites were removed prior to clustering, yielding a total of 96 OTUs for use in cluster analysis. We pruned the resulting cluster dendrogram into four distinct groups of sites that were then used as grouping variables for DFA. These four clusters contained 28, 12, 40, and 24 reference sites, respectively. All 199 OTUs listed in Appendix 1 were used in calculation of probabilities of capture.

Model selection and precision

The all-subsets routine in R produced different models based on combinations of predictor variables for assigning reference site group membership probabilities. The CAL *O*/*E* null model (no predictor variables) had a mean of 1.0 and SD of 0.185, while the *O*/*E* null model for VAL sites had a mean of 1.0 and SD of 0.240. Regardless of the DF model, $O/E_{0.5}$ was not nearly as precise for the 22 VAL reference sites as it was for the CAL sites, likely due to the very low sample size. For the selected model, mean VAL $O/E_{0.5}$ was biased slightly low (0.977) and SD was higher (0.244; Table 2); however, all other models had VAL SD>0.20 and RMSE values approximating the corresponding SDs. We selected the model that had the highest CAL site precision (SD=0.159) and low bias (mean $O/E_{0.5}$=1.024; Table 2); surprisingly that model used only three predictor variables – Julian day, latitude, and an ecoregion 69a dummy variable.

Julian day and latitude were the most important predictors in all DFA models, selected in 92 and 75 % of all potential models, respectively. Table 2 shows discriminant function results for the CAL dataset. Our three-variable DFA model was significant (F=20.81, Wilks λ=0.239, group classification efficiencies of 73.5 and 70.6 % for re-substitution and cross-validation, respectively). Catchment area did not contribute to better DFA models, indicating that expected macroinvertebrate P_c were not strongly related to stream size in this ecoregion; CAL reference site catchment areas ranged from 2.9–74.3 km^2 (Table 1). Final reference site *O*/*E* scores were not correlated to catchment area (r=0.089, p>0.05). The χ^2 test flagged 8 % of the TEST sites as outliers (i.e., most fell out of range of Julian day, rather than latitude); these sites were not assessed and were excluded from further analysis. No reference (CAL or VAL) sites were flagged as outliers. We found no significant difference between WV and KY reference *O*/*E* scores (Mann–Whitney *U* test, p=0.45) and no significant difference among subecoregion reference site *O*/*E* scores (Kruskal–Wallis; p=0.21), indicating that modeling removed any potential geographical or subecoregional interferences. Selection of subecoregion 69a as a dummy variable might infer a longitudinal and elevation influence on assemblage structure in our study area; however, the selection of this dummy variable over more continuous variable representations of longitude and elevation, indicates that community similarity likely changed abruptly from subecoregion 69a (the most easterly ecoregion with the highest elevation) to the adjacent subecoregion (69d). Unquestionably though, Julian day

Table 2 Discriminant function (DF) results for the three-predictor variable model (Julian day, latitude, and ecoregion 69a dummy variable), and $O/E_{0.5}$ and BC index score means and standard deviations (SDs) for calibration (CAL), validation (VAL), and TEST sites

	CAL	VAL	TEST
F	20.81		
Wilks λ	0.239		
DF % accuracy (re-substitution)	73.5		
DF % accuracy (cross-validation)	70.6		
$O/E_{0.5}$ (mean)	1.024	0.977	0.621
$O/E_{0.5}$ (SD)	0.159	0.244	0.256
BC (mean)	0.265	0.275	0.486
BC (SD)	0.053	0.077	0.157

$O/E_{0.5}$ observed/expected model with a probability of capture of >0.5

contributed the most to predicting individual taxon P_c; it was selected in >90 % of all models. By way of example, Fig. 2 shows how several taxon P_c changed along a sample date (Julian day) gradient at one particular site sampled throughout the year reflecting individual taxa life histories (e.g., voltinism, emergence, and multicohort presence) to seasonality. *Ameletus* and *Neophylax* declined sharply throughout the year, while *Dolophilodes*, *Baetis*, and *Maccaffertium* (OTU=*Stenonema*) probabilities increased with time. *Leuctra* and *Diplectrona* maintained similar P_c throughout the year, while *Simulium* slightly increased in summer and then declined to early spring levels by fall. Our model adjusted for this seasonal effect and thus, over the entire Julian day range we found no trends in *O*/*E* scores at CAL sites (r=0.069, p>0.05) or TEST sites (r=0.094, p>0.05). Final modeling R code required to calculate *O*/*E* and BC for new sites can be obtained by contacting the authors.

Model sensitivity

Distributions of $O/E_{0.5}$ scores and the BC index values among reference CAL and VAL sites and the remaining TEST sites are shown in Fig. 3. For $O/E_{0.5}$, the impairment threshold (5th percentile of reference) was 0.77, while the threshold for BC was 0.37 (95th percentile). Both indices showed very good discriminatory power when plotted against TEST sites. The mean CAL BC value was 0.265, and the BC index had high precision (SD=0.054) and slightly better sensitivity than the $O/E_{0.5}$ model. For example, %DE for $O/E_{0.5}$ was 73.4, while %DE for BC was 83.5. The mean $O/E_{0.5}$ score at TEST sites was 0.621 (SD=0.256; Table 2) indicating that, on average, non-reference sites had lost an estimated 38 % of those expected "common" reference taxa (i.e., P_c>0.5).

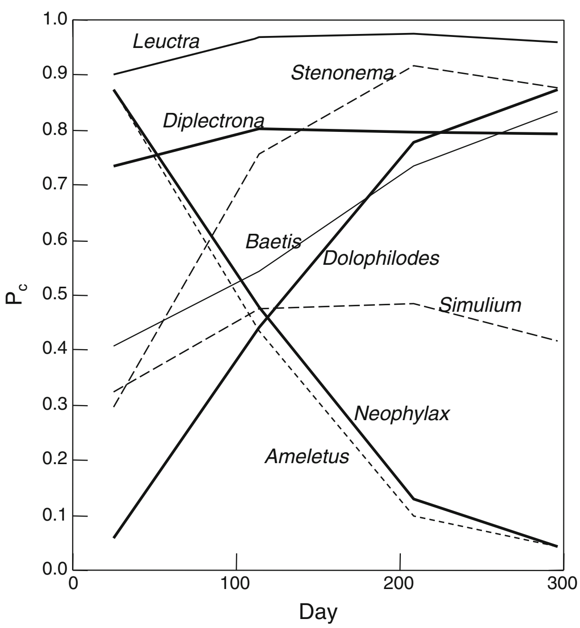


Fig. 2 Relationship between Julian day and modeled site-specific probability of capture (P_c) for several taxa exhibiting seasonal variation (or stability). Example is from Pigeonroost Creek, WV, sampled in the months of February, April, July, and October

Assessing mining and residential impacts with independent datasets

We assessed the independent Pond et al. (2008) macroinvertebrate dataset from WV with $O/E_{0.5}$ and BC by applying the derived impairment thresholds (0.77 and 0.37, respectively) and found that both indices rated nine of the ten unmined sites as unimpaired, while 89 and 93 % of mined sites were rated as impaired with $O/E_{0.5}$ and BC, respectively (Table 3; Fig. 4). Differences in mean $O/E_{0.5}$ and BC values between unmined and mined sites were highly significant (Mann–Whitney *U* test, p<0.001). The $O/E_{0.5}$ and BC indices were similarly responsive to specific conductance (an indicator of dissolved solids from mine pollution), and percent mining land cover (Fig. 5). No significant relationship between habitat score and $O/E_{0.5}$ or BC (both R^2<0.10, p>0.79) was found in the independent WV dataset.

The independent KY macroinvertebrate data showed that $O/E_{0.5}$ and BC clearly depicted biological impairment across RESID, MINED/RESID, and MINED land-use categories (Fig. 6), however, we did not detect significant differences between these three land-use types (Kruskal–Wallis; p>0.4). Among stressor variables, both $O/E_{0.5}$ and BC similarly responded to percent mining, specific conductance, housing density, and RBP habitat score (Fig. 7).

An example of *O*/*E* and BC scoring at select reference versus residential sites are provided in Appendix 2.

Response to acid deposition

The 23 sites affected primarily by acid deposition had a mean pH of 5.37, mean total RBP habitat score of

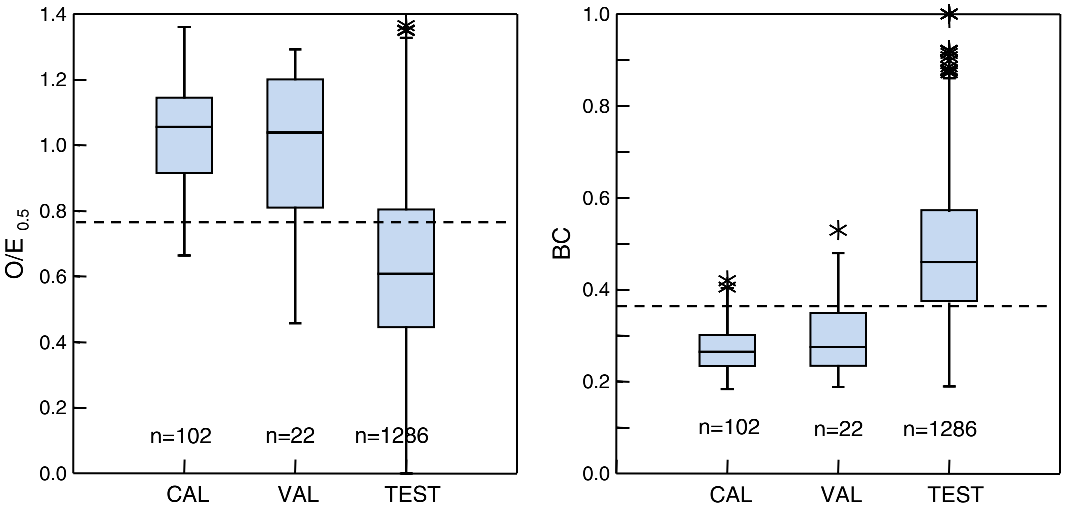


Fig. 3 *Box* and *whisker plots* of $O/E_{0.5}$ and BC index scores for all calibration (*CAL*), validation (*VAL*), and TEST sites from KY and WV. *Dashed lines* indicate impairment thresholds based on 5th percentile (*O/E*) and 95th percentile (*BC*) of calibration reference site distributions. *Boxes* enclose upper and lower quartiles; *vertical line* indicates the median; and *whiskers* represent range minus outliers (*asterisks* and *circles*). $O/E_{0.5}$ observed/expected model with a probability of capture at >0.5

171, mean specific conductance of 26 μS/cm, and mean forest land cover of 98 %. Other than the depressed pH values, these sites were equivalent or better than most reference streams in terms of habitat, forest cover, and other chemical qualities. Within this subset of acidified streams, we found significant ($p<0.001$) pH relationships with $O/E_{0.5}$ ($R^2=0.39$) and BC ($R^2=0.52$; Fig. 8) but these relationships were more variable compared with that found within mining and residential impacted streams. Non-impaired assemblages based on $O/E_{0.5}$ and BC were typically more prevalent when pH was >5.5.

Comparison of state MMIs and *O*/*E*-type indices using independent datasets

State MMIs and our RIVPACS-type indices were strongly correlated in the independent KY dataset ($n=49$; $r=0.87$ for $O/E_{0.5}$ and $r=0.86$ for BC) and WV dataset ($n=37$; $r=0.91$ for $O/E_{0.5}$ and $r=0.89$ for BC). There was good agreement in assessments of the independent sites (i.e., MMIs and *O*/*E* indices agreed between 80 and 100 % of the time) and minimal differences were detected between $O/E_{0.5}$ and BC values (Table 3). A few disagreements occurred between the index pairs in both disturbed and undisturbed stream types (Table 3), but overall, the *O*/*E*-type indices agreed somewhat more frequently with the KY MBI (92 %) versus the WV GLIMPSS (84 %). Table 3 also shows the impairment rates for both independent datasets using all three indices. Greater than 90 % of the sites in the mined, residential, and mined/residential groups of the KY dataset were considered impaired by all three indices—BC rated 100 % of all disturbed sites as impaired, while MBI and $O/E_{0.5}$ were nearly equivalent with impairment rates ranging from 91 to 100 %. In the WV dataset, BC also rated more mined sites as impaired (93 %) when compared with $O/E_{0.5}$ (89 %) and GLIMPSS (85 %).

Discussion

As elsewhere, widespread impacts to the macroinvertebrate assemblages in central Appalachia are a result of region-specific land uses and their associated stressors (Chambers and Messinger 2000; Pond et al. 2008; Pond 2010; Palmer et al. 2010; Bernhardt et al. 2012). Effective bioassessment tools based on native biota are necessary for regulatory agencies to detect stream degradation and to assess aquatic life uses. Although both KY and WV assess stream condition using MMIs, we believe our $O/E_{0.5}$ model and BC index could supplement these existing biomonitoring tools by concentrating on direct taxa loss. The $O/E_{0.5}$ model measures taxonomic completeness, while the BC index measures compositional

Table 3 Percent agreement (impaired or unimpaired) of state multimetric indices (MMIs) versus $O/E_{0.5}$ and BC assessments, and a comparison of overall impairment rates (%) for all indices using each independent dataset

	% agreement with MMI		% impaired		
	$O/E_{0.5}$	BC	MMI	$O/E_{0.5}$	BC
KY MBI					
All (n=49)	92	92			
Reference (n=20)	100	100	5	0	0
Residential (n=6)	83	83	83	100	100
Mined/residential (n=12)	100	100	92	100	100
Mined (n=11)	91	100	100	91	100
WV GLIMPSS					
All (n=37)	84	84			
Unmined (n=10)	80	80	10	10	10
Mined (n=27)	89	89	85	89	93

$O/E_{0.5}$ observed/ expected model with a probability of capture of >0.5, *MBI* Macroinvertebrate Bioassessment Index, *GLIMPSS* Genus-Level Index of Most Probable Stream Status, *KY* Kentucky data from Pond (2010, 2012), *WV* West Virginia data from Pond et al. (2008)

shift away from reference conditions at sites; these indices are becoming more frequent in use as universal indicators of ecological integrity (Hawkins 2006; Van Sickle 2008). While most regional MMIs account for some natural variation (e.g., discrete seasons and ecoregions), *O*/*E*-type models predict taxa along continuous environmental gradients and therefore might better predict deviations in taxonomic composition.

Our Central Appalachian *O*/*E*-type model was precise and responsive to regional stressors. While other *O*/*E*-type models have used several predictor variables, ours could assess new sites relatively easily using only three variables—Julian day, latitude, and an ecoregion 69a dummy variable—and did not require extensive GIS. The higher precision of the predictive model versus the null model indicated that natural abiotic variables contribute to structuring aquatic assemblages (i.e., the predictor variables helped reduce variability in $O/E_{0.5}$). Both the $O/E_{0.5}$ model and BC index were responsive, showing significant assemblage shifts and losses in expected common taxa under mining and residential development land uses. Moderately strong dose response-type relationships were also evident when comparing $O/E_{0.5}$ and BC

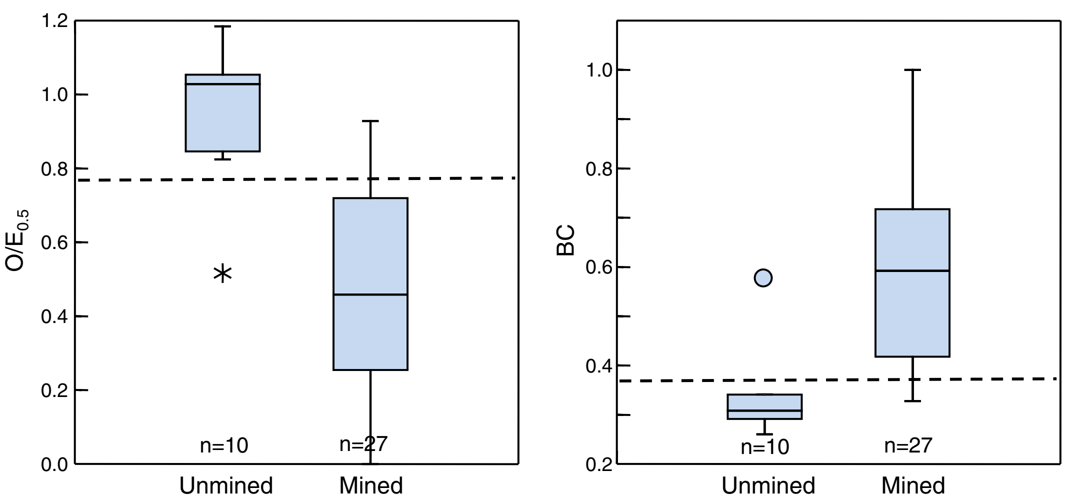


Fig. 4 *Box* and *whisker* plots of $O/E_{0.5}$ and BC index scores among unmined and mined sites sampled in WV. *Dashed lines* represent impairment thresholds derived from the 5th percentile ($O/E_{0.5}$) and 95th percentile (*BC*) of calibration reference sites. *Boxes* enclose upper and lower quartiles; *vertical line* indicates the median; and *whiskers* represent range minus outliers (asterisks). $O/E_{0.5}$ observed/expected model with a probability of capture at >0.5. Data from Pond et al. (2008)

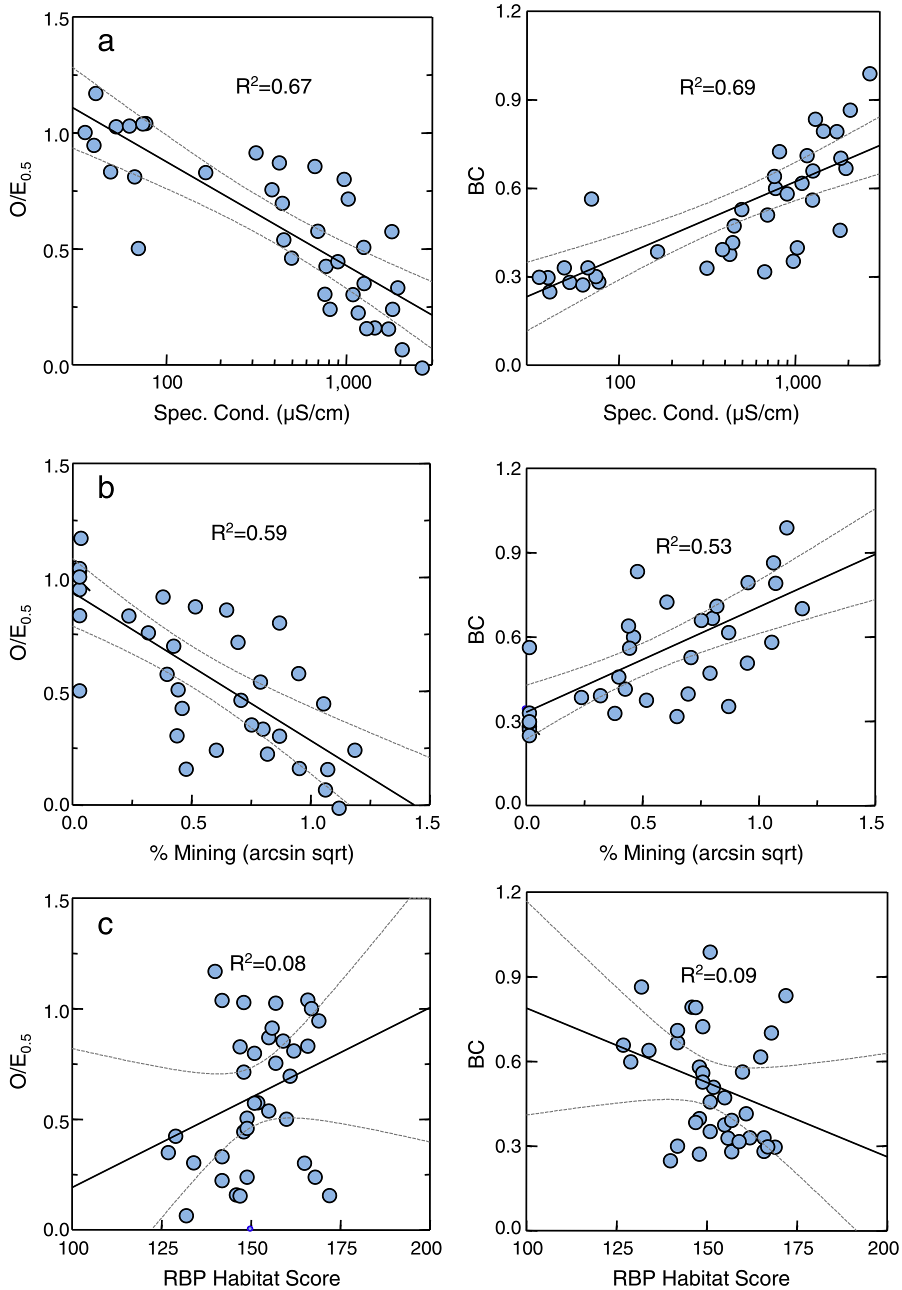


Fig. 5 Scatterplots of $O/E_{0.5}$ and BC index scores versus **a** specific conductance (*Spec. Cond.*), **b** percent mining (*arcsin sqrt*), and **c** rapid bioassessment protocol (*RBP*) habitat score from sites sampled in WV. *Solid lines* indicate linear regression lines; *dashed lines* represent 95 % confidence intervals. $O/E_{0.5}$ observed/expected model with a probability of capture at >0.5. Data are from Pond et al. (2008)

values to percent mining land cover, specific conductance, habitat quality, and housing density. In the acidified stream set (intact forested sites having optimal instream habitat and low conductivity), $O/E_{0.5}$ and BC detected significant acid deposition effects when pH was generally <5.5. In some instances, we found that the BC index was more sensitive than $O/E_{0.5}$ but further testing with additional datasets are necessary. Like many regional MMIs, $O/E_{0.5}$ and BC exhibited an excellent

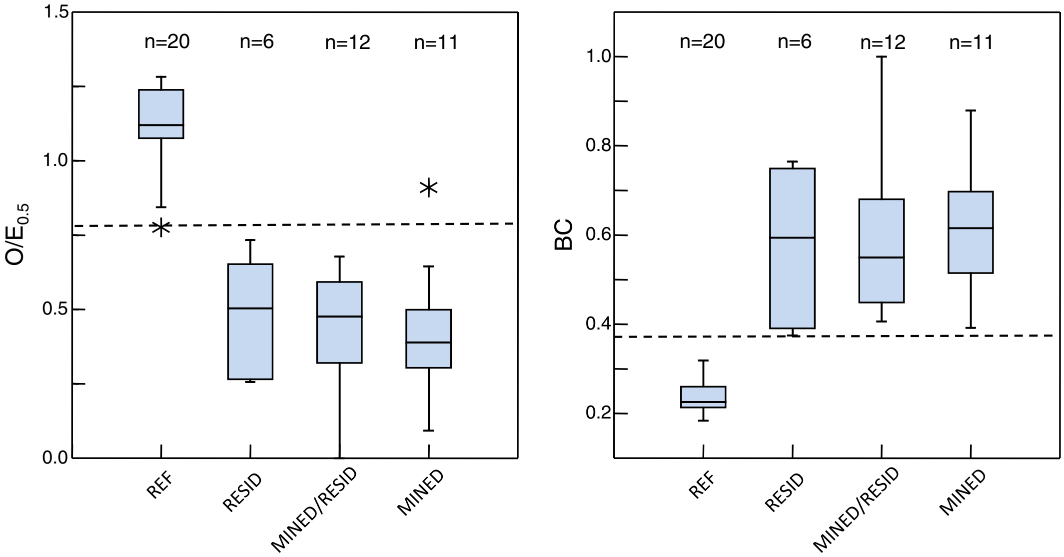


Fig. 6 Box and whisker plots of $O/E_{0.5}$ and BC index scores among land use categories in eastern KY. *Dashed lines* indicate impairment threshold. $O/E_{0.5}$ observed/expected model with a probability of capture at >0.5, *REF* reference, *RESID* residential only, *MINED/RESID* mined plus residential, *MINED* mined only. Data are a subset of Central Appalachian headwater sites from Pond (2010, 2012)

ability to detect deviation of assemblages from reference conditions; but, instead of measuring changes in summary community metrics (e.g., %EPT used in a MMI), *O*/*E*-type models measured direct expected taxa loss and taxonomic shifts not routinely expressed in summary-type metrics.

Among the independent mining datasets from WV and KY, relationships between $O/E_{0.5}$ and BC versus specific conductance and percent mining were equivalent, indicating that assemblage composition responded similarly to these independent variables regardless of location or dataset. However, we believe the stark differences between these two independent datasets in the correlation of $O/E_{0.5}$ and BC values to habitat score was a statistical artifact since the range and distribution of each set of habitat scores differed. For instance, in the independent WV data set, an effort was made to sample sites that had relatively good habitat (i.e., RBP habitat scores ranged from 126 to 171). In contrast, habitat quality was not considered in site selection in the KY mining dataset and KY reference sites had better habitat than the WV unmined sites; thus, a better response was observed across a larger habitat gradient in KY (i.e., RBP habitat scores ranged from 84 to 187). Overall, our *O*/*E*-type models and the state MMIs depicted high rates of impairment (defined using the 5th percentile of reference site distributions) associated with regional land uses in both KY and WV streams. Future analysis should aim to apply these multivariate bioassessment tools to other existing datasets to help confirm their sensitivity, responsiveness, and overall assessment benefits.

A major advantage of using our Central Appalachian models is the ability to compare regional stressor effects and biological degradation across political boundaries where states use different assessment indices. This could prove desirable as researchers continue to assess pollution signatures from common disturbance types (point- and nonpoint sources) shared across this and other ecoregions. These indices could certainly be used with macroinvertebrates assemblages sampled in the adjacent coalfield region of VA (given the clear spatial proximity; see Fig. 1), if collected and processed with similar methods. For instance, EPA collected several sites in VA in April 2009 (unpublished data, but sites are plotted in Fig. 1); these data were within our model's experience (i.e., not flagged as outliers), indicating that the models are applicable in this portion of VA.

Assessing sites using both MMIs and the *O*/*E* and BC provides higher information value and could act as a form of statistical concurrence in assessments.

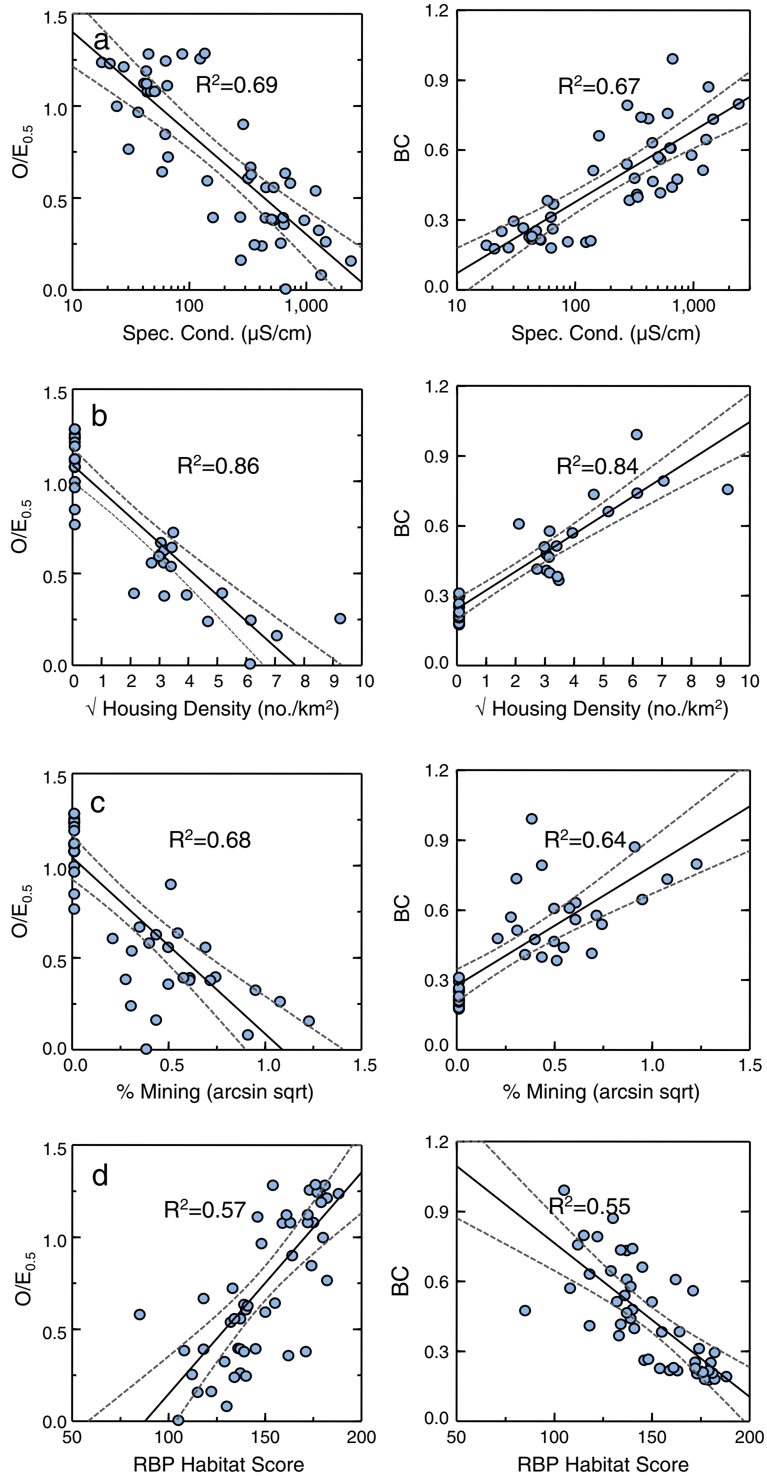


Fig. 7 Scatterplots of $O/E_{0.5}$ and BC index scores versus **a** specific conductance (*Spec. Cond.*), **b** housing density (*sqrt*), **c** *percent* mining (*arcsin sqrt*), and **d** rapid bioassessment protocol (*RBP*) habitat score in Central Appalachian streams of KY. *Solid lines* indicate linear regression lines; *dashed lines* represent 95 % confidence intervals. Mined sites omitted from housing density plot; residential sites omitted from percent mining plot. $O/E_{0.5}$ observed/expected model with a probability of capture at >0.5. Data from Pond (2010, 2012)

O/*E* and BC might also assist in diagnosing stream impacts focusing on individual taxon traits or taxon tolerances to stressors. Although taxonomic composition is the foundation of both MMIs and *O*/*E* indices, these bioassessment models measure different aspects of the assemblage. Hawkins

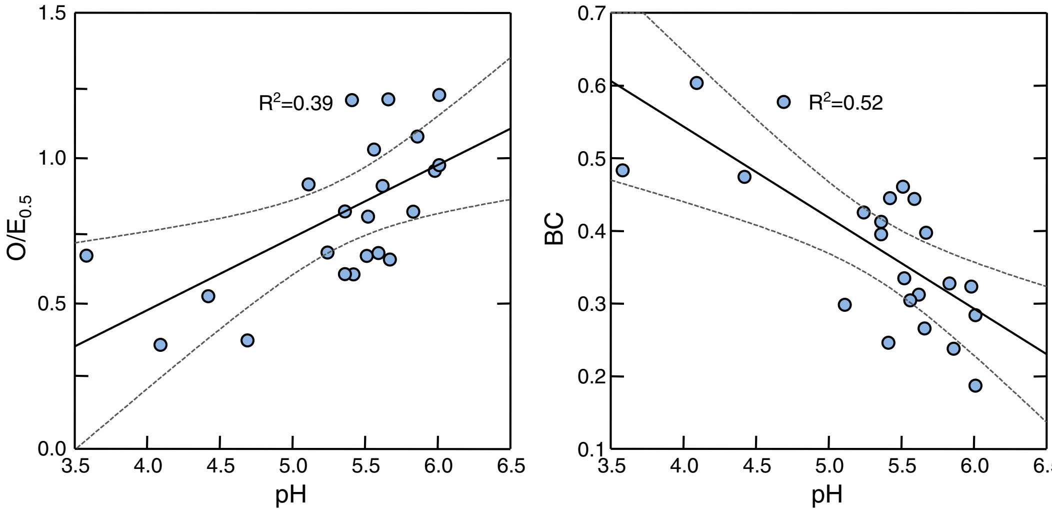


Fig. 8 Scatter plot of $O/E_{0.5}$ and BC index scores versus pH among acid deposition streams in WV and KY (n=23). *Solid lines* indicate linear regression lines; *dashed lines* represent 95 % confidence intervals. $O/E_{0.5}$ observed/expected model with a probability of capture at >0.5

(2006) found that in a multistate comparison, MMI, and *O*/*E* correlation was imperfect (however, see Herbst and Silldorff 2006), and argued that since *O*/*E* indices are based on the raw compositional data from which MMIs are derived, *O*/*E* could serve as the universal indicator. We actually found good agreement between our *O*/*E*-type models and the state's MMIs for the independent study sites, but discrepancies were noted at some sites. Using an *O*/*E* model or BC index alone (which are based on presence/absence) might miss significant changes in community structure where the individual taxon abundances are unaccounted for. In contrast, summary metrics used in MMIs (e.g., %intolerant individuals, %Chironomidae+ Oligochaeta, %Ephemeroptera, or relative abundance of other traits) might help better inform assessments of sites exhibiting dose–response induced shifts in the abundance of certain indicator- or trait-based groups; however, these summary metrics rely on low resolution taxonomy (order or family level), which could give false signals as well. A shortcoming of using summary metrics without some regional or seasonal discretion is the inherent variability associated with metric values in relation to natural abiotic gradients (e.g., seasonality, site-specific environmental features, etc.); however, recent advancements in modeling abiotic gradients for MMI development show promise (Pont et al. 2009; Hawkins et al. 2010). In contrast to traditional MMI development and application, the taxonomic expectations developed from *O*/*E*-type models are specifically standardized to site-specific conditions.

Conclusions

We believe that the Central Appalachian $O/E_{0.5}$ and BC indices can be integrated with KY and WV MMIs to help verify the extent of taxa loss that accompanies biological impairment from regional stressors. To simultaneously use both MMIs and predictive models in assessments, some method of reconciliation must be decided upon (i.e., when they disagree in rating a site as either impaired or unimpaired). One might argue that successfully passing all three indices should be required, while others may weigh one index higher than the others depending on agency policies informed by science and CWA goals of protecting and maintaining biological integrity. Based on the facts that our *O*/*E*-type models directly estimated taxonomic completeness, and were both precise and responsive to known stressors across the region, we recommend evaluating both MMI and *O*/*E*-type model scores at sites to help present a more complete picture of stream conditions throughout the Central Appalachian region.

Acknowledgments This research was conducted jointly by EPA's Environmental Assessment and Innovation Division in Region III and EPA's National Exposure Research Laboratory in the Office of Research and Development. The US Environmental Protection Agency through its Office of Research and Development partially funded and collaborated in the research described here under contract number EP-D-06-096 to Dynamac Corporation. We thank Randy Pomponio and John Forren (Region III, Philadelphia, PA), Joe Flotemersch and Brad Autrey (ORD, Cincinnati, OH), and Justicia Rhodus (Dynamac Corporation) for programmatic support. We also thank John Van Sickle and Karen Blocksom (EPA, ORD, Corvallis, OR) for statistical and software programming advice and reviews. Datasets were supplied by Jeff Bailey and Mike Whitman (WVDEP) and John Brumley and Mark Vogel (KYDEP). GIS support was provided by Ellen D'Amico and Elisabeth Hagenbuch (Dynamac Corporation). Earlier versions of the manuscript were improved by reviews from Maggie Passmore (Region III, Wheeling, WV), John Forren and Stefania Shamet (Region III, Philadelphia, PA), Matt Klasen (EPA Headquarters, Washington, DC), and editing and formatting of the manuscript was performed by Justicia Rhodus (Dynamac Corporation). We also thank two anonymous reviewers who greatly improved the final manuscript. Although this research was supported by EPA, the views and opinions expressed in this article are those of the authors and do not represent the official views or positions of the EPA or the US government.

Appendix 1

Table 4 List of the 199 calibration reference site operational taxonomic units (OTUs) with minimum (min), mean, and maximum (max) probability of capture frequencies across all sites

Family	OTU	Min	Mean	Max
Turbellaria	Turbellaria	0.01	0.03	0.05
Pisidiidae	*Pisidium*	0.00	0.01	0.02
Pisidiidae	*Sphaerium*	0.00	0.01	0.02
Oligochaeta	Oligochaeta	0.21	0.34	0.45
Ameletidae	*Ameletus*	0.04	0.42	0.82
Baetidae	*Acentrella*	0.25	0.32	0.42
Baetidae	*Acerpenna*	0.03	0.07	0.12
Baetidae	*Baetis*	0.44	0.75	0.96
Baetidae	*Centroptilum*	0.00	0.02	0.04
Baetidae	*Diphetor*	0.15	0.20	0.25
Baetidae	*Plauditus*	0.02	0.12	0.25
Baetidae	*Procloeon*	0.00	0.06	0.12
Ephemerellidae	*Attenella*	0.03	0.04	0.06
Ephemerellidae	*Dannella*	0.00	0.01	0.05
Ephemerellidae	*Drunella*	0.17	0.37	0.45
Ephemerellidae	*Ephemerella*	0.09	0.52	0.78
Ephemerellidae	*Eurylophella*	0.11	0.22	0.27
Ephemerellidae	*Serratella*	0.00	0.05	0.12
Ephemeridae	*Ephemera*	0.08	0.13	0.21
Heptageniidae	*Cinygmula*	0.04	0.31	0.52
Heptageniidae	*Epeorus*	0.45	0.69	0.96
Heptageniidae	*Heptagenia*	0.03	0.07	0.10
Heptageniidae	*Leucrocuta*	0.03	0.16	0.45
Heptageniidae	*Nixe*	0.01	0.03	0.05
Heptageniidae	*Stenacron*	0.13	0.23	0.30

Table 4 (continued)

Family	OTU	Min	Mean	Max
Heptageniidae	*Stenonema*	0.45	0.61	0.83
Isonychiidae	*Isonychia*	0.03	0.17	0.29
Leptophlebiidae	*Choroterpes*	0.00	0.01	0.02
Leptophlebiidae	*Paraleptophlebia*	0.66	0.76	0.87
Siphlonuridae	Siphlonuridae	0.00	0.01	0.02
Aeshnidae	*Boyeria*	0.02	0.10	0.21
Cordulegastridae	*Cordulegaster*	0.00	0.06	0.21
Corduliidae	*Macromia*	0.00	0.01	0.02
Gomphidae	Gomphidae	0.05	0.12	0.22
Gomphidae	*Lanthus*	0.08	0.08	0.08
Gomphidae	*Stylogomphus*	0.05	0.12	0.21
Libellulidae	Libellulidae	0.00	0.01	0.02
Capniidae	*Allocapnia*	0.00	0.01	0.04
Capniidae	*Paracapnia*	0.00	0.04	0.08
Chloroperlidae	*Alloperla*	0.00	0.11	0.17
Chloroperlidae	*Haploperla*	0.17	0.29	0.38
Chloroperlidae	*Rasvena*	0.00	0.02	0.06
Chloroperlidae	*Sweltsa*	0.14	0.44	0.55
Chloroperlidae	*Utaperla*	0.00	0.01	0.02
Leuctridae	*Leuctra*	0.93	0.96	1.00
Nemouridae	*Amphinemura*	0.09	0.56	0.83
Nemouridae	*Nemoura*	0.00	0.01	0.04
Nemouridae	*Ostrocerca*	0.00	0.04	0.12
Nemouridae	*Paranemoura*	0.00	0.02	0.06
Nemouridae	*Prostoia*	0.00	0.02	0.05
Nemouridae	*Shipsa*	0.00	0.01	0.02
Nemouridae	*Soyedina*	0.00	0.03	0.08
Peltoperlidae	*Peltoperla*	0.17	0.27	0.42
Peltoperlidae	*Tallaperla*	0.08	0.20	0.41
Perlidae	*Acroneuria*	0.53	0.71	0.87
Perlidae	*Agnetina*	0.00	0.01	0.04
Perlidae	*Eccoptura*	0.02	0.11	0.21
Perlidae	*Paragnetina*	0.00	0.03	0.08
Perlidae	*Perlesta*	0.00	0.02	0.04
Perlodidae	*Clioperla*	0.01	0.03	0.05
Perlodidae	*Cultus*	0.00	0.01	0.02
Perlodidae	*Diploperla*	0.03	0.07	0.15
Perlodidae	*Isoperla*	0.13	0.39	0.58
Perlodidae	*Malirekus*	0.08	0.13	0.17
Perlodidae	*Remenus*	0.00	0.06	0.12
Perlodidae	*Yugus*	0.00	0.29	0.48
Pteronarcyidae	*Pteronarcys*	0.28	0.39	0.50
Taeniopterygidae	*Taenionema*	0.00	0.02	0.05
Taeniopterygidae	*Taeniopteryx*	0.00	0.01	0.02

Table 4 (continued)

Family	OTU	Min	Mean	Max
Gerridae	Gerridae	0.00	0.01	0.02
Veliidae	*Rhagovelia*	0.00	0.05	0.16
Corydalidae	*Corydalus*	0.00	0.01	0.02
Corydalidae	*Nigronia*	0.12	0.33	0.62
Sialidae	*Sialis*	0.00	0.03	0.05
Brachycentridae	*Brachycentrus*	0.00	0.01	0.02
Glossosomatidae	*Agapetus*	0.00	0.05	0.10
Glossosomatidae	*Glossosoma*	0.08	0.18	0.29
Goeridae	*Goera*	0.00	0.04	0.12
Hydropsychidae	*Ceratopsyche*	0.19	0.45	0.83
Hydropsychidae	*Cheumatopsyche*	0.19	0.38	0.70
Hydropsychidae	*Diplectrona*	0.79	0.82	0.92
Hydropsychidae	*Homoplectra*	0.00	0.01	0.02
Hydropsychidae	*Hydropsyche*	0.13	0.24	0.32
Hydroptilidae	*Hydroptila*	0.00	0.01	0.02
Lepidostomatidae	*Lepidostoma*	0.04	0.25	0.40
Limnephilidae	*Pycnopsyche*	0.04	0.10	0.17
Philopotamidae	*Chimarra*	0.00	0.02	0.05
Philopotamidae	*Dolophilodes*	0.13	0.50	0.87
Philopotamidae	*Wormaldia*	0.13	0.19	0.30
Phryganeidae	*Ptilostomis*	0.00	0.01	0.02
Polycentropodidae	*Nyctiophylax*	0.00	0.01	0.02
Polycentropodidae	*Polycentropus*	0.35	0.45	0.54
Psychomyiidae	*Lype*	0.00	0.01	0.04
Psychomyiidae	*Psychomyia*	0.00	0.01	0.02
Rhyacophilidae	*Rhyacophila*	0.65	0.72	0.77
Uenoidae	*Neophylax*	0.04	0.44	0.82
Dryopidae	*Helichus*	0.29	0.31	0.33
Elmidae	*Macronychus*	0.00	0.02	0.06
Elmidae	*Microcylloepus*	0.00	0.05	0.17
Elmidae	*Optioservus*	0.31	0.53	0.67
Elmidae	*Oulimnius*	0.36	0.47	0.58
Elmidae	*Promoresia*	0.00	0.04	0.08
Elmidae	*Stenelmis*	0.10	0.20	0.25
Hydrophilidae	Hydrophilidae	0.00	0.01	0.04
Psephenidae	*Ectopria*	0.31	0.50	0.62
Psephenidae	*Psephenus*	0.11	0.34	0.54
Ptilodactylidae	*Anchytarsus*	0.05	0.06	0.08
Athericidae	*Atherix*	0.00	0.06	0.12
Blephariceridae	*Blepharicera*	0.03	0.06	0.12
Ceratopogonidae	*Atrichopogon*	0.00	0.03	0.07
Ceratopogonidae	*Bezzia/Palpomyia*	0.13	0.17	0.22
Ceratopogonidae	*Ceratopogon*	0.00	0.01	0.02
Ceratopogonidae	*Dasyhelea*	0.00	0.02	0.04

Table 4 (continued)

Family	OTU	Min	Mean	Max
Ceratopogonidae	*Probezzia*	0.00	0.04	0.12
Chironomidae	*Brillia*	0.00	0.05	0.12
Chironomidae	*Cardiocladius*	0.00	0.03	0.10
Chironomidae	*Chaetocladius*	0.04	0.10	0.15
Chironomidae	*Cladotanytarsus*	0.00	0.01	0.02
Chironomidae	*Constempellina*	0.00	0.01	0.05
Chironomidae	*Cricotopus*	0.04	0.08	0.12
Chironomidae	*Cricotopus/Orthocladius*	0.04	0.14	0.27
Chironomidae	*Demicryptochironomus*	0.00	0.06	0.12
Chironomidae	*Diamesa*	0.10	0.15	0.20
Chironomidae	*Diplocladius*	0.00	0.01	0.02
Chironomidae	*Epoicocladius*	0.00	0.03	0.08
Chironomidae	*Eukiefferiella*	0.05	0.25	0.39
Chironomidae	*Heleniella*	0.04	0.09	0.12
Chironomidae	*Heterotrissocladius*	0.00	0.01	0.02
Chironomidae	*Hydrobaenus*	0.00	0.01	0.02
Chironomidae	*Krenosmittia*	0.00	0.03	0.05
Chironomidae	*Larsia*	0.00	0.02	0.04
Chironomidae	*Limnophyes*	0.00	0.02	0.06
Chironomidae	*Lopescladius*	0.00	0.03	0.10
Chironomidae	*Mesocricotopus*	0.00	0.02	0.06
Chironomidae	*Micropsectra*	0.21	0.34	0.45
Chironomidae	*Microtendipes*	0.29	0.40	0.50
Chironomidae	*Nanocladius*	0.00	0.01	0.02
Chironomidae	*Natarsia*	0.00	0.01	0.02
Chironomidae	*Nilotanypus*	0.00	0.02	0.04
Chironomidae	*Orthocladius*	0.02	0.06	0.17
Chironomidae	*Pagastia*	0.00	0.01	0.02
Chironomidae	*Parachaetocladius*	0.13	0.21	0.37
Chironomidae	*Parachironomus*	0.00	0.01	0.02
Chironomidae	*Paracladopelma*	0.00	0.01	0.02
Chironomidae	*Parakiefferiella*	0.00	0.01	0.05
Chironomidae	*Paramerina*	0.00	0.01	0.04
Chironomidae	*Parametriocnemus*	0.51	0.66	0.75
Chironomidae	*Paraphaenocladius*	0.00	0.06	0.10
Chironomidae	*Paratanytarsus*	0.00	0.01	0.05
Chironomidae	*Platysmittia*	0.00	0.03	0.05
Chironomidae	*Polypedilum*	0.26	0.51	0.81
Chironomidae	*Potthastia*	0.00	0.02	0.05
Chironomidae	*Pseudochironomus*	0.00	0.01	0.02
Chironomidae	*Pseudorthocladius*	0.00	0.01	0.02
Chironomidae	*Psilometriocnemus*	0.00	0.01	0.02
Chironomidae	*Rheocricotopus*	0.03	0.06	0.12
Chironomidae	*Rheosmittia*	0.00	0.03	0.06

Table 4 (continued)

Family	OTU	Min	Mean	Max
Chironomidae	*Rheotanytarsus*	0.02	0.19	0.33
Chironomidae	*Stempellina*	0.08	0.10	0.12
Chironomidae	*Stempellinella*	0.08	0.22	0.46
Chironomidae	*Stilocladius*	0.00	0.02	0.05
Chironomidae	*Sublettea*	0.00	0.01	0.02
Chironomidae	*Tanytarsus*	0.33	0.49	0.71
Chironomidae	*Thienemanniella*	0.02	0.05	0.10
Chironomidae	*Thienemannimyia*	0.30	0.49	0.71
Chironomidae	*Tokunagaia*	0.00	0.01	0.02
Chironomidae	*Tvetenia*	0.20	0.30	0.39
Chironomidae	*Zavrelia*	0.00	0.03	0.08
Chironomidae	*Zavrelimyia*	0.00	0.06	0.12
Dixidae	*Dixa*	0.00	0.11	0.21
Empididae	*Chelifera*	0.05	0.12	0.17
Empididae	*Clinocera*	0.00	0.02	0.05
Empididae	*Hemerodromia*	0.02	0.08	0.15
Empididae	*Neoplasta*	0.00	0.01	0.04
Sciaridae	*Corynoptera*	0.00	0.04	0.07
Simuliidae	*Prosimulium*	0.08	0.26	0.59
Simuliidae	*Simulium*	0.33	0.42	0.57
Tabanidae	*Chrysops*	0.00	0.02	0.05
Tabanidae	*Tabanus*	0.00	0.01	0.02
Tipulidae	*Antocha*	0.03	0.09	0.15
Tipulidae	*Brachypremna*	0.00	0.01	0.02
Tipulidae	*Cryptolabis*	0.00	0.02	0.07
Tipulidae	*Dicranota*	0.15	0.29	0.44
Tipulidae	*Gonomyia*	0.00	0.01	0.02
Tipulidae	*Hexatoma*	0.64	0.69	0.77
Tipulidae	*Limnophila*	0.00	0.09	0.19
Tipulidae	*Limonia*	0.00	0.01	0.05
Tipulidae	*Molophilus*	0.00	0.04	0.07
Tipulidae	*Ormosia*	0.00	0.03	0.06
Tipulidae	*Prionocera*	0.00	0.01	0.02
Tipulidae	*Pseudolimnophila*	0.04	0.08	0.12
Tipulidae	*Tipula*	0.21	0.36	0.50
Crangonyctidae	*Crangonyx*	0.00	0.03	0.07
Gammaridae	*Gammarus*	0.04	0.08	0.13
Asellidae	*Caecidotea*	0.00	0.05	0.07
Asellidae	*Lirceus*	0.00	0.02	0.04
Cambaridae	*Cambarus*	0.52	0.58	0.62
Cambaridae	*Orconectes*	0.00	0.01	0.02

Appendix 2

Table 5 Comparison of taxa presence/absence and site-specific probability of captures (P_c) and scoring of $O/E_{0.5}$ and BC at reference vs. residential sites

Stream/type/date	Expected	Observed	P_c	$\lvert O-P_c \rvert$	$O+P_c$
Clemons Fork/reference/10 April 2000[a]	*Leuctra*	1	0.95	0.05	1.95
	Epeorus	1	0.93	0.07	1.93
	Diplectrona	1	0.84	0.16	1.84
	Amphinemura	1	0.81	0.19	1.81
	Rhyacophila	1	0.76	0.24	1.76
	Neophylax	1	0.76	0.24	1.76
	Ephemerella	1	0.75	0.25	1.75
	Ameletus	1	0.75	0.25	1.75
	Hexatoma	1	0.73	0.27	1.73
	Paraleptophlebia	0	0.72	0.72	0.72
	Acroneuria	1	0.70	0.30	1.70
	Ectopria	1	0.60	0.40	1.60
	Isoperla	0	0.56	0.56	0.56
	Parametriocnemus	1	0.55	0.45	1.55
	Baetis	0	0.54	0.54	0.54
	Sweltsa	1	0.53	0.47	1.53
	Cambarus	1	0.53	0.47	1.53
	Cinygmula	1	0.50	0.50	1.50
	Sum	15	12.5	6.1	27.5
Caney Creek/residential/12 April 2000[b]	*Leuctra*	0	0.93	0.93	0.93
	Epeorus	0	0.89	0.89	0.89
	Amphinemura	0	0.80	0.80	0.80
	Diplectrona	0	0.79	0.79	0.79
	Hexatoma	0	0.77	0.77	0.77
	Neophylax	0	0.76	0.76	0.76
	Rhyacophila	0	0.76	0.76	0.76
	Ameletus	0	0.76	0.76	0.76
	Ephemerella	1	0.72	0.28	1.72
	Acroneuria	0	0.68	0.68	0.68
	Paraleptophlebia	0	0.67	0.67	0.67
	Ectopria	0	0.59	0.59	0.59
	Prosimulium	0	0.56	0.56	0.56
	Isoperla	1	0.55	0.45	1.55
	Cambarus	1	0.54	0.46	1.54
	Parametriocnemus	1	0.52	0.48	1.52
	Optioservus	1	0.50	0.50	1.50
	Sweltsa	0	0.50	0.50	0.50
	Sum	5	12.3	11.6	17.3

Example paired sites were selected based on location proximity, collection dates, and similar catchment areas even though models are standardized to site-specific expectations and can be used across the entire gradient of natural predictors

$O/E_{0.5}$ observed/expected model with a probability of capture at >0.5

[a] Observed=15; expected (ΣP_c)=12.5; $O/E_{0.5}$=1.20; BC ($\Sigma|O-P_c|/\Sigma(O+P_c)$)=0.22

[b] Observed=5; expected (ΣP_c)=12.3; $O/E_{0.5}$=0.41; BC ($\Sigma|O-P_c|/\Sigma(O+P_c)$)=0.67

References

Barbour, M. T., Gerritsen, J., Snyder, B. D., & Stribling, J. B. (1999). Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates, and fish, 2nd edition. EPA 841-B-99-002. Washington, DC: US Environmental Protection Agency, Office of Water.

Bernhardt, E. S., Lutz, B. D., King, R. S., Fay, J. P., Carter, C. E., Helton, A. M., et al. (2012). How many mountains can we mine? Assessing the regional degradation of Central Appalachian rivers by surface coal mining. *Environmental Science and Technology, 46*(15), 8115–8122.

Carlisle, D. M., Hawkins, C. P., Meador, M. R., Potapova, M., & Falcone, J. (2008). Biological assessments of Appalachian streams based on predictive models for fish, macroinvertebrate, and diatom assemblages. *Journal of the North American Benthological Society, 27*(1), 16–37.

Chambers, D.B., & Messinger T. (2000). Benthic invertebrate communities and their responses to selected environmental factors in the Kanawha River Basin, West Virginia, Virginia, and North Carolina. Water-Resources Investigations Report 01-4021. US Geological Survey, Charleston, West Virgina.

Clarke, R. T., Wright, J. F., & Furse, M. T. (2003). RIVPACS models for predicting the expected macroinvertebrate fauna and assessing the ecological quality of rivers. *Ecological Modelling, 160*(3), 219–233.

Fry, J., Xian, G., Jin, S., Dewitz, J., Homer, C., Yang, L., et al. (2011). Completion of the 2006 National Land Cover Database for the Conterminous United States. *Photogrammetric Engineering and Remote Sensing, 77*(9), 858–864.

Fritz, K. M., Fulton, S., Johnson, B. R., Barton, C. D., Jack, J. D., Word, D. A., et al. (2010). Structural and functional characteristics of natural and constructed channels draining a reclaimed mountaintop removal and valley fill coal mine. *Journal of the North American Benthological Society, 29*(2), 673–689.

Hargett, E. G., ZumBerge, J. R., Hawkins, C. P., & Olson, J. R. (2007). Development of a RIVPACS-type predictive model for bioassessment of wadeable streams in Wyoming. *Ecological Indicators, 7*(4), 807–826.

Hartman, K. J., Kaller, M. D., Howell, J. W., & Sweka, J. A. (2005). How much do valley fills influence headwater streams? *Hydrobiologia, 532*(1–3), 91–102.

Hawkins, C. P., Norris, R. H., Hogue, J. N., & Feminella, J. W. (2000). Development and evaluation of predictive models for measuring the biological integrity of streams. *Ecological Applications, 10*(5), 1456–1477.

Hawkins, C. P. (2006). Quantifying biological integrity by taxonomic completeness: its utility in regional and global assessments. *Ecological Applications, 16*(4), 1277–1294.

Hawkins, C. P., Cao, Y., & Roper, B. (2010). Method of predicting reference condition biota affects the performance and interpretation of ecological indices. *Freshwater Biology, 55*(5), 1066–1085.

Herbst, D. B., & Silldorff, E. L. (2006). Comparison of the performance of different bioassessment methods: similar evaluations of biotic integrity from separate programs and procedures. *Journal of the North American Benthological Society, 25*(2), 513–530.

Herlihy, A. T., Kaufmann, P. R., Church, M. R., Wigington, P. J., Jr., Webb, J. R., & Sale, M. J. (1993). The effects of acidic deposition on streams in the Appalachian Mountain and Piedmont Region of the Mid-Atlantic United States. *Water Resources Research, 29*(8), 2687–2703.

Hubler, S. (2008). PREDATOR: development and use of RIVPACS-type macroinvertebrate models to assess the biotic condition of wadeable Oregon streams (November 2005 models). DEQ08-LAB-0048-TR Version 1.1. Hillsboro, OR: Oregon Department of Environmental Quality. http://159.121.9.10/lab/techrpts/docs/10-lab-004.pdf. Accessed 13 Aug 2012.

Kentucky Department for Environmental Protection (KYDEP). (2009). Laboratory procedures for macroinvertebrate processing, taxonomic identification, and reporting. Kentucky Energy and Environment Cabinet. Available from http://water.ky.gov/Documents/QA/Surface%20Water%20SOPs/BenthicMacroinvertebratesLabProcessingandIdentificationSOP.pdf

Lindberg, T. T., Bernhardt, E. S., Bier, R. A., Helton, M., Merola, R. B., Vengosh, A., et al. (2011). Cumulative impacts of mountaintop mining on an Appalachian watershed. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 20929–20934.

Merriam, E. R., Petty, J. T., Merovich, G. T., Jr., Fulton, J. B., & Strager, M. P. (2011). Additive effects of mining and residential development on stream conditions in a central Appalachian watershed. *Journal of the North American Benthological Society, 30*(2), 399–418.

Ode, P. R., Hawkins, C. P., & Mazor, R. D. (2008). Comparability of biological assessments derived from predictive models and multimetric indices of increasing geographic scope. *Journal of the North American Benthological Society, 27*(4), 967–985.

Ostermiller, J. D., & Hawkins, C. P. (2004). Effects of sampling error on bioassessments of stream ecosystems: application to RIVPACS-type models. *Journal of the North American Benthological Society, 23*(2), 363–382.

Palmer, M. A., Bernhardt, E. S., Schlesinger, W. H., Eshleman, K. N., Foufoula-Georgiou, E., Hendryx, M. S., et al. (2010). Mountaintop mining consequences. *Science, 327*(5962), 148–149.

Pond, G. J., Call, S. M., Brumley, J. F., & Compton, M. C. (2003). The Kentucky macroinvertebrate bioassessment index: Derivation of regional narrative ratings for assessing wadeable and headwater streams. Frankfort, KY: Kentucky Department for Environmental Protection, Division of Water. Available from http://water.ky.gov/Documents/QA/MBI/Statewide_MBI.pdf. Accessed 13 August 2012.

Pond, G. J., Passmore, M. E., Borsuk, F. A., Reynolds, L., & Rose, C. A. (2008). Downstream effects of mountaintop coal mining: comparing biological conditions using family- and genus-level macroinvertebrate bioassessment tools. *Journal of the North American Benthological Society, 27*(3), 717–737.

Pond, G. J. (2010). Patterns of Ephemeroptera taxa loss in Appalachian headwater streams (Kentucky, USA). *Hydrobiologia, 641*(1), 185–201.

Pond, G. J. (2012). Biodiversity loss in Appalachian headwater streams (Kentucky, USA): Plecoptera and Trichoptera communities. *Hydrobiologia, 679*(1), 97–117.

Pond, G. J., Bailey, J. E., Lowman, B. M., & Whitman, M. J. (2012). Calibration and validation of a regionally and seasonally stratified macroinvertebrate index for West Virginia wadeable streams. *Environmental Monitoring and Assessment*. doi:10.1007/s10661-012-2648-3.

Pont, D., Hughes, R. M., Whittier, T. R., & Schmutz, S. (2009). A predictive index of biotic integrity model for aquatic-vertebrate assemblages of western U.S. streams. *Transactions of the American Fisheries Society, 138*(2), 292–305.

R Development Core Team. (2006). R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Available from http://www.R-project.org. Accessed 15 August 2012.

Rosenberg, D. M., & Resh, V. H. (1993). Introduction to freshwater biomonitoring and benthic macroinvertebrates. In D. M. Rosenberg & V. H. Resh (Eds.), *Freshwater Biomonitoring and Benthic Macroinvertebrates* (pp. 1–9). Norwell, MA: Kluwer Academic.

Stoddard, J. L., Larsen, D. P., Hawkins, C. P., Johnson, R. K., & Norris, R. H. (2006). Setting expectations for the ecological condition of running waters: the concept of reference condition. *Ecological Applications, 16*, 1267–1276.

US Environmental Protection Agency. 2005. Mountaintop mining/valley fills in Appalachia. Final programmatic environmental impact statement. Region 3, US Environmental Protection Agency, Philadelphia, Pennsylvania. Available from http://www.epa.gov/region03/mtntop/eis2005.htm

Van Sickle, J., Huff, D. H., & Hawkins, C. P. (2006). Selecting discriminant function models for predicting the expected richness of aquatic macroinvertebrates. *Freshwater Biology, 51*(2), 359–372.

Van Sickle, J. (2008). An index of compositional dissimilarity between observed and expected assemblages. *Journal of the North American Benthological Society, 27*(2), 227–235.

Van Sickle, J., Larsen, D. P., & Hawkins, C. P. (2007). Exclusion of rare taxa affects performance of the *O*/*E* index in bioassessments. *Journal of the North American Benthological Society, 26*(2), 319–331.

West Virginia Department of Environmental Protection (WVDEP). (2011). Watershed Branch 2011 Standard Operating Procedures. West Virginia Department of Environmental Protection, Division of Water and Waste Management, Watershed Assessment Branch. Charleston, WV. Available from http://www.dep.wv.gov/WWE/watershed/Pages/WBSOPs.aspx

Wigington, P. J., Jr., Baker, J. P., DeWalle, D. R., Kretser, W. A., Murdoch, P. S., Simonin, H. A., et al. (1996). Episodic acidification of small streams in the northeastern United States: episodic Response Project. *Ecological Applications, 6*(2), 374–388.

Woods, A.J., Omernik, J.M., Brown, D.D. & Kiilsgaard, C.W. (1996). Level III and IV Ecoregions of Pennsylvania and the Blue Ridge Mountains, the Ridge and Valley, and the Central Appalachians of Virginia, West Virginia, and Maryland. EPA/600R-96/077. USEPA, ORD, Corvallis, OR

Woods, A. J., Omernik, J. M., Martin, W. H., Pond, G. J., Andrews, W.M., Call, S. M., Comstock, J.A., & Taylor, D. D. (2002). Ecoregions of Kentucky (2 sided color poster with map, descriptive text, summary tables, and photographs): Reston, Va., US Geological Survey (map scale 1:1,000,000).

Wright, J. F., Sutcliffe, D. W., & Furse, M. T. (Eds.). (2000). *Assessing the biological quality of fresh waters: RIVPACS and other techniques*. Ambleside, Cumbria, UK: Freshwater Biological Association.

Yuan, L. L., Hawkins, C. P., & Van Sickle, J. (2008). Effects of regionalization decisions on an *O*/*E* index for the US national assessment. *Journal of the North American Benthological Society, 27*(4), 892–905.

Aquatic Toxicology 97 (2010) 109–115

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

The effects of total dissolved solids on egg fertilization and water hardening in two salmonids—Arctic Grayling (*Thymallus arcticus*) and Dolly Varden (*Salvelinus malma*)

Kevin V. Brix[a,b,*], Robert Gerdes[a], Nathan Curry[c], Amanda Kasper[a], Martin Grosell[a]

[a] RSMAS, University of Miami, Miami, FL, USA
[b] EcoTox, Key Biscayne, FL, USA
[c] US Department of Agriculture, USA

ARTICLE INFO

Article history:
Received 1 August 2009
Received in revised form 7 December 2009
Accepted 10 December 2009

Keywords:
Total dissolved solids
Salmonid
Fertilization success
Water hardening

ABSTRACT

Previous studies have indicated that salmonid fertilization success may be very sensitive to elevated concentrations of total dissolved solids (TDS) with effects at concentrations as low as 250 mg l^{-1} being reported. However, interpretation of these studies is complicated by poor control performance and variable concentration response relationships. To address this, a series of experiments were performed to evaluate TDS effects on Arctic Grayling (*Thymallus arcticus*) and Dolly Varden (*Salvelinus malma*) fertilization success and identify possible mechanisms for previously observed test variability and any observed effects of TDS. Results indicate that some of the experiments reported here were likely confounded by extended milt holding times prior to experiment initiation. Milt holding times >6 h were shown to significantly reduce control fertilization and corresponding concentration response relationships were variable. When milt holding time was minimized during fertilization experiments, consistent control performance with >90% control fertilization was achieved and consistent concentration response relationships were observed for both species examined. Experiments performed under these conditions indicate that Arctic Grayling and Dolly Varden fertilization success is not sensitive to elevated TDS with EC20s (concentration causing 20% effect) of >2782 and >1817 mg l^{-1} (the highest concentrations tested), respectively. However, TDS was shown to significantly affect embryo water absorption during the water hardening phase immediately following fertilization. The lowest observable effect concentrations (LOECs) for this endpoint were 1402 and 964 mg l^{-1} for Arctic Grayling and Dolly Varden, respectively. The effect of reduced embryo turgidity, due to impaired water absorption, on resistance to mechanical damage under real world conditions needs further investigation in order to understand the implications of this observed effect.

1. Introduction

Teck Cominco's Red Dog Mine (RDM) is located north of Kotzebue, Alaska. The RDM is a lead–zinc mine with on-site milling operations. In the milling process, RDM generates tailings that, together with mine drainage and otherwise impacted waters are deposited in an impoundment. Mining activities accelerate the naturally occurring oxidation of sulfide minerals such as pyrite (FeS_2) and sphalerite (ZnS), which results in the mine drainage water containing high levels of dissolved metal sulfates. Typical of many hard rock mining operations, the RDM utilizes a lime treatment plant for the removal of heavy metal contamination in the tailings impoundment water. The treatment plant removes the dissolved metals from solution and replaces them with calcium resulting in a high total dissolved solids (TDS) concentration in the whole effluent (comprised primarily of $CaSO_4$) of approximately 3300 mg l^{-1} (ranging between 2400 and 3900 mg l^{-1}). TDS is typically defined as the sum of major cations (Ca^{2+}, Na^+, Mg^{2+}, K^+) and anions ($SO_4{}^{2-}$, Cl^-, $HCO_3{}^-$) present in water.

The effluent is discharged to Red Dog Creek, a first order tributary of the Ikalukrok River, which is part of the larger Wulik River drainage. This drainage supports large populations of several salmonids including Dolly Varden (*Salvelinus malma*) and Arctic Grayling (*Thymallus arcticus*) which spawn in the upper drainage, including Red Dog Creek and the upper Ikalukrok River. As a result, the elevated TDS concentrations in the RDM discharge are of potential concern with respect to the protection of these fish.

The effects of elevated TDS, or the specific ions comprising TDS, on freshwater aquatic organisms have largely been limited to acute

* Corresponding author at: University of Miami, RSMAS, Marine Biology and Fisheries, 4600 Rickenbacker Causeway, Miami, FL 33149, USA. Tel.: +1 904 210 6562.
E-mail address: kbrix@rsmas.miami.edu (K.V. Brix).

0166-445X/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2009.12.011

lethal testing of several standard test organisms (e.g., *Ceriodaphnia dubia*, *Daphnia magna*, and *Pimephales promelas*). Data from these studies have allowed development of statistical models investigating the sublethal toxicity of TDS to aquatic biota (Mount et al., 1997; Tietge et al., 1997). Specific to fish, these studies have investigated the effects of elevated TDS on egg fertilization, water hardening, and early survival.

Early studies on the effects of high TDS (13 mM $CaSO_4$; 1597 mg l^{-1} TDS) showed that egg fertilization was significantly impacted and that Ca^{2+} was the primary cause of observed effects (Ketola et al., 1988). More recently and specific to the RDM, Chapman et al. (2000) investigated the effects of elevated TDS on rainbow trout (*Oncorhynchus mykiss*). They simulated the ionic composition of the RDM effluent and a second mining effluent and evaluated the effects of increasing TDS concentrations on *O. mykiss* embryo viability as well as fry survival and growth during a 7-d exposure. No significant effects were observed for either simulated effluent at any concentration tested (up to 2000 mg l^{-1} TDS) for any of the endpoints.

Based on this study and a review of the extant literature, the State of Alaska granted RDM a site-specific water quality standard for TDS of 1500 mg l^{-1} in the mainstem Red Dog Creek during periods when salmonids are not spawning. During spawning periods, the limit was set at 500 mg l^{-1} TDS. The 500 mg l^{-1} TDS limit during periods of salmonid spawning is based on current State of Alaska Water Quality Regulations. However, a series of unpublished (see Weber-Scannell and Duffy (2007) for summary review) toxicity studies conducted by Stekoll et al. (2003a,b), has recently raised questions regarding the validity of the State Water Quality Standards for TDS.

Stekoll et al. (2003a) conducted several types of studies evaluating various endpoints including fertilization, early embryonic development, and long-term embryonic development. The first studies evaluated fertilization and development in 96-h assays with coho salmon (*Oncorhynchus kisutch*). By exposing eggs or embryos to elevated TDS before, after or during both fertilization and development the researchers determined which life stage was most sensitive. Results from these experiments clearly showed that eggs exposed to elevated TDS during fertilization were most sensitive.

In another set of experiments, Stekoll et al. (2003b) further evaluated TDS toxicity to site-specific populations of Arctic Grayling, Dolly Varden and chum salmon. The TDS composition was a simulation of RDM effluent. In the Arctic Grayling assay, a significant difference in fertilization was observed between the controls and the lowest concentration tested of 500 mg l^{-1}. However, there was no effect at the next two higher concentrations. In the chum salmon assay, Stekoll et al. reported there was strong evidence for fertilization effects at 250 mg l^{-1}; however, fertilization was also low in the controls. Finally, in the Dolly Varden assay, the authors could make no conclusions on the effects of TDS to this species due to low fertilization rates in all treatments including the control.

Overall, the results for all three species were inconclusive due to interrupted concentration-response relationships or poor fertilization rates in the controls. However, they raise the possibility of effects on salmonid spawning at TDS concentrations below the site-specific criterion issued for mainstem Red Dog and Ikalukrok Creeks of 500 mg l^{-1} TDS currently applied to RDM effluent during salmonid spawning periods. Given the intra- and inter-species variability observed in the Stekoll et al. studies, and the fact that some of the effects thresholds estimated approach background TDS concentrations where salmonids successfully spawn, further research is needed on the effects of TDS on salmonid embryo fertilization. Ideally, additional studies would be conducted to determine effect thresholds with greater precision than has been achieved to date.

The objectives of the present studies were to provide a better understanding of the potential effects of TDS on salmonid fertilization success using the two species known to spawn near in the Red Dog and upper Ikalukrok creeks, *T. arcticus* and *S. malma*. Further, if significant effects were observed, we sought to gain an improved understanding of the mechanism(s) by which elevated TDS may affect the early life stages of salmonids. While the current study is site-specific, discharge of high TDS hard rock mining effluent into streams where salmonids spawn is relatively common in western North and South America giving broader applicability to the results and implications of this study.

2. Methods and materials

2.1. General

Studies on Arctic Grayling were conducting in May–June 2004 during their spawning season while Dolly Varden experiments were performed in September 2004 during their corresponding spawning season. As discussed below, relatively high variability in the Arctic Grayling results prompted additional studies on this species in May 2005.

The Stekoll et al. studies evaluated the relative sensitivity of several different endpoints/life stages. The first endpoint, termed the fertilization endpoint, included fertilization, water hardening and embryo development through 50% epiboly. Other endpoints included embryo development from 50% epiboly up to hatching, hatching success, and larval growth and survival. Although there were significant uncertainties regarding what concentration of TDS caused effects, these studies were relatively conclusive in demonstrating that the fertilization endpoint (fertilization, water hardening and development through 50% epiboly) was the most sensitive of those evaluated. Based on these results our experiments focused on this endpoint as well. However, in addition to the standardized tests that generally copied the Stekoll et al. methodology, we also conducted experiments to evaluate the effects of elevated TDS on water absorption and net ion flux during the water hardening phase of development, as well as experiments evaluating TDS effects and milt storage time on sperm longevity. The objective of these experiments was to potentially elucidate physiological mechanisms by which any observed toxicity might be manifested.

2.2. Fish collection

The movement of adult fish upstream into the upper Ikalukrok and Red Dog creeks was closely monitored to facilitate collection as soon as they reached spawning beds, when gametes are at their optimum quality. Adult Arctic Grayling were collected from Bons Pond using hook and line and from North Fork Red Dog Creek using a fyke net. Sufficient fish were collected over a 2-d period in 2004 to conduct a total of 4 toxicity tests. After this period, additional females collected from Red Dog creek were either partially or completely spawned out, making them unsuitable for use in additional toxicity testing. In 2005, Arctic Grayling were collected from the same two locations allowing 4 additional toxicity tests to be performed with gametes from these animals.

Dolly Varden were collected from upper Ikalukrok Creek and on the Wulik River approximately 43 km upstream of the confluence with Ikalukrok Creek. All fish were collected by beach seine in September 2004. Adult fish not used the day of capture were held at the collection site in hoop nets (males and females kept in separate nets). A total of 7 toxicity tests were conducted on Dolly Varden over the course of the study period.

Collected fish were spawned in the field with gametes from individual males and females collected separately into 50 ml polypropylene test tubes and placed on ice for transport back to the laboratory. Once in the laboratory, both eggs and milt were stored in an environmental chamber maintained at the test temperature used for each of the species. Milt was carefully inspected for quality prior to experimentation. When excessive blood or feces were present, the milt was discarded. Once in the laboratory, milt quality was further evaluated by placing a small subsample on a microscope slide, adding a drop of freshwater and observing motility (Environment Canada, 1998). For the Arctic Grayling testing, only highly active milt was used in testing and the sperm density ($0.66–1.35 \times 10^{10}$ sperm ml^{-1}) for pooled milt samples used to conduct the toxicity tests were within a factor of 2 of each other for all tests conducted.

In contrast to this, Dolly Varden milt generally had low or no activity in all males sampled. Milt used in only one of the Dolly Varden experiments had activity that approached what was typically observed for Arctic Grayling. Low sperm motility has previously been observed for Dolly Varden (F. Decicco, personal communication) that still produced high fertilization rates. Given this, we did not use sperm motility as a screening tool for milt quality in the Dolly Varden testing. Sperm density for the Dolly Varden used in toxicity testing was also more variable than for Arctic Grayling, varying in density by a factor of 5.5 ($0.25–1.37 \times 10^{10}$ sperm ml^{-1}).

2.3. Toxicity tests

The general experimental design was similar for all tests. All testing was conducted on-site at Teck Cominco's RDM in a building separate from the mine/mill facilities. Approximately 30–50 eggs were placed in 30 ml polypropylene cups along with 20 μl of milt. Care was taken to ensure milt and eggs did not contact each other (i.e., no dry fertilization was allowed). To this, 5 ml of the test solution was added in a manner that rapidly mixed the milt and eggs together. Eggs were allowed to fertilize for 2 min after which they were rinsed twice with 10 ml of fresh solution and then transferred to 1 l beakers. Each test beaker contained 500 ml of test solution and was placed on gentle aeration (~100 bubbles/min.) in a temperature controlled environmental chamber at 6 °C for the Arctic Grayling and 5 °C for the Dolly Varden.

For each test conducted in 2004, nominal TDS concentrations of 125, 250, 500, 750, 1000, and 2000 mg l^{-1} were evaluated. The 125 mg l^{-1} treatment served as the control group for all toxicity tests. The exception to this was the first Arctic Grayling test (AG1) in which the 2000 mg l^{-1} TDS treatment was omitted due to the limited number of eggs available for testing. In 2005, higher nominal TDS concentrations of 150, 300, 500, 750, 1500, and 3000 mg l^{-1} TDS were tested based on 2004 results where no significant effects were observed at the highest TDS concentration tested in tests with acceptable control performance (see Section 3).

All salts used to make the test waters were either technical or reagent grade (Sigma Chemicals, St. Louis, MO). Temperature, dissolved oxygen and pH were measured at test initiation and termination. Samples were collected from each treatment for measurement of ionic composition. Each treatment was tested in either triplicate or quadruplicate depending on the amount gametes available.

Because of the difficulty in discerning fertilization in Arctic Grayling embryos at 24 h, the exposure period was extended to 72 h after which the embryos were fixed in Stockard solution (5% formaldehyde, 4% glacial acetic acid, 6% glycerin) for later inspection. In contrast, fertilization was easily discernable at 24 h for Dolly Varden embryos, and all tests were terminated at this time. Embryos for both species were scored as fertilized/unfertilized at the University of Miami using a dissecting microscope.

2.4. Characterization of water uptake in embryos

To characterize the potential effects of elevated TDS on water hardening of the newly fertilized embryos, a separate experiment was performed in which embryo mass was determined as a function of TDS exposure concentration. It was assumed that changes in embryo mass would primarily be a function of water absorption during initial development. In the experiment with Arctic Grayling, embryos were exposed to measured TDS concentrations of 145, 784, 1402, and 1381 mg l^{-1} for up to 840 min after fertilization. At 0, 5, 10, 20, 40, 60, 90, 120 and 840 min after fertilization, 10 embryos were sampled from each treatment and individual embryo mass was determined to the nearest 0.1 mg. The Dolly Varden experiment used the same design as described above, but measured TDS concentrations were 250, 585, 964, and 1789 mg l^{-1} in this experiment.

Table 1
2004 Arctic Grayling test media ionic composition (mM).

Parameter	Nominal TDS concentration (mg l^{-1})					
	125	250	500	750	1000	2000
Ca^{2+}	0.90	1.82	3.24	3.67	4.34	8.33
K^+	0.03	0.07	0.15	0.20	0.28	0.51
Mg^{2+}	0.10	0.20	0.38	0.62	0.74	1.60
Na^+	0.11	0.23	0.48	0.87	0.87	1.65
Cl^-	0.03	0.06	0.19	0.28	0.38	0.70
HCO_3^-	0.13	0.25	0.48	0.69	0.85	1.82
SO_4^{2-}	0.83	1.57	3.26	5.01	6.63	8.53
pH	7.1	7.3	7.7	7.8	7.6	7.8
TDS (mg l^{-1})	132	254	503	719	921	1381
Hardness (mg l^{-1})	100	202	362	429	509	1002

2.5. Analytical chemistry

Cations and anions were measured using atomic absorption (Varian SpectrAA 220FS) and ion chromatography (DIONEX DX-120), respectively, with the exception of bicarbonate, which was measured by double endpoint titration in the Arctic Grayling testing and using a total CO_2 analyzer (Corning 965) in the Dolly Varden tests. These two methods for determining bicarbonate concentrations have been cross validated to reveal excellent agreement (Grosell et al., 1999). Hardness was determined from measured Ca^{2+} and Mg^{2+} concentrations. Total dissolved solids were determined as the sum of measured ion concentrations.

2.6. Data analysis

The no observable effect concentration (NOEC), lowest observable effect concentration (LOEC), chronic value (geometric mean of the NOEC and LOEC) and concentration causing a 20% and 50% effect (EC20 and EC50) were determined in each test. The NOEC and LOEC were determined using analysis of variance after appropriate transformations and checks for normality and homogeneity of variance. The EC20 and EC50 (and their 95% confidence limits) were estimated using linear regression techniques (linear interpolation or probit analysis, as appropriate to the data). Data analysis was performed on measured TDS concentrations (not nominal) using ToxCalc Version 5.0 (Tidepool Scientific, McKinleyville, CA, USA).

3. Results

3.1. Water quality

Test temperature was maintained at 6 and 5 °C throughout all of the studies conducted, for the Arctic Grayling and Dolly Varden tests, respectively. Dissolved oxygen was maintained near saturation (~12 mg l^{-1}) in all tests. The individual measured ion concentrations for each of the test treatments are summarized in Tables 1 and 2 for Arctic Grayling (2004 and 2005), and Table 3 for Dolly Varden. In general, measured ion concentrations closely

Table 2
2005 Arctic Grayling test media ionic composition (mM).

Parameter	Nominal TDS Concentration (mg l^{-1})					
	150	300	500	750	1500	3000
Ca^{2+}	0.62	1.20	1.87	3.27	6.06	13.77
K^{+}	0.04	0.08	0.12	0.19	0.36	0.66
Mg^{2+}	0.15	0.21	0.28	0.40	0.66	1.19
Na^{+}	0.05	0.28	0.57	1.09	2.09	4.18
Cl^{-}	0.08	0.12	0.18	0.31	0.48	0.93
HCO_3^{-}	0.15	0.31	0.41	0.69	1.15	2.05
SO_4^{2-}	1.06	2.17	3.48	5.81	10.36	20.02
pH	6.8	6.8	6.9	7.1	7.3	7.6
TDS (mg l^{-1})	145	294	465	784	1402	2782
Hardness (mg l^{-1})	77	141	214	367	671	1497

Table 3
Dolly Varden test media ionic composition (mM).

Parameter	Nominal TDS concentration (mg l^{-1})					
	125	250	500	750	1000	2000
Ca^{2+}	0.70	1.30	2.52	3.74	4.72	9.81
K^{+}	0.05	0.05	0.05	0.05	0.05	0.05
Mg^{2+}	0.58	0.58	0.58	0.58	0.58	0.58
Na^{+}	0.96	1.04	1.04	1.04	1.04	1.09
Cl^{-}	0.05	0.05	0.05	0.06	0.06	0.05
HCO_3^{-}	1.05	1.11	1.23	1.16	1.20	1.23
SO_4^{2-}	1.36	2.10	3.73	5.19	6.80	13.25
pH	7.7	7.7	7.7	7.7	7.7	7.7
TDS (mg l^{-1})	263	363	576	761	957	1784
Hardness (mg l^{-1})	127	187	310	432	529	1036

approximated nominal values but were slightly higher at low TDS concentrations due to low levels of TDS in the on-site deionized water.

3.2. Toxicity test results

A total of 8 toxicity tests were successfully conducted with Arctic Grayling and 7 with Dolly Varden embryos (Table 4). All 8 of the Arctic Grayling tests resulted in >80% control fertilization while 5 of 7 Dolly Varden tests resulted in control fertilization >80%. The two Dolly Varden tests that did not achieve acceptable control fertilization are discussed further below.

Results from all tests are summarized in Table 4 and Figs. 1 and 2. Considerable inter-test variability was observed in the 2004 Arctic Grayling experiments with 2 tests exhibiting high control fertilization (≥97%) and no significant effect of TDS up to the highest concentration tested (Fig. 1a). In contrast, the other two tests, one of which had lower (83%) control fertilization, exhibited a U-shaped concentration response similar to that observed in some of the Stekoll et al. experiments. Less variability was observed in the 2004 Dolly Varden studies with 5 of the 7 tests exhibiting high control fertilization (≥90%) and no significant effect of TDS up to the highest concentration tested (Fig. 2). However, for 2 tests, an inverse

Table 4
Toxicity testing results with Arctic Grayling and Dolly Varden (mg l^{-1} TDS).

Test	NOEC	LOEC	EC20	EC50
AG1	921	>921	>921	>921
AG2	1381	>1381	>1381	>1381
AG3	254	503	748	>1381
AG4	132	254	202	>1381
AG5	2782	>2782	>2782	>2782
AG6	2782	>2782	>2782	>2782
AG7	2782	>2782	>2782	>2782
AG8	2782	>2782	>2782	>2782
DV1	1817	>1817	>1817	>1817
DV2	1789	>1789	>1789	>1789
DV3	1704	>1704	>1704	>1704
DV4[a]	1762	>1762	>1762	>1762
DV5	1777	>1777	>1777	>1777
DV6	1796	>1796	>1796	>1796
DV7[a]	1808	>1808	>1808	>1808

[a] Inverse dose response relationship observed in this test.

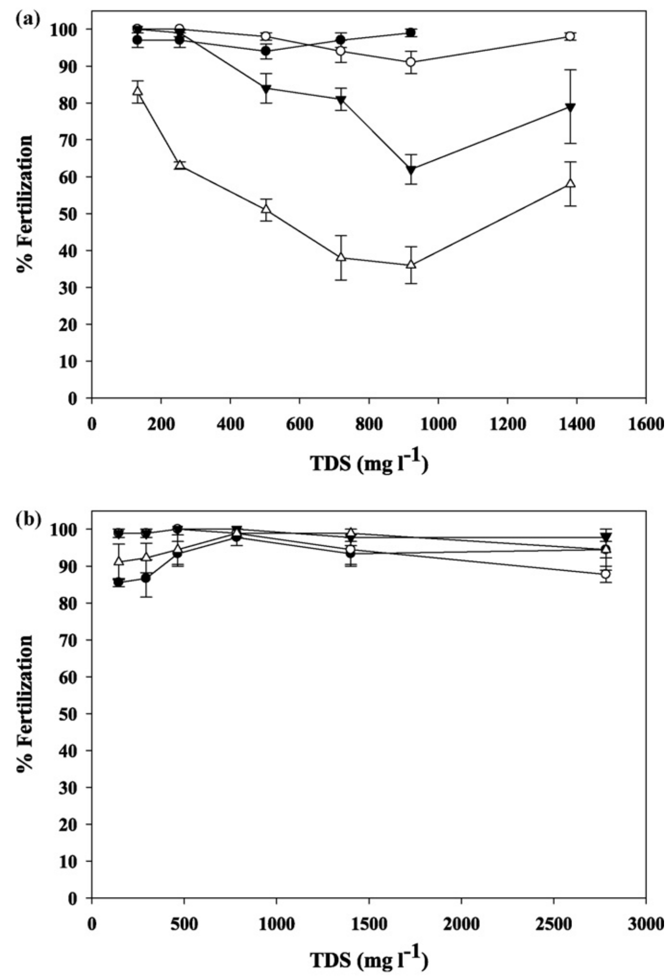


Fig. 1. (a) Arctic Grayling embryo fertilization as a function of TDS concentration. Results of 4 individual tests performed in 2004. (b) Arctic Grayling embryo fertilization as a function of TDS concentration. Results of 4 individual tests performed in 2005.

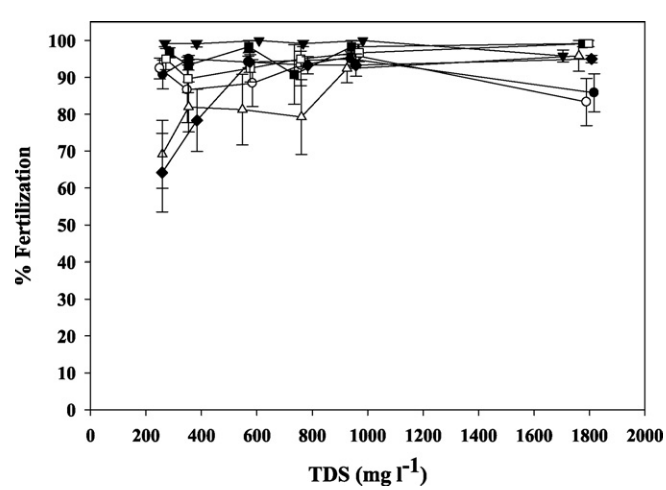


Fig. 2. Dolly Varden embryo fertilization as a function of TDS concentration. Results of 7 individual tests performed in 2004.

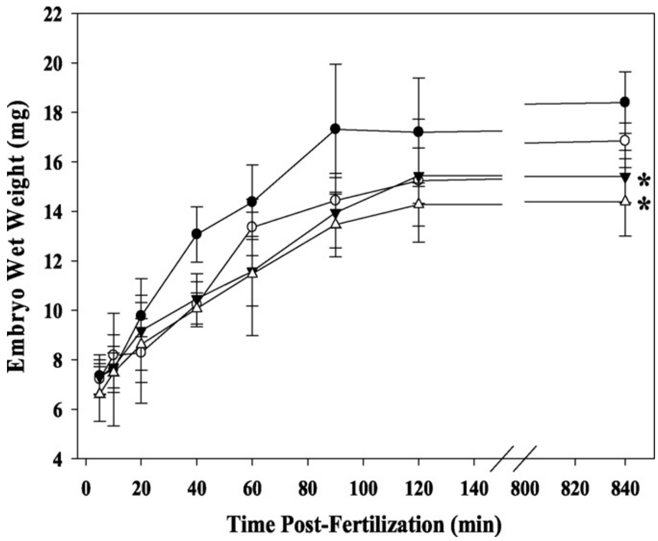


Fig. 3. Arctic Grayling embryo water absorption as a function of TDS concentration ($n = 10$ for each sampling point). (●) 145 mg l^{-1}, (○)784 mg l^{-1}, (▼) 1402 mg l^{-1}, and (△) 2782 mg l^{-1} TDS. *Significantly different from control ($p < 0.05$). Statistical analysis of only the last time point is shown.

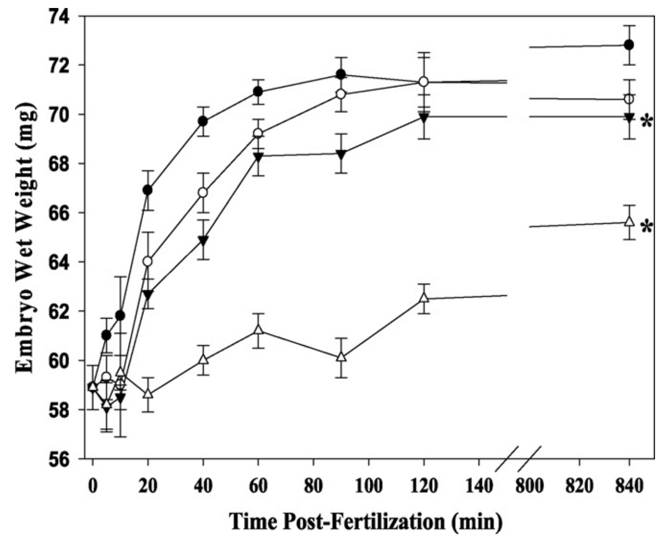


Fig. 4. Dolly Varden embryo water absorption as a function of TDS concentration ($n = 10$ for each sampling point). (●) 250 mg l^{-1}, (○) 585 mg l^{-1}, (▼) 964 mg l^{-1}, and (△) 1789 mg l^{-1} TDS. *Significantly different from control ($p < 0.05$). Statistical analysis of only the last time point is shown.

concentration response relationship was observed with relatively low control fertilization (64% and 69%), but fertilization comparable to the other 5 tests at higher TDS concentrations. All 4 of the Arctic Grayling experiments performed in 2005 were similar, with high control fertilization and no effects of TDS observed up to the highest concentration tested (Fig. 1b).

3.3. Water uptake in embryos

In Arctic Grayling, water absorption in control embryos appeared to reach steady-state 90 min after fertilization, with no significant change in wet weight from 90 min until the end of the exposure at 840 min (Fig. 3). At elevated TDS concentrations, steady-state water absorption appeared to be slightly delayed requiring between 90 and 120 min. After the full 840 min exposure, embryo wet weight was significantly ($p < 0.05$) lower in the 1402 and 2782 mg l^{-1} TDS treatments compared to the control. Although we did not perform a quantitative analysis, qualitatively, embryos from these higher TDS treatments exhibited an obvious reduction in turgidity.

Similar to Arctic Grayling, water absorption in Dolly Varden control embryos appeared to reach steady-state approximately 90 min after fertilization (Fig. 4). Steady-state water absorption was not significantly delayed at 585 or 964 mg l^{-1} TDS, but was significantly delayed in the 1789 mg l^{-1} TDS treatment with a significant ($p < 0.05$) increase in embryo wet weight between the 120 and 840 min sampling points. Additionally, again similar to Arctic Grayling, embryo wet weights were significantly lower in the two highest TDS treatments at the end of the 840 min exposure period, suggesting impairment of water absorption at elevated TDS concentrations. A reduction in embryo turgidity, although not quantified, was not as obvious in Dolly Varden embryos.

4. Discussion

4.1. Toxicity test results

The objective of this study was to resolve some of the uncertainties associated with previous studies conducted by Stekoll et al. on Arctic Grayling and other salmonids. Stekoll et al. (2003b) had previously reported an NOEC of 250 mg l^{-1} and LOEC of 500 mg l^{-1} TDS when testing Arctic Grayling embryos using methods similar to those reported here. However, mean control fertilization (∼68%) was below what is normally considered acceptable in embryo studies (ASTM, 1998). Additionally, although statistically significant effects on fertilization were observed at 500 mg l^{-1} TDS, they did not observe statistically significant effects at 750 and 1250 mg l^{-1} TDS, creating uncertainty as to where the true effect level occurred.

In comparison, in 2004 all of the Arctic Grayling tests conducted in the present study achieved >80% control fertilization. However, results from the 2004 studies did not resolve the uncertainty associated with previous studies as two of the tests conducted indicated no significant effect of TDS on fertilization success up to the highest concentration tested while the other two tests did indicate effects with one test having effects below the 500 mg l^{-1} TDS water quality standard. Further, for the two tests where effects were observed, a reduced effect was observed in the highest TDS concentration tested, repeating the unusual concentration-response previously observed by Stekoll et al. (2003b).

The observed variability in the 2004 tests may be the result of natural differences in the quality and sensitivity of Arctic Grayling embryos to TDS. The increased sensitivity to TDS in the second two tests from 2004 generally corresponded to reduced control fertilization suggesting the embryos were less robust than in the first two tests. The increased sensitivity in the second two tests also corresponded with an increase in ambient temperature from which the adults were collected. Finally, the increased sensitivity also corresponded with the end of the spawning window for the Arctic Grayling. One or more of these factors may have contributed to the observed variability. It is also possible that the variability in the 2004 tests is the result of an artifact in the test method, a possibility discussed further below.

The Dolly Varden studies were much more conclusive than the 2004 Arctic Grayling studies. None of the experiments on Dolly Varden indicate a significant effect on fertilization success even up to the highest concentration tested. However, two of the Dolly Varden tests did exhibit an inverse concentration response relationship with the lowest fertilization success in the controls and highest fertilization success in the highest TDS treatment. We hypothesize this inverse concentration response relationship may be a result of extended milt holding time. When milt holding time is plotted

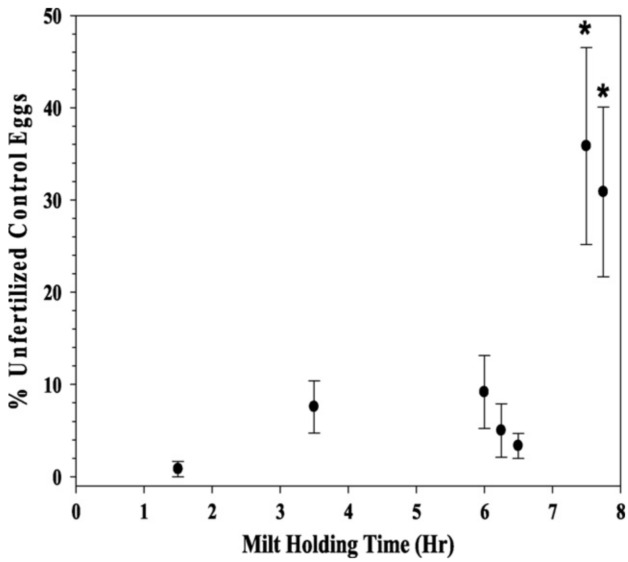


Fig. 5. Effect of milt holding time on Dolly Varden control fertilization. *Statistically different from control ($p < 0.05$).

against control fertilization, there appears to be a clear effect on milt quality when the holding time exceeds 6 h (Fig. 5).

Salmonid sperm is typically viable for several days when held at 5–6 °C (Scott and Baynes, 1980). However, it has also been shown that it is critical that sperm be well oxygenated during holding as cellular respiration is occurring to some extent in unactivated sperm. Consistent with the Stekoll et al. studies, milt was not actively aerated during holding, rather sperm were maintained in 50 ml polypropylene test tubes that were loosely capped to reduce evaporation and provide good air exchange. It is possible that these holding conditions did not provide sufficient oxygen for the sperm and the threshold for viability was being reached at approximately 7 h. Several previous studies have shown that sperm rapidly lose viability (within 3–5 h) if sufficient oxygen supply is not available (Smith and Quistorff, 1943; Henderson and Dewar, 1959). Interestingly, it has also been shown that exposure to high concentrations (10 mM) of Ca^{2+} and Mg^{2+} can counteract the effects of low oxygen supply, though the mechanism for this effect is unknown (Pautard, 1962). Given this, it is possible that the elevated Ca^{2+} in the higher TDS concentrations counteracted the oxygen depletion effect, providing a mechanism for the observed inverse concentration response relationship observed in several experiments during this study.

Based on the results of the Dolly Varden experiments, we hypothesized that the variable results observed in the 2004 Arctic Grayling experiments may also have been caused by excessive holding time for the milt. Exact records were not taken regarding milt holding time in the 2004 Arctic Grayling experiments, but in general holding time was >4 h and exceeded 8 h in at least one test. To address this issue, holding times for milt in the 2005 experiments were <3 h for all studies and <2 h for 3 of the experiments. With the exception of reducing the holding time, milt in the 2005 studies was treated exactly the same as in the 2004 studies. Similar to the Dolly Varden experiments, the 2005 Arctic Grayling experiments provided very consistent data indicating no effects on fertilization success up to the highest TDS concentration tested (2782 mg l^{-1}).

4.2. Effect of TDS on embryo water absorption

Because we were assessing fertilization success on embryos that have reached the epiboly stage of development, we hypothesized that any observed effects on fertilization success might actually be an effect on early embryo development. In particular, given the results of previous studies, we hypothesized that elevated TDS might interfere with water hardening. While the fertilization success experiments show rather conclusively that elevated TDS is not affecting Arctic Grayling and Dolly Varden fertilization, significant effects on water absorption were observed (Figs. 3 and 4).

There were interesting differences between the two species under control conditions. The small (6 mg wet weight) Arctic Grayling eggs underwent a nearly 3-fold increase in mass during water hardening while the comparatively larger (59 mg wet weight) Dolly Varden egg mass only increased by ~25%. Despite these inherent differences in water absorption, the two species appeared to be roughly similar in sensitivity with respect to the effect of TDS on water absorption.

The long-term impact of observed reductions in water absorption during the water hardening phase is unclear. In addition to the short-term fertilization assays, Stekoll et al. (2003a) performed longer exposures evaluating hatching success and early larval development. These life stages were less sensitive than fertilization suggesting that the long-term impacts of reduced water absorption may not be significant. However, these experiments obviously do not simulate real world conditions where embryo turgidity and corresponding resistance to mechanical damage are likely to be much more important. Given the data in this study indicate that fertilization success is not very sensitive to elevated TDS for Arctic Grayling and Dolly Varden, effects on water hardening appear to be the most sensitive endpoint evaluated to date and further research on this endpoint is needed.

5. Conclusions

Results from the experiments presented in this paper suggest that previous studies (and some of our own experiments) showing variable but relatively high sensitivity of salmonid fertilization success in high TDS waters may have been confounded by variation in milt holding time. When milt holding time is minimized, more consistent results are achieved indicating that TDS does not have a significant impact on Arctic Grayling and Dolly Varden fertilization up to the highest concentrations evaluated (2782 and 1817 mg l^{-1}, respectively). However, elevated TDS did significantly affect embryo water absorption at concentrations as low as 964 mg l^{-1} TDS (NOEC of 585 mg l^{-1}) and the ecological implications of this effect on embryo survival under real world conditions is worth further investigation. Given these results, the current site-specific water quality standard of 500 mg l^{-1} TDS during salmonid spawning periods and 1500 mg l^{-1} during other periods appears adequate for protection of salmonid reproduction. However, the 500 mg l^{-1} TDS standard during salmonid spawning periods should not be increased based on results from the fertilization study given the observed sensitivity of embryo water absorption to elevated TDS.

Acknowledgements

This study could not have been completed without the generous support of Al Townsend (ADNR) and Fred DeCicco (ADF&G) in assisting with the collection of adult Arctic Grayling and Dolly Varden. Lillian Herger (USEPA) assisted in the conduct of the Dolly Varden fertilization experiments. The authors also acknowledge Mark Thompson (Teck Cominco) for arranging logistical support. This study was funded by a research grant from Teck Cominco Alaska.

References

ASTM, 1998. Standard guide for conducting early life-stage toxicity tests with fishes. Standard E1241-98. In: Annual Book of ASTM Standards: Biological Effects and Environmental Fate; Biotechnology; Pesticides. American Society for Testing and Materials, Philadelphia, Pennsylvania, 11.05, pp. 587–614.

Chapman, P.M., Bailey, H.C., Canaria, E., 2000. Toxicity of total dissolved solids associated with two mine effluents to chironomids larvae and early life stages of rainbow trout. Environ. Toxicol. Chem. 19 (1), 210–214.

Environment Canada, 1998. Biological Test Method: Toxicity Tests Using Early Life Stages of Salmonid Fish (Rainbow Trout). Environment Canada, Ottawa, Ontario, 100 pp.

Grosell, M., De Boeck, G., Johannsson, O., Wood, C.M., 1999. The effects of silver on intestinal ion and acid-base regulation in the marine teleost fish, *Parophrys vetulus*. Comp. Biochem. Physiol. 124C (3), 259–270.

Henderson, N.E., Dewar, J.E., 1959. Short-term storage of brook trout milt. Prog. Fish Cult. 21, 169–170.

Ketola, H.G., Longacre, D., Greulich, A., Phetterplace, L., Lashomb, R., 1988. High calcium concentration in water increases mortality of salmon and trout eggs. Prog. Fish Cult. 50 (3), 129–135.

Mount, D.R., Gulley, D.D., Hockett, J.R., Garrison, T.D., Evans, J.M., 1997. Statistical models to predict the toxicity of major ions to *Ceriodaphnia dubia*, *Daphnia magna* and *Pimephales promelas* (fathead minnows). Environ. Toxicol. Chem. 16 (10), 2009–2019.

Pautard, F.G.E., 1962. Biomolecular aspects of spermatozoan motility. In: Bishop, D.W. (Ed.), Spermatozoan Motility. American Association for the Advancement of Science, Washington, D.C., pp. 189–232.

Scott, A.P., Baynes, S.M., 1980. A review of the biology, handling and storage of salmonid spermatozoa. J. Fish Biol. 17, 707–739.

Smith, R.T., Quistorff, E., 1943. Experiments with the spermatozoa of the steelhead trout, *Salmo gairdneri*, and the Chinook salmon, *O. tshawytscha*. Copeia 3, 164–167.

Stekoll, M., Smoker, W., Wang, I., Failor, B., 2003a. Final report for ASTF grant #98-1-012. Salmon as a bioassay model of effects of total dissolved solids. University of Alaska - Fairbanks, Juneau, Alaska, 87 pp.

Stekoll, M., Smoker, W., Wang, I., Hayes, W., 2003b. Final report on the effects of total dissolved solids on fertilization rates of salmonids in the Red Dog Mine area. University of Alaksa - Fairbanks, Juneau, Alaska, 27 pp.

Tietge, J.E., Hockett, J.R., Evans, J.M., 1997. Major ion toxicity of six produced waters to three freshwater species: application of ion toxicity models and TIE procedures. Environ. Toxicol. Chem. 16 (10), 2002–2008.

Weber-Scannell, P.K., Duffy, L.K., 2007. Effects of total dissolved solids on aquatic organisms: a review of literature and recommendations. Am. J. Environ. Sci. 3 (1), 1–6.

Title: Limiting total dissolved solids to protect aquatic life
Author(s): Rod Bodkin , Arthur J. Butt , James Kern , Charles Martin and Phillip McClellan
Source: ***Journal of Soil and Water Conservation.*** 62.3 (May-June 2007): p57A.
Document Type: Article

Full Text:

WHAT IS TDS?

"Total dissolved solids" (TDS) is a measure of all of the dissolved constituents in water with the exception of dissolved gases. The primary anions are carbonates, chlorides, sulfates, and nitrates. The primary cations are sodium, calcium, potassium, and magnesium.

TDS and conductivity are often related because conductivity is the ability of an aqueous solution to carry an electric current. This relationship depends on the concentration of different ions present, their valence, and the temperature of the solution.

WHY IS TDS A CONCERN?

TDS and elevated conductivity have been identified as a probable cause of biological impairments in high gradient mountain streams in the southern coalfield areas of Virginia, Kentucky, and West Virginia.

In these streams, conductivity is strongly and significantly correlated to degrading biological conditions and impairment. In some of these streams, no other stressor was identified. Typically, streams in the Central Appalachian ecoregion are very dilute with conductivities often less than 40 [micro]mhos [cm.sup.-1]. Furthermore, the benthic macroinvertebrate communities that inhabit these streams are dominated by highly sensitive pollution intolerant species. In areas impacted by mine drainage, conductivities are often elevated and range from 500 to 2,000 [micro]mhos [cm.sup.-1] (USEPA 2006).

Land disturbance such as surface mining increases the dissolved mineral content of natural waters by exposing large areas of fractured rock formations to direct weathering (figure 1). In addition, the water discharged from deep mining operations contains high dissolved ion concentrations. However, there is no way to efficiently and cheaply remove TDS from mine drainage. Therefore, treatment of mine drainage for TDS to date is not a practical option.

The intent of this paper is to review the current state of research with TDS impacts on the aquatic community and to summarize state efforts to address the issue in terms of water quality standards and total maximum daily load (TMDL) development.

LITERATURE REVIEW

Since the late 1980s, TDS has received considerable research attention due to its role as a toxicant. Most of that research involved laboratory toxicity tests using traditional species such as fathead minnows and crustaceans (Cladocera) (organisms approved for testing by the United States Environmental Protection Agency [USEPA] Toxic Management Program protocols) rather than benthic macro-invertebrates. Published toxicity studies have been performed with benthic macroinvertebrates such as Isonychia bicolor and Chironomous tentans (Kennedy 2002; Chapman et al. 2000).

However, inherent difficulties exist in extrapolating the laboratory studies to predict field responses. Organisms used in toxicity tests must be able to withstand the stress that comes from handling them in a laboratory situation or the results of the tests could be compromised. In addition, the short duration of many of these tests makes it unlikely that chronic impacts on sensitive organisms will be detected. Furthermore, not enough is known about the most sensitive life stage of benthic macroinvertebrate organisms to develop protocols to run appropriate laboratory tests.

A case study in Alaska demonstrated the importance of species and life stage when conducting laboratory toxicity tests. Tests on vulnerable life stages of rainbow trout (Oncorhynchus mykiss), the fertilized egg, and swim-up fry were conducted using synthetic effluent from two Alaskan mines. The results found no adverse effects with early life stages up to a TDS concentration of 2,000 mg [L.sup.-1] (Chapman et al. 2000). Subsequent tests on unfertilized Salmon eggs found reduced fertilization rates in king, Coho, and pink salmon at TDS concentrations as low as 250 mg [L.sup.-1] (Stegkoll et al. 2003). The Alaska studies clearly illustrate the need to test multiple species at the most sensitive life stage of the organism needing to be protected.

Many laboratory researchers attribute the observed toxicity of TDS concentrations to the species and concentrations of the specific ions that make up TDS. Evaluations have been performed to determine the most toxic ions to freshwater invertebrates using Ceriodaphnia dubia. According to a 1997 study reported by the Society of Environmental Toxicology (SETAC 2004), the relative toxicity of ions in concentrations of dissolved solids from most toxic to least is [K.sup.+] >

HC[O.sub.3.sup.-] > [Mg.sup.2+] > [Cl.sup.-] > S[O.sub.4.sup.2-].

Research also indicates that the likely mechanism(s) of TDS benthic macroinvertebrate mortality is from gill and internal tissue dehydration, salt accumulation, and compromised osmoregulatory function. In fact, the rate of change in TDS concentrations may be more toxic to benthic macroinvertebrates than the TDS alone (Kennedy 2002). The chronic impacts (rather than the toxicity) that TDS concentrations have on benthic macroinvertebrates could play a very important role in benthic macroinvertebrate impairments.

A technical issue paper noted that the stress caused by the energy expenditure of organisms trying to regulate water and ions can affect growth and reproduction (SETAC 2004). If growth and reproduction are chronically impacted over a significant portion of organisms' life cycles, it is possible for more (salinity) tolerant organisms to replace less tolerant organisms and result in a shift in the community structure toward more pollution-tolerant organisms or an impaired condition. Therefore, TDS has the ability to cause benthic macroinvertebrate impairments by chronic nonlethal effects.

Much of the accumulated knowledge on TDS toxicity is based on acute responses (lethality) due to the standard laboratory procedures used to predict the responses of fish and invertebrates. These standard tests are very limited in their ability to predict the impacts of increasing TDS concentrations on benthic macroinvertebrate communities. They cannot consider the potential indirect impacts of TDS on the functional characteristics of an ecosystem. In a study by Leland and Fend (1998), benthic macroinvertebrate communities were analyzed along a gradient of TDS (55 to 1,700 mg [L.sup.-1]), and species-specific variation in response to concentrations of sulfates and bicarbonates were measured. The findings were not consistent with laboratory toxicity tests (Clements 2002). At the present time, not enough available information exists on the chronic and sublethal responses by benthic macroinvertebrate communities to increasing concentrations of TDS (Clements 2002).

Other possibilities for potential impacts on benthic macroinvertebrate organisms may be on the early instar nymphs or on the eggs (USEPA 2006). Toxicity tests using benthic macroinvertebrate organisms rely on older larvae. Data from small headwaters streams in West Virginia and Kentucky coalfields areas suggest that 90% to 100% of macroinvertebrate Index of Biotic Integrity (IBI) scores were below established impairment thresholds (based on the reference condition) when conductivity is greater than 500 [micro]mhos [cm.sup.-1] (USEPA 2006).

Mayflies (Ephemeroptera; figure 2) appear to be highly sensitive to TDS in these areas (figure 3). A possible explanation for mayflies' sensitivity to TDS is the fact that they have more exposed cells that are not protected by the epithelial layer making them more permeable than many other benthic macroinvertebrates (Kennedy 2002). Researchers were careful to screen the Kentucky and West Virginia data for pH (no benthic data used when pH was less than 6.0 standard units) and for habitat (no benthic data used when total habitat scores were less than optimum). The purpose was to eliminate common covariant stressors such as dissolved metals (metals solubility increases at lower pHs) and poor habitat quality.

Field studies in Kentucky (Pond 2004) and West Virginia (Green et al. 2000) statistically compared land uses to biological indexes and the metrics of which they are composed as well as habitat scores and individual habitat metrics. Together, the studies used 120 sites, including reference sites. Correlations between the benthic metrics and selected physical and chemical variables indicated that the strongest and most significant associations were between biological condition and conductivity (Pond 2004). West Virginia's aggregate bioassessment index, as well as the mayfly taxa richness metric, were also the most strongly correlated with median conductivity (Green et al. 2000). While conductivity was shown to be the primary stressor based on statistical correlation, sediment and habitat were also significantly correlated to lower macroinvertebrate index scores. It is interesting to note that the community composition of benthic macroinvertebrates impacted solely by residential land uses (conductivities typically less than 500 [micro]mhos [cm.sup.-1]) was different from those impacted solely by high conductivities from mining land use in the Kentucky study (Pond 2004).

TMDL DEVELOPMENT AND STANDARDS

Problems caused by high TDS concentrations in freshwater have become more important in many states and in every USEPA region.

USEPA Region 3 was recently awarded a two-year Regional Applied Research Effort (RARE) grant to work with the Duluth, Minnesota, USEPA Office of Research and Development and the U.S. Geological Survey to provide guidelines to the states such as protective TDS thresholds for Appalachian benthic macroinvertebrate communities.

States within USEPA Region 3 have identified TDS as the primary stressor causing benthic impairments in a number of watersheds, primarily in the southern coalfields area of the region. The Virginia Department of Environmental Quality has identified TDS as a primary stressor of benthic macroinvertebrate communities in seven TMDL watershed studies to date in the coalfields area of southwestern Virginia. All seven TMDLs have been approved by the USEPA. Total dissolved solids will likely be identified as a primary stressor as more benthic macroinvertebrate TMDL studies are completed.

The Commonwealth of Virginia has decided to explore development of a TDS water quality standard because the TMDL studies have demonstrated that TDS is a significant pollutant in some geographic areas of the state. USEPA Region 3 recommended that Virginia base its water quality standard on a statistical analysis of suitable field data because of the uncertainties surrounding laboratory toxicity test results.

The West Virginia Department of Environmental Protection has identified TDS as the primary stressor in approximately a dozen streams (D. Montali, personal communication, August 7, 2006). Numerous other streams have been indicated as impaired because of "conditions not allowed." Many of these streams are probably also stressed by TDS.

Both Mississippi and Arkansas have completed TMDLs for conductivity and/or TDS. TMDLs for TDS have been completed and approved by the USEPA in some western states that have high salinity issues due to the arid climate in much of the region.

Table 1 contains examples of numeric and/or narrative water state quality standards for the protection of aquatic life, conductivity, or TDS. Arkansas has by far the most comprehensive water quality standards for TDS. Its standards define a reference concentration for TDS based on ecoregion, and they also list site-specific concentrations for many of the streams in the state. In addition, a narrative provides a process to determine whether a TDS concentration would contravene a standard for a stream that has not been specifically listed.

SUMMARY AND CONCLUSIONS

Total dissolved solids can affect both the survival and reproductive capability of aquatic organisms. The correlation between mayflies and conductivity in the Kentucky study (Pond 2004) provides the most compelling evidence of the negative impact that TDS can have on sensitive benthic

macroinvertebrates at concentrations much lower than predicted by conventional toxicity testing.

Total dissolved solids can impact benthic organisms in more ways than just specific ion toxicity. TDS can cause chronic stress to benthic macroinvertebrates by requiring excess expenditures of energy when regulating water and ions. This leads to reduced growth and reproduction and ultimately a decrease in the ability to compete for resources. These impacts can cause shifts in community composition from less tolerant to more tolerant organisms, which results in biological impairments.

Other chronic or sublethal impacts from TDS have not been fully researched, but negative impacts at other macroinver-tebrate life stages are likely to exist.

Of the states with water quality standards referenced in this paper, Arkansas has the most comprehensive and protective water quality standards for TDS. Because of the mounting evidence that TDS impacts in the field are greater than the impacts predicted by laboratory tests, other state regulatory agencies are developing water quality standards for TDS that will be protective of benthic macroinver-tebrate community health independent of the standard laboratory toxicity testing procedures.

Questions still exist as to the level of TDS that is protective of aquatic communities, and the answer may vary among ecosystems, but it is becoming increasingly evident that standard laboratory toxicity testing procedures alone are not adequate for setting water quality criteria that are protective. Additional field testing of a variety of organisms at varying life stages, under a range of conditions of toxicity, temperature, and duration is necessary to fully resolve this question.

REFERENCES

Chapman, P.M., H. Bailey, and E. Canaria. 2000. Toxicity of total dissolved solids associated with two mine effluents to Chironomid larvae and early lifestages of rainbow trout. Environmental Toxicology and Chemistry 19:210-214.

Clements, W.H. 2002. Final Comments on BLM Draft Environmental Impact Statement Related to Water Quality. Sheridan, WY: Powder River Basin Resource Council. http://www.powder-riverbasin.org/cbm/expert_clements.shtml.

Green, J., M. Passmore, and H. Childers. 2000. A Survey of the Condition of Streams in the Primary Region of Mountaintop Mining/Valley Fill Coal Mining. Philadelphia: PA: United States Environmental Protection Agency Region 3.

Kennedy, A.J. 2002. An Ecotoxicological Assessment of a Treated Coal-mining Effluent in the Leading Creek Watershed, Meigs County, Ohio. MS thesis, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Pond, G.J. 2004. Effects of Surface Mining and Residential Land Use on Headwater Stream Biotic Integrity. Division of Water, Frankfort, KY: Kentucky Department of Environmental Protection.

SETAC (Society of Environmental Toxicology and Chemistry). 2004. Whole effluent toxicity testing: Ion imbalance. Technical issue paper. Pensacola FL: SETAC.

Stegkoll, M.S., W.W. Smoker, I.A., Wang, and B.J., Failor. 2003. Salmon as a Bioassay Model of Effects of Total Dissolved Solids. Alaska: Alaska Science and Technology Foundation.

USEPA (United States Environmental Protection Agency). 2006. Regional Science Workshop on Headwaters and Associated Wetlands in the Mid-Atlantic Highlands Region. Philadelphia, PA: USEPA Region 3.

Rod Bodkin is an environmental scientist, James Kern is the chief operations officer, and Phillip McClellan is the president of MapTech Inc. Arthur J. Butt and Charles Martin work in the Watershed Programs Section, Office of Water Quality Programs, Virginia Department of Environmental Quality.

Table 1 State water quality standards for conductivity or total dissolved solids.

State	Parameter	Standard	Purpose
Arkansas	Total dissolved solids	Site and ecoregion specific standards that range from 70 to 1,600 mg [L.sup.-1]	Protect designated uses
Illinois	Total dissolved solids	1,500 mg [L.sup.-1]	Protect indigenous aquatic life
Kentucky*	Total dissolved solids	Total dissolved solids shall not be changed to the extent that the indigenous aquatic community is adversely affected.	Protect indigenous aquatic life
Mississippi	Conductivity	There shall be no substances added to increase the conductivity above 1,000 [micro]mhos [cm.sup.-1] for freshwater streams.	Protect indigenous aquatic life
Ohio	Total dissolved solids	1,500 mg [L.sup.-1]	Statewide protection of aquatic life

State	Reference
Arkansas	Arkansas. 2006. Arkansas Pollution Control and Ecology Commission: Regulation No. 2--Regulation Establishing Water Quality Standards for Surface Waters of the State of Arkansas. Little Rock, AR: State of Arkansas.
Illinois	Illinois Pollution Control Board. 2005. Environmental Regulations for the State of Illinois: Title 35 of the Illinois Administrative Code. Part 302.402. Springfield, IL: Illinois Pollution Control Board, http://www.ipcb.state.il.us.
Kentucky*	USEPA (United States Environmental Protection Agency). 2005. Kentucky Administrative Regulations Title 401: Chapter 5, Water Quality. Washington, DC: Office of Water, Environmental Protection Agency. http://www.epa.gov/waterscience/standards/wqslibrary/ky/ky_4_wqs.pdf.
Mississippi	Mississippi Department of Environmental Quality. 2004. TMDL for Conductivity Long Branch, Pascagoula River Basin, Clark County, Ms. Biloxi, MS: Office of Pollution Control, Mississippi Department of Environmental Quality.
Ohio	Ohio Environmental Protection Agency. 2005. Water use designations and statewide criteria. Columbus, OH: Ohio Environmental Protection Agency. http://www.epa.state.oh.us/dsw/rules/01-07.pdf.

* Kentucky is in the process of developing numeric TDS criteria based upon field data.

Bodkin, Rod^Kern, James^McClellan, Phillip^Butt, Arthur J.^Martin, Charles

American Journal of Environmental Sciences 3 (1): 1-6, 2007
ISSN 1553-345X

Effects of Total Dissolved Solids on Aquatic Organisms: A Review of Literature and Recommendation for Salmonid Species

[1]Phyllis K. Weber-Scannell and [2]Lawrence K. Duffy
[1]Scannell Technical Services, 1235 Schodack Landing Road, Schodack Landing, NY 12156
[2]Department of Chemistry and Biochemistry, University of Alaska Fairbanks
Fairbanks, AK 99775-6160

Abstract: Total dissolves solids (TDS) are naturally present in water or are the result of mining or some industrial treatment of water. TDS contain minerals and organic molecules that provide benefits such as nutrients or contaminants such as toxic metals and organic pollutants. Current regulations require the periodic monitoring of TDS, which is a measurement of inorganic salts, organic matter and other dissolved materials in water. Measurements of TDS do not differentiate among ions. The amount of TDS in a water sample is measured by filtering the sample through a 2.0 µm pore size filter, evaporating the remaining filtrate and then drying what is left to a constant weight at 180ºC. The concentration and composition of TDS in natural waters is determined by the geology of the drainage, atmospheric precipitation and the water balance (evaporation-precipitation). The mean salinity of the world's rivers is approximately 120 mg L^{-1} and the major anion found in natural waters is bicarbonate. The most commonly occurring cation in fresh water is calcium. Changes in TDS concentrations in natural waters often result from industrial effluent, changes to the water balance (by limiting inflow, by increased water use or increased precipitation), or by salt-water intrusion. It is recommended that different limits for individual ions, rather than TDS, be used for salmonid species. These limits should be based on the effect of the ion on fertilization and egg development.

Key words: Total Dissolved Solid, TDS, water standards, aquatic organisms, Alaska, salmon

INTRODUCTION

Total Dissolved Solid (TDS) is a measurement of inorganic salts, organic matter and other dissolved materials in water[1]. Measurements of TDS do not differentiate among ions. The amount of TDS in a water sample is measured by filtering the sample through a 2.0 µm pore size filter, evaporating the remaining filtrate and then drying what is left to a constant weight at 180ºC[2]. The concentration and composition of TDS in natural waters is determined by the geology of the drainage, atmospheric precipitation and the water balance (evaporation-precipitation)[3]. The mean salinity of the world's rivers is approximately 120 mg L^{-1} and the major anion found in natural waters is bicarbonate, with a mean for all North American river waters of 68 mg L^{-1}[3] The second most common anion is sulfate, with a mean concentration of 20 mg L^{-1}. The most commonly occurring cation in fresh water is calcium, with a mean of all North American river waters for which data were available, of 21 mg L^{-1}; the next most commonly occurring cations are sodium and silica, each with an average concentration of 9 mg L^{-1}[3]. Water with total dissolved solids concentrations greater than 1000 mg L^{-1} is considered to be "brackish". Changes in TDS concentrations in natural waters often result from industrial effluent, changes to the water balance (by limiting inflow, by increased water use or increased precipitation), or by salt-water intrusion.

Total dissolved solids cause toxicity through increases in salinity, changes in the ionic composition of the water and toxicity of individual ions. Increases in salinity have been shown to cause shifts in biotic communities, limit biodiversity, exclude less-tolerant species and cause acute or chronic effects at specific life stages. Bierhuizen and Prepas[4] found a significant and negative correlation between concentrations of chlorophyll-a (an estimate of primary production) and concentrations of Na^+, Mg^{2+}, SO_4^{2-}, HCO_3^- and CO_3^{2-}. Hallock and Hallock[5] reported substantial changes in marsh communities. When TDS increased from 270 to 1170 mg L^{-1}, both coontail (*Ceratophyllus demersum*) and cattails (*Typha* sp.) were nearly eliminated. Derry *et al*.[6] reported that salinity and aquatic biodiversity are inversely related in lake water.

Changes in the ionic composition of water can exclude some species while promoting population growth of others. For example, Derry *et al*.[6] found that the rotifer *Brachionus plicatilis* and the harpactacoid copepod *Cletocamptus* sp. prevailed in lakes with Cl-

Corresponding Author: Lawrence K. Duffy, Department of Chemistry and Biochemistry, Box 756160, University of Alaska Fairbanks, Fairbanks, AK 99775-6160, Tel: 907-474-7525, Fax: 907-474-5101

dominated water. In contrast, the calanoid copepods *Leptodiaptoums sicillis* and *Diaptomus nevadensis* were dominant in the SO_4^{2-}/CO_3^{2-}-dominated lake water. Mount *et al*.[7] stated that the composition of specific ions determined toxicity of elevated TDS in natural waters. In general, they found relative ion toxicity was $K^+ > HCO_3^- = Mg^{2+} > Cl^- > SO_4^{2-}$. Ca^{2+} and Na^+ did not produce significant toxicity. For *C. dubia* and *D. magna*, toxicity of Cl^-, SO_4^{2-} and K^+ were reduced in solutions containing more than one cation.

The diversity of aquatic species decline as osmotic tolerances are exceeded with increasing salinity[6]. Concentrations of specific ions may reach toxic levels for certain species of life history stages. Stekoll *et al*.[8] identified Ca^{2+} as the primary ion responsible for inhibiting hatch of salmonid eggs exposed during fertilization. Erickson *et al*.[9] found that the addition of potassium chloride markedly increased copper toxicity, while addition of calcium chloride and sodium chloride substantially reduced it. Stekoll *et al*.[8] reported that spermatozoa activity was inhibited when small quantities of potassium chloride (19.2 mg L^{-1}) or potassium carbonate (106.2 mg L^{-1}) were added. The current standards of using TDS might be reconsidered to monitor specific ions in light of future risk assessments.

MATERIALS AND METHODS

In order to assess gaps in knowledge and new developments in methodology regarding TDS in Alaska waters, we examined the peer-reviewed literature and official reports to compile available data on toxicity related to TDS. Over forty reports, abstracts and papers were examined which document the effects of elevated TDS on fish spawning and rearing, aquatic invertebrates and aquatic vertebrates. The information is summarized in tables reporting the toxicity of TDS, including the species and life stage tested, the concentration producing the effect and the endpoint. This framework and interpretation of the literature is based on the long experience of the authors.

RESULTS

Invertebrates: Authors have reported a wide range of toxicity (either EC50 or LC50) for aquatic invertebrates, depending on species and especially, on the type of ion (Table 1 and 2). Chapman *et al.*[9] exposed chironomid (*Chironomus tentans*) larvae to two synthetic TDS mixtures modeled after the ionic composition of two mine effluents from Alaskan mining operations. The TDS was primarily $CaSO_4$. They reported significant effects in the chironomid larvae above 1100 mg L^{-1}. Hoke *et al*.[10] reported a 48-h LC50 of 735 mg L^{-1} for *C. dubia* exposed to $NaHCO_3$ and a 48-h LC50 >5000 mg L^{-1} for *Daphnia magna* exposed to NaCl.

Mount *et al*.[7] reported a wide range of toxicities for *C. dubia* and *D. magna*, depending on the ionic composition (Table 1). The researchers reported that mixtures of $KHCO_3 + K_2SO_4$ had the lowest 24-h and 48-h LC50 concentrations for *C. dubia* (390 mg L^{-1} for both 24-h and 48-h). Mixtures of $CaSO_4$ and K_2CO_4 resulted in 24-h LC50 of 1140 mg L^{-1} and 48-h LC50 of 1130 for *C. dubia*. Other mixtures of ions resulted in LC50 concentrations in the range of 2,000 to 4,000 mg L^{-1} and with some mixtures, even higher[7].

Fish: Tests on salmonidae (trout, char, salmon, grayling, whitefish) exposure to high levels of TDS have yielded mixed results, depending upon when exposure occurred[10-14]. Chapman *et al*.[9] exposed embryonic and juvenile rainbow trout (*O. mykiss*) to two synthetic TDS mixtures modeled after the ionic composition of two mine effluents from Alaskan mining operations. No significant effects of the exposures were found on the rainbow trout up to 2000 mg L^{-1}. Their results are consistent with the results of Stekoll *et al*.[8,11] for exposures after fertilization.

Stekoll, *et al*.[15] exposed coho salmon embryos to elevated TDS during different life stages, from post fertilization to button-up fry. They found no significant increase in mortalities with higher concentrations of TDS and concluded that these life stages were unaffected by TDS exposure in either the short or long term. However, when the coho salmon (*O. kisutch*) were exposed at fertilization, higher concentrations resulted in reduced hatch rates and delayed hatch, as well as long-term effects on growth and development. They found coho salmon to be sensitive to TDS exposure at fertilization but not at other embryonic life stages or the juvenile stages from alevin to button- up. Eggs exposed at fertilization that hatched showed effects in later development, i.e., eggs exposed to higher concentrations (1875 and 2500 ppm TDS) had high mortality rates between the eyed and alevin stages. In the 2500-ppm concentration range, they found 50% mortality of the 50% that had been fertilized.

Brix and Grosell[16] conducted similar studies on Dolly Varden (*Salvelinus malma*) and Arctic grayling (*Thymallus arcticus*). They reported an LOEC for Arctic grayling ranging from 254 to >2782 mg L^{-1} TDS and an LOEC for Dolly Varden ranging from >1704 to >1817. Their results for Dolly Varden are similar to the results of Stekoll *et al*.[11] for Arctic char; Stekoll *et al* reported an LOEC of 1875[8]. The wide range in the LOEC for Arctic grayling is possibly related to the ripeness of the fish when eggs and milt were taken.

Table 1: Studies of effects of elevated TDS on freshwater aquatic invertebrates

Species		TDS Components	Effects Unit	Effects Concentration mg L^{-1}	Reference
Chironomus tentans	Diptera larvae	$CaSO_4$	Growth reduced by 45%	2,089	Chapman *et al*.[9]
C. tentans	Diptera larvae	$CaSO_4$	Reduced survival	1,750 and 2,240	Chapman *et al*.[9]
C. tentans	Diptera larvae	$CaSO_4$	10 day, LC50[1]	2,035	USEPA[22]
C. tentans	Diptera larvae	$CaSO_4$	IC_{20}	1,598	USEPA[23]
Cricotopus trifascia	Diptera larvae	K^+	LC50	1567	Hamilton 1975, cited in ENSR[24]
C. trifascia	Diptera larvae	CL^-	LC50	1406	Hamilton 1975, cited in ENSR[24]
Hexagenia bilineata	Insect: mayfly	K, Li, Mg, Mo, Na, SO_4, NO_3	15 day test, 80% survival	2,270	Woodward *et al*.[25]
H. bilineata	Insect: mayfly	K, Li, Mg, Mo, Na, SO_4, NO_3	30 day test, 70% survival	1,230	Woodward *et al*.[25]
Hydroptila angusta	Insect: caddisfly	K^+	LC50	2316	Hamilton 1975, cited in ENSR[24]
Hydroptila angusta	Insect: caddisfly	Cl^-	LC50	2077	Hamilton 1975, cited in ENSR[[24]
Dugesia gonocephala	flatworm	Cl^-	Mortality	1230	Palladina 1980, cited in ENSR[24]

Tubifex tubifex	segmented worm		K^+	EC50[1]	2000	Khangarot 1991, cited in ENSR[24]
Tubifex tubifex	segmented worm		Ca^{+2}	EC50	814	Khangarot 1991, cited in ENSR[24]
Cyclops abyssorum prealpinus	cyclopoid copepod		Mg^{+2}	EC50	280	Baudoin 1974, cited in ENSR[24]
C.abyssorum prealpinus	cyclopoid copepod		Ca^{+2}	EC50	7000	Baudoin 1974, cited in ENSR[24]
C. dubia	zooplankton			LC50	1,692	Tietge and Hockett[26]
C. dubia	zooplankton		NaCl	48-hr, LC50	835	Hoke *et al*.[10]
C. dubia	zooplankton		NaCl	48-hr, LC50	735	Hoke *et al*.[10]
Cladoceran	zooplankton		$CaSO_4$	LC50, 48-h	>1,910	Mount *et al*.[7]
D. pulex	zooplankton		Ca, ion	EC50, 48-h	499	Goodfellow *et al*.[27]
D. magna	zooplankton			LC50	1,692	Tietge and Hockett[25]
D. magna	zooplankton	<24 h	NaCl	48-hr, LC50	5015	Hoke *et al*.[10]
D. magna	zooplankton	<24 h	NaCl	48-hr, LC50	5000	Hoke *et al*.[10]
D. magna	zooplankton	4th instar	NaCl	48-hr, LC50	4000	Hoke *et al*.[10]
D. magna	zooplankton	<24 h	$NaHCO_3$	48-hr, LC50	1400	Hoke *et al*.[10]
D. magna	zooplankton	<24 h	$NaHCO_3$	48-hr, LC50	1150	Hoke *et al*.[10]
D. magna	zooplankton	7 day	$NaHCO_3$	48-hr, LC50	1780	Hoke *et al*.[10]
D. magna	zooplankton	7 day	$NaHCO_3$	48-hr, LC50	2200	Hoke *et al*.[10]
D. magna	zooplankton	7 day	$NaHCO_3$	48-hr, LC50	1250	Hoke *et al*.[10]
D. magna	zooplankton	<24 h	$NaHCO_3$	48-hr, LC50	1160	Hoke *et al*.[10]
D. magna	zooplankton	<24 h	$NaHCO_3$	48-hr, LC50	1000	Hoke *et al*.[10]
Mysidopsis bahia	mysid shrimp		Ca, ion	LC50, 96-h	927	Goodfellow *et al*.[27]

LC50 = Lethal Concentration 50, or concentration causing 50% mortality
IC0 = Inhibition Concentration 0, or concentration causing inhibition of 0% of the population.
EC50 = Effects Concentration, or concentration effecting 50% of the population.

Ketola *et al*.[12] found that exposing salmonid embryos to high concentrations of calcium (520 mg L^{-1} or greater) during water hardening (post-fertilization) decreased survival rates of several salmonid species. They[12] reported 38% survival at eye up for *Salvelinus fontinalis* exposed to 2229 mg L^{-1} $CaSO_4$, 35% survival

Table 2: Studies of effects of elevated TDS on aquatic plants, algae and bacteria reported in published literature

Species	Effects Concentration mg/L	TDS Components	Effects Unit	Notes	Reference
Algae, species not given	>1400	Not specified		Decline in productivity	Kerekes and Nursall[28] in Sorensen *et al.*[19]
Selanastrum capricornutum	551.3	CaSO4	EC20	All sample concentrations resulted in toxic effects	LeBlond[20]
S. capricornutum	250 – 500			Inhibition of growth	Cleave *et al*. 1976, in Sorensen *et al.*[19]
S. capricornutum	$\geq$2020	$CaCO_4$	Growth inhibition	No toxic effects at 99, 664, 1180, or 1640	EVS Environment Consultants[29]
Nitrogen-fixing bluegreen bacteria	~2450	TDS		Nitrogen fixation limited	Evans and Prepas[22]
Vibrio fischeri	1960	$CaSO_4$	EC20	Inhibited growth	LeBlond and Duffy[21]
Ceratophyllus demersu,	1170			elimination of sensitive species	Hallock and Hallock[5]
Typha sp	1170			elimination of sensitive species	Hallock and Hallock[5]

Table 3: The most toxic ions or combinations of ions identified by Mount et al (1997). Ions are ordered from most toxic to least toxic for each species

Ceriodaphnia dubia	*Daphnia magna*	Fathead minnow
24-h test	24-h test	96-h test
$KHCO_3 + K_2SO_4$	$KHCO_3 + K_2SO_4$	$KHCO_3$
$KHCO_3 + KCl$	$KHCO_3$	K_2SO_4
$K_2SO_4 + KCl$	KCl	$KHCO_3 + K_2SO_4$
KCl	$K_2SO_4 + KCl$	$KHCO_3 + NaHCO_3$
$KHCO_3$	$KHCO_3 + KCl$	$K_2SO_4 + KCl$
K_2SO_4	K_2SO_4	$KHCO_3 + KCl$
$MgCl_2 + KHCO_3$		$NaHCO_3$
$KHCO_3 + NaHCO_3$		KCl
$MgSO_4 + KHCO_3$		

for *Salmo solar* exposed to 1395 mg L^{-1} CaCL and 4% survival for *O. mykiss* exposed to 1500 mg L^{-1} $CaSO_4$. In Ketola *et al*.'s study,[12] eggs were dry fertilized (fertilized in the presence of ovarian and seminal fluids only), while embryos in Stekoll *et al*.'s study[11] were fertilized in control or exposure waters. Both studies rinsed fertilized embryos in exposure waters.

Brannock *et al*.[17] examined the individual ionic components of a TDS mixture and the effect of those ions on the fertilization rates of king and pink salmon (*O. gorbuscha*). The ions were tested individually at levels equivalent to Stekoll *et al*.'s 2500-ppm simulation,[11] also at one quarter of the concentration and at four times the concentration. Fertilization rates in both the king and pink salmon were significantly lower with exposure to either calcium or sulfate at 2500 ppm TDS equivalent. Potassium and magnesium ions showed no detectable differences from the control at 2500 ppm TDS equivalent. This work pointed to calcium or possibly sulfates as being the likely cause of lowered fertilization rates.

Mount *et al*.[7] examined the toxicity of different combinations of ions to fathead minnows, in test similar to those conducted with *C. dubia* and *D. magna*. Results with the fathead minnows were similar to results with the invertebrates, producing a wide range of LC50 values, depending on ionic composition. Of the 30 combinations of ions reported by Mount *et al*., one (KHCO3) had a 96-h LC50 <510 mg L^{-1}, 7 combinations resulted in 96-h LC50 concentrations that were less than 1000 mg L^{-1} and a number of ionic combinations resulted in 96-h LC50 values higher than 2000 mg L^{-1}. Mount *et al*.[7] did not test toxicity on reproduction or early development; their tests were limited to mortality of mature fish.

Aquatic plants, algae and bacteria: Few studies were found that documented effects of elevated TDS or of different ions on aquatic plants and algae (Table 2). Kerekes and Nursall (cited in Sorensen *et al*.[18]) found lower productivity in algae at TDS concentrations >1400 mg L^{-1}. LeBlond[19,20] reported an EC20 = 551.3 for *Selanastrum capricornutum*. Evans and Prepas[21] reported decreased nitrogen fixation in bluegreen bacteria exposed to approximately 2450 mg L^{-1} TDS. Hallock and Hallock[5] reported the near elimination of coontail (*Ceratophyllus demersum*) and cattails (*Typha* sp.) in water with 1170 mg L^{-1} TDS.

DISCUSSION

The measurement of TDS integrates all anions and cations in the sample and some ions or combinations of ions are substantially more toxic than other ions or combinations of ions. A species might be more sensitive to TDS toxicity at certain life stages, as many fish are during fertilization. Therefore, a water quality standard for TDS can take several approaches: 1) The standard can be set low enough to protect all species and life stages exposed to the most toxic ions or combination of ions; 2) The standard can be set to protect most species and life stages for most ions and combinations of ions; or 3) Different limits can be defined for different categories of ions or combinations of ions, with a lower limit during fish spawning, if salmonid species that have been shown to be sensitive to TDS during fertilization and egg development are present.

Approach (1) may be unnecessarily restrictive, although simpler to define and implement. Approach (2), although less restrictive, may lead to adverse effects to aquatic communities. Approach (3) is more complicated to define and would require that the potential discharger determine the composition of the effluent and which species and life stages are present downstream of the effluent. Overall, Approach (3) would provide the greatest protection to aquatic species and the least unnecessary restriction to potential dischargers. The research of Mount *et al*.[7] provides information on toxicity of different ions and ion combinations. Of the ions and combinations of ions tested by Mount, *et al*.,[7] the most toxic to *C. dubia, D. magna and* fathead minnows are shown on Table 3, ordered from most toxic to less toxic. All tests with these ions resulted in LC50 values less than 1,000 mg L^{-1}.

The research of Stekoll *et al*.[11] and Brix and Grosell[17] provide information on toxicity of TDS, mostly in the form of $CaSO_4$, to some Alaska fish species, especially at fertilization. Using the results of Stekoll *et al*.[10] and Brix and Grosell[17] the fish species can be ordered from most sensitive to least sensitive (to $CaSO_4$ TDS): *O. keta (* chum Salmon) > *O. mykiss* (steelhead salmon) > *Thymallus arcticus* (Arctic grayling) > *Salvelinus malma* (Dolly Varden) > *Salvelinus alpinus* (arctic char).

ACKNOWLEDGEMENTS

The need for research on toxicity of total dissolved solids (TDS) arose from the concerns of the Alaska Departments of Fish and Game and Environmental Conservation, working in concert with the Alaska Council of Producers to revise discharge limits for total dissolved solids. Resource managers and representatives of industry were instrumental in initiating research projects to investigate possible effects of TDS to Alaska's important salmon resources. McKie Campbell led the team, which secured funding for the initial research from the Alaska Science and Technology Foundation (ASTF). Dr. Alvin G. Ott, now Operations Manager, of Office of Habitat Management and Permitting, Alaska Department of Natural Resources, recognized egg fertilization and early development as the most probable sensitive life stage of salmonid fish. Dr. Mike Stekoll and his fellow researchers from the University of Alaska Southeast designed test systems for salmon egg fertilization and early development. They also developed a short-term fertilization/initial egg development test that could be used to test toxicity of different ionic combinations. They also identified fish species that were most sensitive to elevated TDS. Their research remains a valuable contribution. Mr. Tom Irwin and Mr. Bill Jeffress represented the interests of the hard rock mining industry; without their concern about TDS toxicity and encouragement the initial ASTF studies would not have occurred. This review and report was funded by a grant from Alaska Department of Environmental Conservation, through the University of Alaska Fairbanks. Thanks go to Nancy Sonafrank, ADEC, for facilitating the grant, to Judie Triplehorn, UAF, for her library searches and for supplying many of the papers in the report.

REFERENCES

1. U.S. Environmental Protection Agency, Office of Water, 1986. Quality Criteria for Water (Gold Book). EPA 440/5-86-001. Washington D.C.
2. American Public Health Association (APHA), 1992. Standard Methods for the Examination of Water and Wastewater. 18th Edn.
3. Wetzel, R.G., 1983. Limnology. Sec. Ed.n. Saunders College Publishing, NY, pp: 767.
4. Bierhuize,n J.F.H. and E.E. Prepas, 1985. Relationship between nutrients, dominant ions and phytoplankton standing crop in prairie saline lakes. Can. J. Fish. Aquat. Sci., 42: 1588-1594.
5. Hallock, R.J. and L.L. Hallock (Ed.), 1993. Detailed Study of Irrigation Drainage in and near Wildlife Management Areas, West-Central Nevada, 1987-90. Part B. Effect on Biota in Stillwater and Fernley Wildlife Management Areas and other Nearby Wetlands. US Geological Survey, Water Resources Investigations Report 92-4024B.
6. Derry, A.M., E.E. Prepas and P.D.N. Hebert, 2003. A comparison of zooplankton communities in saline lakewater with variable anion composition. Hydrobiologia, 505: 199-215.
7. Mount, D.R., D.D. Gulley, J.R. Hockett, T.D. Garrison and J.M. Evans, 1997. Statistical models to predict the toxicity of major ions to *C. dubia, Daphnia magna* and *Pimephales promelas* (Fathead Minnows). Environ. Toxicol. Chem., 16: 2009-2019.
8. Stekoll, M., W. Smoker, I. Wang and B. Failor, 2003a. Final report for ASTF grant #98-1-012. Salmon as a bioassay model of effects of total dissolved solids. Juneau, Alaska, University of Alaska – Fairbanks, pp: 87.

9. Chapman, P.M., H. Bailey and E. Canaria, 2000. Toxicity of total dissolved solids associated with two mine effluents to chironomid larvae and early lifestages of rainbow trout. Environ. Toxicol. Chem., 19: 210-214.
10. Hoke, R.A., W.R. Gala, W.R., J.B. Drake, J.P. Geisy and S. Fleger, 1992. Bicarbonate as a potential confounding factor in cladoceran toxicity assessments of pore water from contaminated sediments. Can. J. Fish. Aquat. Sci., 49: 1633-1640.
11. Stekoll, M., W.Smoker, I. Wang and W. Hayes, 2003b. Final report on the effects of total dissolved solids on fertilization rates of salmonids in the Red Dog Mine area. Juneau, Alaska, University of Alaska – Fairbanks, pp: 27.
12. Ketola, H.G., D. Longacre, A. Greulich, L. Phetterplace and R. Lashomb, 1988. High Calcium Concentration in Water Increases Mortality of Salmon and Trout Eggs. Progressive Fish-Culturist, 50: 129-135.
13. Weber Scannell P. and L. Jacobs. 2001. Effects of Total Dissolved Solids on Aquatic Organisms: A Literature Review. Alaska Dept. of Fish and Game Tech. Report 01-06. Juneau, AK, pp: 68.
14. Erickson, R.J., D.A. Benoit, V.R. Mattson, H.P. Nelson Jr. and E.N. Leonard, 1996. The effects of water chemistry on the toxicity of copper to fathead minnows. J. Environ. Toxicol. Chem., 15: 181-193.
15. Stekoll, M., W. Smoker, I. Wang and B. Failor, 2001. Fourth Quarter 2000 Report for ASTF Grant #98-012, Project: Salmon as a Bioassay Model of Effects of Total Dissolved Solids. 17 January 2001.
16. Brix, K.V. and M. Grosell, 2005 Report on the effects of total dissolved solids on arctic grayling and dolly varden fertilization success. Teck Cominco Red Dog Mine, Kotzebue, AK.
17. Brannock, P., M.S. Stekoll, B. Failor and I. Wang. 2002. Salt and salmon: The effects of hard water ions on fertilization. Aquatic Sciences Meeting. American Society of Limnology and Oceanography. Feb. 11-15, pp: 142.
18. Sorensen, D.L., M. McCarthy, E.J. Middlebrooks and D.B. Porcella, 1977. Suspended and dissolved solids effects on freshwater biota: A review. US Environmental Protection Agency, EPA-600/3-77-042.
19. LeBlond, J.B., 2000. A Toxicity Assessment of Total Dissolved Solid Ions in Mine Effluent Using Two Common Bioassays: The 22-hr MicroTox® Assay and a S. Capricornutum Growth Assay. April 2000. M. S. Thesis, University of Alaska Fairbanks, Fairbanks, Alaska.
20. LeBlond, J.B. and L.K. Duffy, 2001. Toxicity assessment of total dissolved solids in effluent of Alaskan mines using 22-h chronic Microtox® and *Selenastrum capricornutum* assays. Sci. Tot. Environ., 1-3: 49-59.
21. Evans, J.C. and E.E. Prepas, 1996. Potential effects of climate change on ion chemistry and phytoplankton communities in prairie saline lakes. Limnol. Oceanogr., 41: 1063-1076.
22. U.S. Environmental Protection Agency, Office of Water, 1999. National Recommended. Water Quality Criteria – Correction. EPA 822-Z-99-001. April 1999.
23. U.S. Environmental Protection Agency, Region 10 Laboratory, February 19, 1999. Memorandum from M.K. Parker Manchester Laboratory Chemist, to Mike Letourneau, EPA Region 10 Project Manager, conveying a quality assurance data review of chemistry analyses conducted at Manchester Laboratory.
24. ENSR, 1997. Review of toxicity studies conducted on Kensington Mine effluent. Doc. No. 2012-001-200.
26. Teitge, J.E. and J.R. Hockett, and J.M. Evans, 1996. Major Ion Toxicity of Six Produced Waters to Three Freshwater Species: Application of Ion Toxicity Models and TIE Procedures. Environ. Toxicol. Chem., 16: 2002-2008.
25. Woodward, D.F., R.G. Riley, M.G. Henry, J.S. Meyer, and T.R. Garland, 1985. Leaching of Retorted Oil Shale: Assessing the Toxicity to Colorado Squawfish, Fathead Minnows, and Two Food-Chain Organisms. Trans. Am. Fish. Soc., 114: 887-894.
27. Goodfellow, W.L., L.W. Ausley, D.T. Burton, D.L. Denton, P.B. Dorn, D.R. Grothe, M.A. Heber, T.J. Norberg-King, and J.H. Rodgers Jr., 2000. Major ion toxicity in effluents: a review with permitting recommendations. Issn: 1552-8618. J. Environ. Toxicol. Chem., 19: 175-182.
28. Kerekes, J. and J.R. Nursall, 1966. Eutrophication and senescence in a group of Prairie-Parkland Lakes in Alberta, Canada. Verhandlungen der Internationalen Vereinigung fur theoretische und angewandte Limnologie, 16: 65-73.
29. EVS Environment Consultants, 1997c. Toxicity of Total Dissolved solids (TDS) in Coeur Mine Effluent: *Selenastrum capricornutum*. Laboratory Report prepared for Coeur Alaska, Juneau, AK, pp: 10.

Environmental Toxicology and Chemistry, Vol. 32, No. 12, pp. 2826–2835, 2013

Printed in the USA

USE OF RECONSTITUTED WATERS TO EVALUATE EFFECTS OF ELEVATED MAJOR IONS ASSOCIATED WITH MOUNTAINTOP COAL MINING ON FRESHWATER INVERTEBRATES

JAMES L. KUNZ,*† JUSTIN M. CONLEY,‡ DAVID B. BUCHWALTER,‡ TERESA J. NORBERG-KING,§ NILE E. KEMBLE,† NING WANG,† and CHRISTOPHER G. INGERSOLL†
†US Geological Survey, Columbia Environmental Research Center, Columbia, Missouri
‡North Carolina State University, Raleigh, North Carolina
§US Environmental Protection Agency, Duluth, Minnesota

(Submitted 18 January 2013; Returned for Revision 2 April 2013; Accepted 21 August 2013)

Abstract: In previous laboratory chronic 7-d toxicity tests conducted with the cladoceran *Ceriodaphnia dubia*, surface waters collected from Appalachian sites impacted by coal mining have shown toxic effects associated with elevated total dissolved solids (TDS). The objective of the present study was to evaluate the effects of elevated major ions in chronic laboratory tests with *C. dubia* (7-d exposure), a unionid mussel (*Lampsilis siliquoidea*; 28-d exposure), an amphipod (*Hyalella azteca*; 28-d exposure), and a mayfly (*Centroptilum triangulifer*; 35-d exposure) in 3 reconstituted waters designed to be representative of 3 Appalachian sites impacted by coal mining. Two of the reconstituted waters had ionic compositions representative of alkaline mine drainage associated with mountaintop removal and valley fill-impacted streams (Winding Shoals and Boardtree, with elevated Mg, Ca, K, SO_4, HCO_3), and a third reconstituted water had an ionic composition representative of neutralized mine drainage (Upper Dempsey, with elevated Na, K, SO_4, and HCO_3). The waters with similar conductivities but, with different ionic compositions had different effects on the test organisms. The Winding Shoals and Boardtree reconstituted waters were consistently toxic to the mussel, the amphipod, and the mayfly. In contrast, the Upper Dempsey reconstituted water was toxic to the mussel, the amphipod, and the cladoceran but was not toxic to the mayfly. These results indicate that, although elevated TDS can be correlated with toxicity, the specific major ion composition of the water is important. Moreover, the choice of test organism is critical, particularly if a test species is to be used as a surrogate for a range of faunal groups. *Environ Toxicol Chem* 2013;32:2826–2835. © 2013 SETAC

Keywords: Invertebrate toxicology Mussels Mayfly Major ion toxicity

INTRODUCTION

Salinization of freshwater as a result of increased concentrations of major cations or major anions is a growing global concern that can result from a wide variety of human activities [1]. Major ions contributing to elevated total dissolved solids (TDS; often estimated using conductivity as a surrogate measurement) may include various combinations of Ca, Mg, Na, K, sulfate (SO_4), chloride (Cl), and bicarbonate (HCO_3), as well as other ions [2]. In North America, examples of activities that result in increased TDS include mountaintop mining of coal (and resultant valley filling [3]), coal processing [4,5], and the use of de-icing agents and road salts [6]. Although previous work has highlighted the potential for elevated TDS to be toxic to aquatic organisms [2,7–9], general understanding of TDS toxicity is currently inadequate, given the complexity of ionic matrices constituting high-TDS–impacted surface waters and the diversity of aquatic organisms inhabiting these waters.

Elevated concentrations of TDS in streams and rivers are widespread throughout the coal mining regions of Appalachia associated with mountaintop removal and valley fill [10,11]. During mountaintop coal mining and other surface mining, overburden layers are removed to mine the underlying coal seams. The overburden is returned to the mined areas or is deposited in headwater valleys, forming valley fills [12]. Natural streams in this region are dilute, with conductivity generally below 100 μS/cm. However, conductivity is greater than 1000 μS/cm in streams with surface mining activities in the absence of other major TDS sources. Elevated conductivity found in surface mining streams can result from precipitation and ground water percolating through the pulverized overburden containing sulfate and carbonate materials, creating alkaline mine drainage [13].

Studies of macroinvertebrate communities from Kentucky, Virginia, West Virginia, and Pennsylvania impacted by mountaintop removal have shown that conductivity explains a substantial amount of the variance associated with various benthic assessment metrics [10]. Because macroinvertebrates inhabit dilute streams in the region, increased concentrations of major ions or ionic imbalance has been suggested to be contributing to this impairment of benthic communities. At other sites across North America, conductivity above 2000 μS/cm or TDS above about 1340 mg/L reportedly represents conditions that may adversely affect freshwater organisms [2,8,9]. In contrast, more than 90% of the sites in West Virginia and Kentucky receiving runoff water from coalfields exhibit impaired macroinvertebrate index of biotic integrity (IBI) scores when conductivity is greater than 500 μS/cm [10]. Mayflies (Ephemeroptera) are reportedly highly sensitive to TDS in these locations. Component major ions that elevate conductivity in alkaline mine drainage in these areas include Mg, Ca, K, HCO_3, and SO_4.

In the absence of any other toxicant, major ions can be acutely or chronically toxic to aquatic life [9]. However, the correlation between increasing TDS or conductivity and toxicity varies with

All Supplemental Data may be found in the online version of this article.
* Address correspondence to jkunz@usgs.gov
Published online in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.2391

ionic composition; therefore, TDS or conductivity measurements alone may not provide a basis to estimate reliably the toxicity of samples with differing ionic balances [9]. Moreover, ionic strength and ionic imbalance can both contribute to the toxicity of water to freshwater organisms [14,15].

The toxicity of major ions has been evaluated in 24-h to 96-h exposures with 3 commonly tested freshwater species (the cladocerans *Ceriodaphnia dubia* and *Daphnia magna* and fathead minnow *Pimephales promelas*) [7]. The relative ion toxicity was $K > HCO_3 \approx Mg > Cl > SO_4$, with *C. dubia* tending to be more sensitive compared with *D. magna* or *P. promelas* [7]. However, laboratory toxicity tests with reconstituted waters have not been conducted with test organisms more representative of taxa inhabiting freshwater Appalachian streams impacted by mountaintop removal and valley fill, including mayflies and mussels [13].

The objective of the present study was to evaluate the effects of elevated major ions in chronic laboratory tests with 4 aquatic invertebrates exposed to dilutions of 3 reconstituted waters designed to be representative of 3 Appalachian sites impacted by coal mining in southwestern West Virginia with high TDS downstream of valley fill: Winding Shoals Branch, Boardtree Branch, and Upper Dempsey Branch [13]. These 3 test sites were chosen from among multiple field-collected site waters previously demonstrated to be toxic to *C. dubia* in chronic 7-d laboratory exposures and exhibit impaired populations of benthic invertebrates [13]. Two of these waters (Winding Shoals and Boardtree) had ionic signatures representative of alkaline mine drainage associated with streams affected by mountaintop removal and valley fill with elevated Mg, Ca, K, HCO_3, and SO_4), whereas Upper Dempsey had a somewhat different ionic composition, representing neutralized mine drainage or mine drainage that has experienced some cation exchange (elevated Na, K, SO_4, and HCO_3; Table 1 and Supplemental Data, Table S1 [13]).

The 3 reconstituted waters were used to conduct the chronic toxicity tests with a unionid mussel (*Lampsilis siliquoidea*; 28-d exposure), an amphipod (*Hyalella azteca*; 28-d exposure), and a cladoceran (*C. dubia*; 7-d exposure). Subsamples of the reconstituted waters were also provided to North Carolina State University, Raleigh, North Carolina, USA, for conducting exposures with a mayfly (*Centroptilum triangulifer*; 35-d exposure).

MATERIALS AND METHODS

Reconstituted test waters

The reconstituted waters were prepared at the Columbia Environmental Research Center in Columbia, Missouri, USA, by adding reagent-grade salts (K_2SO_4, $CaSO_4$, $NaHCO_3$, $MgSO_4$, NaCl, $CaCl_2$, or Na_2SO_4; Supplemental Data, Table S2) to well water or diluted well water to match the site water chemistry as closely as possible (Supplemental Data, Tables S1 and S3). The base water for Winding Shoals and Boardtree reconstituted waters was 25% well water and 75% deionized water, whereas the base water was 100% well water for Upper Dempsey reconstituted water (Supplemental Data, Table S2; the 100% well water was of approximately 300 mg/L hardness as $CaCO_3$, with a pH of approximately 8). Salt addition recipes for the reconstituted waters were calculated to minimize differences among individual major cations or anions (i.e., attempting to match each major cation or major anion within about 20% of the site water), with no attempt to adjust alkalinity or pH (Supplemental Data, Table S1). The initial recipes were modified (e.g., by reducing $NaHCO_3$ and not attempting to control alkalinity) to eliminate unwanted increases in pH and to ensure that the salts stayed in solution for the duration of the tests. Each salt was initially mixed individually in a 10-L jar with 8 L of the appropriate base water. The 10-L jars were placed on a stir plate and mixed for up to 24 h (depending on the solubility of individual salts). After the salts had been visibly dissolved, all salt solutions for a given reconstituted water were combined in 200-L plastic containers with a circulating pump and brought to a final volume of 130 with the appropriate base water. A similar process was used to prepare smaller 40-L samples of each reconstituted water for the second 2 wk of the exposures conducted at the Columbia Environmental Research Center or for use by North Carolina State University to conduct exposures with *C. triangulifer*.

Exposures with *L. siliquoidea, H. azteca*, and *C. dubia* were conducted in dilutions of Winding Shoals, Upper Dempsey, and Boardtree reconstituted waters (100%, 33%, and 10%, and a dilution water control). The *C. dubia* exposure with the Upper Dempsey reconstituted water had an additional dilution of 5% tested (in an attempt to define better the potential effects at the lower exposure concentration). All 3 reconstituted waters prepared at Columbia Environmental Research Center used

Table 1. Mean water quality characteristics of waters across all chronic toxicity tests in exposures conducted by the Columbia Environmental Research Center[a]

Treatment	Dissolved oxygen (mg/L)	pH	Conductivity (μS/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	Ammonia (mg N/L)	Major cations and anions (mg/L)					
							Ca	Mg	Na	K	Cl	SO_4
Winding Shoals												
0%	6.9 (0.8)	8.3 (0.2)	275 (25)	96 (9)	107 (10)	0.2 (0.2)	30 (2)	10 (1)	12 (2)	2 (1)	14 (2)	22 (3)
10%	7.2 (0.8)	8.2 (0.5)	504 (54)	93 (8)	223 (37)	0.3 (0.1)	42 (4)	38 (6)	14 (2)	4 (1)	13 (2)	141 (12)
33%	6.9 (1.1)	8.1 (0.1)	947 (48)	98 (3)	472 (13)	0.2 (0.1)	65 (3)	93 (3)	18 (1)	10 (1)	12 (1)	386 (33)
100%	7.2 (0.8)	8.1 (0.1)	1906 (162)	99 (6)	1154 (58)	0.3 (0.1)	109 (19)	216 (42)	24 (3)	21 (5)	12 (2)	1023 (138)
Boardtree												
0%	6.9 (0.8)	8.3 (0.2)	275 (25)	96 (9)	107 (10)	0.2 (0.2)	30 (2)	10 (1)	12 (2)	2 (1)	14 (2)	22 (3)
10%	7.0 (0.8)	8.1 (0.1)	565 (36)	84 (8)	262 (26)	0.3 (0.2)	55 (6)	41 (7)	13 (2)	4 (1)	12 (1)	180 (37)
33%	6.9 (1.1)	8.1 (0.1)	1121 (127)	84 (11)	585 (42)	0.7 (0.6)	100 (4)	96 (7)	12 (2)	8 (1)	12 (1)	489 (33)
100%	7.0 (0.8)	8.0 (0.2)	2367 (54)	72 (8)	1408 (37)	0.7 (0.1)	241 (4)	260 (6)	12 (2)	21 (1)	11 (2)	1580 (12)
Upper Dempsey												
0%	6.9 (0.8)	8.3 (0.2)	275 (25)	96 (9)	107 (10)	0.2 (0.2)	30 (2)	10 (1)	12 (2)	2 (1)	14 (2)	22 (3)
10%	6.9 (0.9)	8.3 (0.2)	448 (40)	110 (15)	122 (18)	0.3 (0.1)	33 (2)	13 (1)	52 (5)	3 (1)	15 (1)	78 (8)
33%	7.1 (0.8)	8.4 (0.1)	789 (55)	157 (14)	144 (13)	0.3 (0.1)	38 (2)	17 (1)	125 (6)	5 (1)	21 (3)	175 (17)
100%	6.8 (1.3)	8.4 (0.2)	1813 (157)	279 (20)	209 (36)	0.4 (0.1)	42 (9)	28 (1)	350 (16)	11 (1)	39 (2)	640 (77)

[a] Values are means with standard deviation in parenthesis ($n = 4$ to 15).

the same dilution water (control water) and were prepared by diluting well water with deionized water to a hardness of about 100 mg/L (as $CaCO_3$), alkalinity of 85 mg/L (as $CaCO_3$), and pH of approximately 8.3 (Supplemental Data, Table S3). Supplemental Data, Table S1, summarizes the target, nominal, and measured water-quality characteristics of the 3 test waters before dilution (i.e., at 100%). The *C. triangulifer* exposures were conducted in 2 rounds. Round 1 consisted of Winding Shoals, Upper Dempsey, and Boardtree waters at 100% and 50% and a dilution water control (50:50, well water:reconstituted soft water [16]; Supplemental Data, Table S3). Round 2 consisted of Winding Shoals water tested at 100%, 66%, and 33% and a dilution water control (30:70, well water:deionized water; Supplemental Data, Table S3). The first round of exposures with *C. triangulifer* was conducted to determine whether this organism would be responsive to the reconstituted waters. Given the results of round 1, the second round of *C. triangulifer* exposures was conducted to test the repeatability of results from round 1 (i.e., complete mortality at 100% strength) as well as to test additional dilutions of Winding Shoals water (i.e., 33% and 66%).

For exposures conducted at the Columbia Environmental Research Center, water-quality parameters (dissolved oxygen, pH, conductivity, hardness, alkalinity, ammonia) were determined in the control and all concentrations for each of the test waters at the beginning and the end of the tests, following standard methods [17]. In addition, dissolved oxygen and conductivity were measured weekly during the exposures. Concentrations of major ions (Ca, Mg, Na, K, Cl, SO_4) were determined in the control, and all concentrations were measured at the beginning and end of the test (except for the *C. dubia* test, because of limited water volume). Major ions were also measured in the water samples collected from newly prepared batches of reconstituted test waters. The analyses of the major ions were conducted by Engineering Surveys and Services Testing Laboratories at Columbia, Missouri, using a Perkin Elmer AAnalyst 800 atomic absorption spectrometer and a Thermo Scientific Genesys 20 visible spectrophotometer in accordance with standard methods [17]. For exposures conducted with *C. triangulifer* at North Carolina State University, water-quality parameters (pH, conductivity) were measured weekly for the duration of the exposures. Dissolved oxygen was not measured, because past studies at North Carolina State University have indicated that dissolved oxygen consistently remains at approximately 6 mg/L to 8 mg/L over this period, and *C. triangulifer* do not display evidence of oxygen limitation until dissolved oxygen reaches approximately 1 mg/L to 2 mg/L (D.B. Buchwalter, unpublished data). At the end of round 2 for *C. triangulifer*, 20-mL water samples were collected from each exposure, filtered through a 0.22-μm syringe filter (Fisherbrand), and shipped on ice to the Columbia Environmental Research Center for analyses of major ions. Mean measured concentrations of these water-quality parameters of the 3 reconstituted waters used in all toxicity tests are summarized in Table 1. More details on water-quality measurements for each test with a test species are summarized in Supplemental Data, Tables S1, S3, and S4.

Test organisms

Lampsilis siliquoidea (approximately 2 mo old) were obtained from laboratory cultures at Missouri State University, Springfield (see Wang et al. [18] for mussel culture method). *Hyalella azteca* (approximately 7 d old) and *C. dubia* (>24 h old) used to start the exposures were obtained from laboratory cultures at the Columbia Environmental Research Center (see Besser et al. [19] for methods used to culture *H. azteca* and Wang et al. [18] for methods used to culture *C. dubia*). These 3 species of test organisms were acclimated to the control dilution water and temperature for a minimum of 24 h before the start of the exposures [16,20–22]. Eggs of *C. triangulifer* (WCC-2 clone) were obtained from culture at Stroud Water Research Center, Avondale, Pennsylvania, USA. Exposures with *C. triangulifer* started with newly hatched larvae (first instar, ≤48 h old) placed directly into test water at ambient laboratory temperature of 21 °C.

Mussel testing (Lampsilis siliquoidea)

Static-renewal exposures with mussels were conducted for 28 d in accordance with methods outlined ASTM International [20] (Supplemental Data, Table S5). Toxicity endpoints included survival and shell length. Ten juvenile mussels exhibiting foot movement were impartially transferred into each of 4 replicate 300-mL glass beakers containing 100%, 33%, and 10% test water or into 8 replicates of the dilution water control at 23 °C. Each beaker had a 2.5-cm hole in the side covered with 50-mesh (279-μm width opening) stainless steel screen and contained 200 mL water. Mussels were fed 1 mL of an algal mixture [23] twice daily on Monday through Friday and were fed 2 mL once daily on the weekends. About 2 volumes of test water were added to each beaker with flow splitters once on Mondays, Wednesdays, and Fridays [24]. Mussels were transferred into new test beakers each week. Survival of mussels was determined at the end of the exposures based on foot movement within a 5-min observation period using a dissecting microscope [20]. Surviving mussels were isolated on day 28 and preserved in 8% formalin for subsequent shell length measurement. The maximum shell length of surviving mussels was measured to the nearest 0.001 mm using a digitizing system with video micrometer software (Image Caliper, Resolution Technology).

Amphipod testing (Hyalella azteca)

Static-renewal exposures with amphipods were conducted over 28 d in accordance with methods outlined by ASTM International [21] (Supplemental Data, Table S6). Toxicity endpoints included survival and biomass. At the beginning of the exposures, 10 amphipods were impartially transferred using a pipet into each of 4 replicate 300-mL glass beakers containing about 200 mL of 100%, 33%, and 10% test water or into 8 replicates of the dilution water control at 23 °C. A thin layer of substrate (5 mL fine sand) was added to each beaker. About 2 volumes of test water were added to each beaker with flow splitters once on Mondays, Wednesdays, and Fridays [24]. Amphipods were transferred into new test beakers each week. During exposure, amphipods in each beaker were fed 1.0 mL of yeast–cerophyll–trout chow (YCT; 1.7–1.9 g/L in a water suspension) daily. On day 28, the surviving amphipods were counted and preserved in 8% sugar formalin for subsequent length measurement. Lengths of surviving amphipods were measured from the base of the first antenna to the tip of the third uropod along the curve of the dorsal surface using a digitizing system with video micrometer software (Image Caliper, Resolution Technology) connected to a computer and a microscope. The biomass of surviving amphipods from each replicate was estimated as the sum of individual amphipod weights calculated from the empirical relationship: weight (mg) = $([0.177 \times \text{length (mm)}] - 0.0292)^3$ [25,26].

Cladoceran testing (Ceriodaphnia dubia)

Static-renewal, 3-brood exposures with the cladoceran were conducted for 7-d in accordance with ASTM International [22] (Supplemental Data, Table S7). Toxicity endpoints included survival and reproduction. Test organisms were transferred to test chambers by placing 1 organism in each of 10 30-mL replicate cups containing 15 mL of 100%, 33%, and 10% test water or into 10 replicates of the dilution water control at 25 °C. The Upper Dempsey exposure had an additional dilution of 5%. Each day before water renewal, first-generation organisms were recorded as alive or dead, and live organisms were then transferred to a new test chamber containing fresh solution. Cladocerans were fed 0.1 mL of YCT (1.7–1.9 g/L in a water suspension) and 0.1 mL algal concentrate (*Pseudokirchneriella subcapitata*, 3.0×10^7 cell/mL; Aquatic Biosystems) after daily water renewal. Exposures were conducted for 7 d or until >60% of the control organisms had produced 3 broods. The number of young released from first-generation females over each 24-h period was recorded daily.

Mayfly testing (Centroptilum triangulifer)

Static, nonrenewal exposures with *C. triangulifer* were conducted for a full life cycle (approximately 35 d; Supplemental Data, Table S8) [27–30]. The toxicity endpoints were adult survival and biomass. For each of the 2 rounds of experiments, nymphs were hatched from a single adult female clutch (clonal) to start all exposures. Two rounds of exposures were conducted. Round 1 consisted of controls and Boardtree, Upper Dempsey, and Winding Shoals waters at 100% and 50% strength. Round 2 consisted of controls and Winding Shoals water at 100%, 66%, and 33% strength. All treatments were conducted in triplicate, except in round 1, in which Upper Dempsey water exposures were conducted in duplicate because of a limited number of periphyton plates, and in round 2, in which 1 control and 1 33% Winding Shoals replicate developed a fungal growth on the periphyton surface and were omitted (resulting in $n = 2$ replicates). In round 1, each replicate received 20 nymphs; in round 2, each replicate received 15 nymphs. Mayflies were fed a natural periphyton diet grown at the Stroud Water Research Center by allowing fresh stream water from White Clay Creek, Pennsylvania, USA (39°51′47″N, 75°47′07″W) to flow continuously over acrylic plates ($6.5 \times 23 \times 0.15$ cm) in a greenhouse. Plates were cultivated until the periphyton had reached a thickness of about 1 to 2 mm and relatively uniform coverage, at which time the acrylic plates were shipped on ice overnight to North Carolina State University. Exposures were conducted in 2-L glass bottles with 1.8 L of exposure/control water, and nymphs were fed 2 sets of periphyton plates staggered 22 wk apart (i.e., days 0 and 14). Subimagos (i.e., subadults) emerged after hatching into mesh-lined collection lids during midafternoon to late afternoon. Subimagos were kept overnight in humid chambers containing moist paper towels to facilitate the final molt to adulthood. Gravid adults were stimulated to release eggs by wetting the abdomen in 3.5-cm Petri dishes containing 4.5 mL reconstituted soft water [16]. Adults (now postpartum) were stored in individual microcentrifuge tubes at −20 °C. Once all surviving organisms had emerged and the exposure was completed, the postpartum adults were removed from the freezer and dried at 65 °C for 48 h. Dried mayflies were individually weighed on a microbalance (Sartorius model CPA2P) to the nearest 0.001 mg.

Data analysis

Mean survival, individual length, or biomass (total dry wt of surviving organisms per replicate) of *L. siliquoidea* and *H. azteca* were calculated for each control and exposure replicate. Survival of *C. dubia* was calculated as number of individuals surviving in each treatment. Reproduction of *C. dubia* was calculated as the mean number of offspring across the control or exposure replicates.

Biomass of *C. triangulifer* was calculated based on the product of the mean body weight of surviving organisms and the number of survivors within a given replicate. Mean survival and mean biomass were calculated as the average across replicates for each control or treatment level, which had at least 3 replicates. Survival and biomass measures for controls in rounds 1 and 2 were pooled for comparison with Winding Shoals water because control performance was not significantly different between rounds. Mean survival and mean biomass were not calculated for exposures that had only 2 replicates (Upper Dempsey 50%, Upper Dempsey 100%, and Winding Shoals 33%); however, the biomass and survival of each of the 2 replicates are reported (Table 2).

The no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC) for all endpoints were determined with TOXSTAT software (version 3.5; Western EcoSystems Technology) by one-way analysis of variance (ANOVA) with pairwise comparison performed using Williams' test. If the data were not normally distributed or did not have equal variances, Steel's many-one rank test or Wilcoxon rank sum test with Bonferroni adjustment was used for the determinations of NOEC and LOEC values [31]. An exception was that Wilcoxon rank sum test with Bonferroni adjustment was used to compare mean survival and biomass between the control and the Winding Shoals 50% or Boardtree 50% exposure treatments for *C. triangulifer*, because these tests had only 1 exposure treatment with partial mortality and at least 3 replicates (Table 2). The level of statistical significance was set at $\alpha = 0.05$. In most cases, no 20% effect concentration (EC20) could be estimated because the data from the toxicity test did not meet the conditions for any logistic regression analysis [32]. A 25% effect concentration (EC25) for *C. dubia* was estimated using the TOXSTAT software to compare responses of *C. dubia* in the present study and a previous study [13].

RESULTS

Water chemistry

Concentrations of major cation and major anions and relevant water-quality characteristics of site waters (target field chemistry reported by the US Environmental Protection Agency [USEPA] [13]) are presented in Supplemental Data, Table S1 as well as the nominal (predicted) and measured concentrations of the reconstituted waters. The quality of the reconstituted waters approximated the intended ionic compositions found in site waters (Supplemental Data, Table S1). The reconstituted Winding Shoals and Boardtree waters were largely similar to each other, with elevated concentrations of Ca, Mg, K, SO_4, and HCO_3, as opposed to Upper Dempsey water, which was high in K, Na, SO_4, and HCO_3. Relative to the dilution control water, the 100% Winding Shoals target water had proportionally higher concentrations of Ca (5.4×), Mg (24×), K (8.5×), SO_4 (44×), HCO_3 (19×), and conductivity (7.8×; Supplemental Data, Table S1). Relative to the dilution control water, the 100% Boardtree target water had proportionally higher concentrations of Ca (8.0×), Mg (25×), K (7.5×), SO_4 (69×), HCO_3 (6.0×), and conductivity (8.9×; Supplemental Data, Table S1). Relative to the dilution control water, the 100% Upper Dempsey target water had proportionally higher concentrations of Na (32×), K

Table 2. Responses of cladoceran (*Ceriodaphnia dubia*; 7-d exposures), mussel (*Lampsilis silquoidea*; 28-d exposures), amphipod (*Hyalella azteca*; 28-d exposure), and mayfly (*Centroptilum triangulifer*; ~35-d exposures) to 3 reconsituted water samples[a]

	Cladoceran		Mussel[b]		Amphipod[b]		Mayfly	
Treatment	Survival (%)	No. of young	Survival (%)	Length (mm)	Survival (%)	Biomass (mg)	Survival (%)[c]	Biomass (mg)[c]
Winding Shoals								
Control	90	28 (10)	89 (6)	2.93 (0.6)	86 (13)	3.21 (0.8)	84 (11)	8.5 (2.0)
10%	100	31 (5)	18 (17)*	2.79 (0.3)[d]	83 (29)	3.25 (2.1)	—	—
33%	90	30 (7)	8 (5)*	2.49 (0.7)[d]	73 (17)	1.27 (0.2)*	27/33[e]	1.9/2.2[e]
50%	—[f]	—	—	—	—	—	12 (20)*	1.0 (1.7)*
66%	—	—	—	—	—	—	0*	0*
100%	100	33 (4)	3 (5)*	ND	65 (26)	2.08 (0.9)*	0*	0*
NOEC	>100%	>100%	<10%	>100%	>100%	10%	<33%[g]	<33%[g]
LOEC	>100%	>100%	10%	>100%	>100%	33%	33%[g]	33%[g]
Boardtree								
Control	90	28 (10)	89 (6)	2.93 (0.6)	86 (13)	3.21 (0.8)	80 (10)	8.9 (2.7)
10%	80	23 (13)	45 (29)*	3.01 (0.3)	80 (18)	2.67 (1.3)	—	—
33%	80	21 (12)	10 (8)*	3.52 (0.5)[d]	73 (5)	2.53 (0.3)	—	—
50%	—	—	—	—	—	—	37 (13)*	3.7 (1.8)*
100%	100	22 (7)*	30 (25)*	2.91 (0.6)[d]	75 (10)	1.78 (0.2)*	0*	0*
NOEC	>100%	33%	<10%	>100%	>100%	33%	<50%	<50%
LOEC	>100%	100%	10%	>100%	>100%	100%	50%	50%
Upper Dempsey								
Control	100	40 (3)	89 (6)	2.93 (0.6)	86 (13)	3.21 (0.8)	80 (10)	8.9 (2.7)
5%	100	38 (6)	—	—	—	—	—	—
10%	100	36 (7)	25 (24)*	2.99 (0.5)	98 (5)	4.53 (0.3)	—	—
33%	90	36 (13)	13 (10)*	3.12 (0.4)[d]	95 (6)	3.55 (0.3)	—	—
50%	—	—	—	—	—	—	95/70[e]	11/7.9[e]
100%	40*	18 (13)*	15 (6)*	2.86 (0.5)	95 (10)	2.52 (0.2)*	100/80[e]	11/10[e]
NOEC	33%	33%	<10%	>100%	>100%	33%	>100%	>100%
LOEC	100%	100%	10%	>100%	>100%	100%	>100%	>100%

[a] Values are means with standard deviation in parentheses ($n = 10$ for the cladoceran and $n = 4$ for other species unless otherwise noted).
[b] Mean starting sizes (and standard deviations) were: mussels, 1.9 mm/individual (0.3, $n = 21$); and amphipods, 1.89 mm/individual (0.25, $n = 20$).
[c] $n = 3$, except for the control ($n = 5$), 33% ($n = 2$), and 100% ($n = 6$) treatments in the Winding Shoals water, the 50% and 100% treatments ($n = 2$) in the Upper Dempsey water.
[d] Based on 3 replicates as a result of 100% mortality in 1 replicate at these exposure concentrations.
[e] Two values were from the 2 replicates of this treatment and not used for statistical analysis.
[f] Species was not tested at this concentration.
[g] The 33% water was considered to be an effect concentration because the survival and biomass at this concentration was more than 60% less than those in the controls.
* Significant reduction relative to control ($p < 0.05$).
NOEC = no-observed-effect concentration; LOEC = lowest-observed-effect concentration; ND = not determined (because of limited number of organisms for growth measurement).

(4.8×), SO_4 (21×), HCO_3 (40×), and conductivity (7.9×; Supplemental Data, Table S1).

The 3 reconstituted waters were designed to replicate the target waters, although in some cases an adjustment of the composition of reconstituted waters was necessary, as described in *Materials and Methods*. With limited exceptions, the nominal concentrations of major cations and major anions in all 3 reconstituted waters were within 30% of field target waters. Differences between nominal concentrations and final measured concentrations were generally within 20% for all 3 reconstituted waters (Supplemental Data, Table S1). The most notable difference between the reconstituted waters and the 100% target waters was in alkalinity (no attempt was made to control alkalinity), the reconstituted waters being below target levels of alkalinity by 72% for Winding Shoals water, 38% for Boardtree water, and 57% for Upper Dempsey water. Measured concentrations of K and Cl were consistently higher in reconstituted waters compared with target waters or nominal reconstituted waters, but the absolute differences in concentrations were generally small (i.e., <5 mg/L).

The charge balance between major cations and major anions was relatively similar (within 2.7%) between target site water quality and measured reconstituted water quality for the Upper Dempsey and Boardtree waters (Supplemental Data, Table S1). However, for the Winding Shoals water, there was a 6.6% positive charge imbalance in the target site water and a 3.2% positive charge imbalance in the measured reconstituted water quality (Supplemental Data, Table S1). This imbalance indicates that combined concentrations of major anions in the Winding Shoals water were low (either analytical error or an omission of measuring other anions beyond HCO_3, Cl, or SO_4). Mean water-quality characteristics for each dilution averaged across all species exposures (Table 1) were similar to mean water-quality characteristics for each dilution for individual species exposures (Supplemental Data, Table S4).

Exposures

Control survival of *L. siliquoidea, H. azteca*, *C. dubia*, and *C. triangulifer* met the test acceptability criteria of >80% mean survival (Table 2). Reproduction of *C. dubia* also met the test acceptability criteria of >15 young/surviving female in the control and >60% of surviving females in controls producing 3 broods.

Dilutions of the Winding Shoals reconstituted waters were toxic to *L. siliquoidea* and *C. triangulifer* at the lowest concentrations tested (i.e., LOEC of 10% Winding Shoals for *L. siliquoidea* survival and LOEC of 33% Winding Shoals for *C. triangulifer* survival and biomass; Table 2). Survival of *L.*

siliquoidea and biomass of *C. triangulifer* decreased with increasing percentage of Winding Shoals reconstituted water (Figures 1 and 2). The Winding Shoals reconstituted water was also toxic to *H. azteca* (LOEC of 33% Winding Shoals for biomass; Table 2 and Figures 1 and 2). The Winding Shoals reconstituted water was not toxic to *C. dubia* (LOEC of >100% Winding Shoals; Table 2). By comparison, the USEPA [13] reported a 7-d EC25 for *C. dubia* of 32% Winding Shoals site water. The 100% site water from Winding Shoals had a conductivity of 2147 μS/cm [13], which is comparable to the

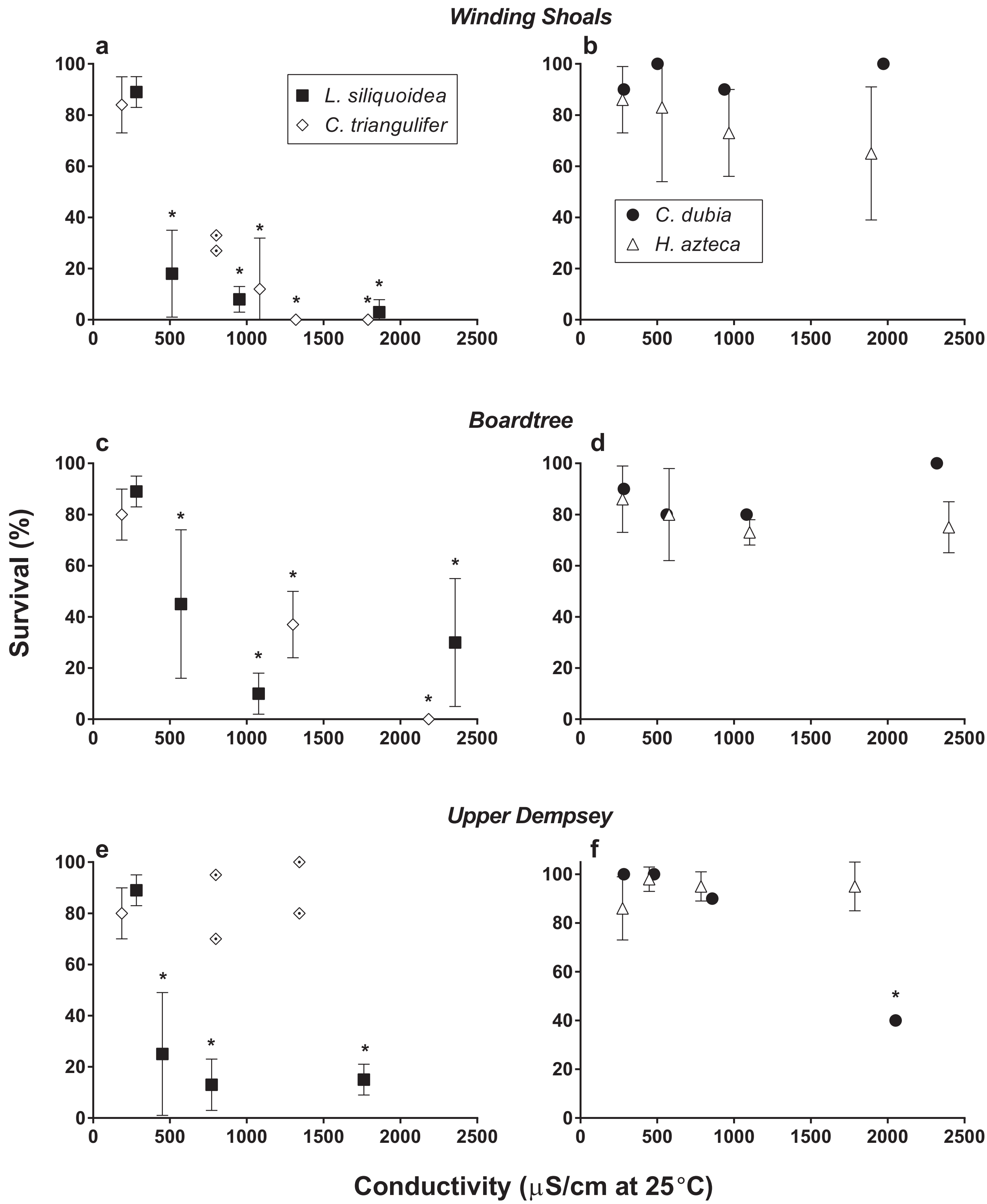


Figure 1. Mean survival of 4 freshwater invertebrates— *Lampsilis siliquoidea*, *Centroptilum triangulifer*, *Ceriodaphnia dubia*, and *Hyalella azteca*—versus conductivity of reconstituted waters. An asterisk (*) indicates significant difference compared with control group ($p \leq 0.05$). Error bars indicate standard deviation. The *C. dubia* data points do not have error bars, because survival was calculated as the percentage surviving among 10 individually exposed organisms. The *C. triangulifer* data points marked with a dot represent individual replicate responses for situations in which there were <3 replicates and a standard deviation could not be calculated.

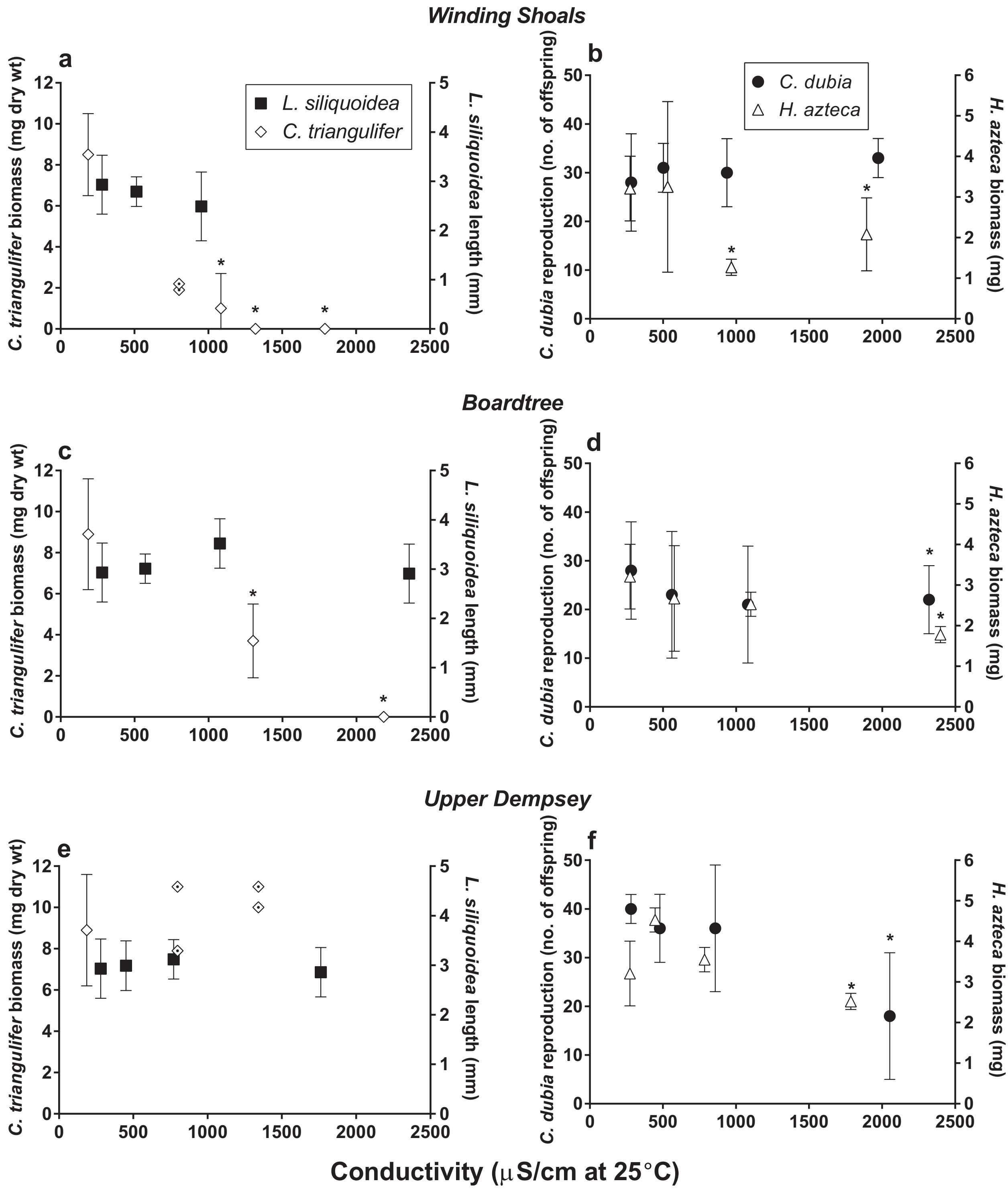


Figure 2. Sublethal response of 4 freshwater invertebrates— *Lampsilis siliquoidea*, *Centroptilum triangulifer*, *Ceriodaphnia dubia*, and *Hyalella azteca*—versus conductivity of reconstituted waters. An asterisk (*) indicates significant difference compared with control group ($p \leq 0.05$). Data points represent the mean of replicates for a given treatment. Error bars indicate standard deviation. The *C. triangulifer* data points marked with a dot represent individual replicate responses for situations in which there were <3 replicates and standard deviation could not be calculated.

conductivity of 1906 μS/cm in the 100% Winding Shoals reconstituted water (Table 1). Relative to the dilution water control, the 10% dilution of Winding Shoals reconstituted water that was toxic to *L. siliquoidea* had relatively higher proportional concentrations of Mg (3.8×), SO_4 (6.4×), and conductivity (1.8×; Supplemental Data, Table S1).

Dilutions of the Boardtree reconstituted waters were toxic to *L. siliquoidea* and to *C. triangulifer* at the lowest concentrations tested (LOEC of 10% Boardtree for *L. siliquoidea* survival and LOEC of 50% Boardtree for *C. triangulifer* survival or biomass; Table 2). Survival of *L. siliquoidea* and survival or biomass of *C. triangulifer* decreased with increasing percentage of Boardtree reconstituted water (Figures 1 and 2). The Boardtree reconstituted water was also toxic to *H. azteca* (LOEC of 100% Boardtree for biomass) and *C. dubia* (LOEC of 100% Boardtree for reproduction; Table 2). By comparison, the USEPA [13] reported a 7-d EC25 for *C. dubia* of 15% Boardtree site water. The 100% Boardtree site water had conductivity of 2582 μS/cm [13], which is comparable to the conductivity of 2367 μS/cm in the 100% Boardtree reconstituted water (Table 1). Relative to the dilution water control, the 10% dilution of Boardtree reconstituted water that was toxic to *L. siliquoidea* had relatively higher proportional concentrations of Mg (4.1×), SO_4 (8.2×), and conductivity (2.1×; Supplemental Data, Table S1).

The Upper Dempsey reconstituted waters were toxic to *L. siliquoidea*, *H. azteca*, and *C. dubia* (LOEC of 10% Upper Dempsey for *L. siliquoidea* survival and LOECs of 100% Upper Dempsey for *H. azteca* biomass and *C. dubia* survival or reproduction; Table 2). Survival of *L. siliquoidea*, survival and biomass of *H. azteca* and reproduction of *C. dubia* decreased with increasing percentage of Upper Dempsey reconstituted water (Figures 1 and 2). The Upper Dempsey reconstituted water was not toxic to *C. triangulifer* (LOEC >100% Upper Dempsey; Table 2). A 7-d EC25 for *C. dubia* reproduction of about 54% for Upper Dempsey reconstituted water was estimated in the present study. By comparison, the USEPA [13] reported a 7-d EC25 of 25% Upper Dempsey site water for *C. dubia*. The 100% Upper Dempsey site water had a conductivity of 2143 μS/cm [13], which is comparable to the conductivity of 1813 μS/cm in the 100% Upper Dempsey reconstituted water (Table 1). Relative to the dilution water control, the 10% dilution of Upper Dempsey reconstituted water that was toxic to *L. siliquoidea* had relatively higher proportional concentrations of Na (4.3×), SO_4 (3.5×), and conductivity (1.6×; Supplemental Data, Table S1).

DISCUSSION

Salinization of freshwaters is a growing problem on a global scale [1]. With 4 major cations (Na, K, Ca, and Mg) and 3 major anions (Cl, HCO_3, and SO_4) all potentially contributing to the ionic matrix of a high-TDS water, the composition of any one site can vary dramatically from the next, particularly across geographic regions. For example, freshwater streams impacted by marine encroachment display elevated Na and Cl [33], whereas some streams affected by uranium mining display elevated Mg and SO_4 [34]. The ionic composition of streams in Kentucky, Virginia, and West Virginia, USA, impacted by mountaintop removal associated with coal mining are characterized by mixtures of elevated Ca, Mg, K, HCO_3, and SO_4 (as represented by Winding Shoals and Boardtree waters), although Na is not typically elevated compared with reference streams [10,35]. Elevated Na in mine drainage (as represented by the Upper Dempsey water) could be due to the following: 1) treating mine effluents with sodium hydroxide (NaOH) or sodium carbonate (soda ash, Na_2CO_3) to reduce Mn; 2) cation exchange processes in overburden that exchange Ca for Na; or 3) the presence of sodium-rich shale layers in the overburden (M. Passmore, USEPA, Wheeling, WV, USA, personal communication).

The reconstituted waters in the present study were generated to closely match site waters from the Winding Shoals, Boardtree, and Upper Dempsey Branches in West Virginia, with the exception of HCO_3, which was below the field target concentrations by 38 to 72% (Supplemental Data, Table S1). This is important because bicarbonate can contribute to toxicity to *C. dubia* [7]. Research is ongoing to develop methods for preparing reconstituted waters with varying concentrations of HCO_3 using CO_2 gas to equilibrate relatively insoluble salts ($CaCO_3$ or $MgCO_3$; D. Mount, USEPA, Duluth, MN, USA, personal communication). Use of this new approach should result in developing reconstituted waters that better represent the alkalinity and HCO_3 of site waters of interest.

Sites impacted by elevated TDS may also have elevated concentrations of trace elements such as Se and Mn [10] and As, Co, and Cu [15]. Therefore, results of whole-effluent toxicity testing can be difficult to interpret with regard to which component of the effluent matrix is contributing to the toxicity of elevated TDS mixtures [14]. Investigators concerned with elevated TDS independent of other toxic elements may have to generate reconstituted waters that approximate the ionic matrix of concern as closely as possible. Importantly, the high-TDS reconstituted waters, created with the addition of elevated major ions without the addition of trace elements, were toxic to test organisms in the present study, indicating that trace elements associated with site waters were not likely substantial contributors to the toxicity observed in previous ambient-water exposures to *C. dubia* [13]. Moreover, ambient water–quality criteria for trace metals were exceeded only sporadically, and these overages were not related to the toxicity of the ambient waters to *C. dubia* [13].

Previous laboratory toxicity tests with ambient water samples from the 3 sites were identified as toxic to *C. dubia* [13]. Two of the 3 reconstituted waters tested (Boardtree and Upper Dempsey) in the present study were also identified as toxic to *C. dubia* (Table 2). Winding Shoals reconstituted water with an ionic composition similar to that of Boardtree reconstituted water was not toxic to *C. dubia*. In previous study, a 7-d EC20 of 996 mg SO_4/L was observed for reproduction of *C. dubia* at a water hardness of about 100 mg/L (as $CaCO_3$; N. Wang, unpublished data). Perhaps the slightly higher concentration of SO_4 in the 100% Boardtree reconstituted water (1450 mg/L) contributed to the reproductive toxicity observed in *C. dubia* compared with the concentration of SO_4 in the 100% Winding Shoals reconstituted water (1108 mg/L) that was not toxic to *C. dubia*, given that all other major ions were relatively similar between the Winding Shoals and Boardtree reconstituted waters (Table 1). Sulfate might have contributed to the toxicity but likely was not the sole cause of the toxicity observed to *L. siliquoidea* in the 10% dilutions of the reconstituted water samples (ranging from 144 to 172 mg SO_4/L in the 10% dilution waters; Table 1 and Supplemental Data, Table S4). A 28-d EC20 of 696 mg SO_4/L (as Na_2SO_4) was observed for biomass of the pink mucket (*L. abrupta*) at a water hardness of about 100 mg/L (as $CaCO_3$; N. Wang, unpublished data). Potassium might also have contributed to the toxicity but likely was not the sole cause of the toxicity observed to *L. siliquoidea* in the 10% to 100% dilution waters (ranging up to 21 mg/L; Table 1 and Supplemental Data, Table S4). A 28-d LC20 of >25 mg K/L was observed for survival of the *L. siliquoidea* at a water hardness of about 100 mg/L (N. Wang, unpublished data). Additional testing of reconstituted waters with varying proportions of major ions is needed to identify the toxicity thresholds for individual ions or to determine whether major ions such as Cl, Ca, or K might ameliorate the toxicity of major ions such as SO_4 [5,36].

Toxicity tests have rarely been conducted with taxa resident to naturally dilute, freshwater Appalachian streams such as mayflies, stoneflies, and caddisflies (i.e., Ephemeroptera, Plecoptera, Trichoptera taxa) or with freshwater mussels. One study investigated the toxicity of high-TDS water on *C. dubia* and a mayfly (*Isonychia bicolor*) and found that *I. bicolor* was substantially more sensitive than *C. dubia* [37] in whole-effluent toxicity testing with ambient waters. Similarly, *C. triangulifer* and the *L. siliquoidea* in the present study were more responsive to the Winding Shoals and Boardtree reconstituted waters compared with the *C. dubia* and the *H. azteca*. Furthermore, all 3 waters were toxic to *L. siliquoidea* and *H. azteca*, whereas *C. triangulifer* was unaffected by exposure to Upper Dempsey water, and *C. dubia* was unaffected by exposure to Winding Shoals water. Therefore, toxicity testing conducted with test organisms such as *C. dubia* may underrepresent the sensitivity of species present in the Appalachian streams of interest. This highlights the need for developing standard methods for testing of multiple taxa in laboratory toxicity tests that represent the sensitivity of resident taxa. Standard methods have been developed for all of the organisms tested in the present study, except for *C. triangulifer*.

Natural streams in the central Appalachian region are dilute, with conductivity generally between 40 and 100 μS/cm; however, mining-impacted streams display conductivities of about 1000 to 2500 μS/cm [10,35]. Because many macroinvertebrates have evolved in and are adapted to dilute streams in this region, overall ionic strength is a potential physiological mechanism of impairment deserving additional study. Field studies have reported declines in abundance and diversity of aquatic insects (particularly mayflies) in mountaintop mining-impacted streams across Appalachia associated with elevated TDS [9,10,35]. Based on the distributions of benthic invertebrates along conductivity gradients in sites within the Appalachian region, the USEPA [35] proposed a regional conductivity benchmark of 300 μS/cm for protection of aquatic life. The benchmark of 300 μS/cm was designed to protect 95% of the 162 genera that were used to develop the benchmark. The benchmark for conductivity applies to waters in the central Appalachians and Allegheny Plateau with circumneutral to mildly alkaline pH where the elevated conductivity is dominated by HCO_3 and SO_4. However, no laboratory toxicity studies have investigated the potential effects of elevated conductivity associated with mountaintop mining on resident taxa (e.g., aquatic insects). The Winding Shoals and Boardtree reconstituted waters were toxic to *C. triangulifer* at a conductivity of about 800 to 1300 μS/cm (Supplemental Data, Table S4) with elevated concentrations of Mg, Ca, Na, K, SO_4, or HCO_3. It is interesting to note that the regional 95% extirpation concentration (XC95) based on conductivity for the genus *Centroptilum* in the benthic community field surveys was determined to be 1092 μS/cm [35]. However, the genus *Centroptilum* was not among the more sensitive taxa used to derive the regional benchmark (i.e., 56 of the native taxa had XC95 values <1092 μS/cm [35]).

The Winding Shoals and Boardtree reconstituted waters were toxic to *L. siliquoidea*, *H. azteca*, or *C. dubia* at conductivities ranging from about 500 to 2400 μS/cm (Table 1). The Upper Dempsey reconstituted water at conductivities ranging from about 500 to1800 μS/cm with elevated concentrations of Na, K, SO_4, and HCO_3 was toxic to *L. siliquoidea*, *H. azteca*, and *C. dubia* but was not toxic to *C. triangulifer* at conductivity as high as about 1800 μS/cm (Supplemental Data, Table S1). Hence, it might be informative to establish toxicity thresholds based on single major ions or mixtures of major ions to individual taxa rather than to depend solely on the use of surrogate regional benchmarks such as TDS or conductivity. On the other hand, conductivity benchmarks developed within a region for specific ion matrices where natural background conductivity levels are comparable can be useful screening tools for measuring potential toxicity in the field.

Currently, the toxic mechanism of high-TDS waters is largely unknown. It has been speculated that osmoregulatory stress is potentially responsible for adverse effects in sensitive taxa as well as disruption of hemolymph pH or osmolarity [38]. Many surficial ionoregulatory pumps on aquatic insects use adenosine triphosphate to move ions against a concentration gradient [39]. It is possible that exposure to high TDS incurs bioenergetic costs as manifested by developmental delays observed in mayflies (D. Funk, Stroud Water Research Center, Avondale, PA, USA, personal communication). Soucek [40] found that *C. dubia* displayed reduced feeding and oxygen consumption when exposed to elevated concentrations of sodium sulfate. Given the large number of ions, the even larger number of ion combinations, and wide range of invertebrate physiologies, there is much to be learned about why ionic stress is toxic to aquatic organisms.

In the present study, 2 central ideas in the assessment of toxicity associated with major ions were reinforced: 1) specific ionic composition of the water is critical, and 2) selection of laboratory test species is also critical for relating major ion toxicity to field data. For example, survival of *C. triangulifer* and *L. siliquoidea* was reduced in all dilutions of Boardtree and Winding Shoals reconstituted waters (with elevated Mg, Ca, K, SO_4, and HCO_3), yet *C. triangulifer* did not exhibit adverse effects with exposure to Upper Dempsey reconstituted water (with elevated in Na, K, SO_4, and HCO_3) at conductivity comparable to the toxic dilutions of Boardtree and Winding Shoals reconstituted waters. Moreover, effects were observed on both *L. siliquoidea* and *H. azteca* with exposure to all 3 reconstituted waters. Conversely, *C. dubia* displayed no adverse effects of exposure to Winding Shoals reconstituted water. The ionic composition of the Winding Shoals and Boardtree reconstituted waters are characteristic of mountaintop-mining-impacted streams, and *C. triangulifer* is representative of native Appalachian taxa, albeit more tolerant to elevated conductivity based on benthic community survey data [35]. Future studies should focus on identifying the primary toxic ions or, conversely, determine whether a characteristic ionic matrix is necessary to produce toxicity. Additionally, future studies should focus on conducting toxicity tests with environmentally relevant and sensitive species, including mayflies and mussels, in order to make stronger links to benthic community survey data.

SUPPLEMENTAL DATA

Tables S1–S8. (115 KB PDF).

Acknowledgment—We thank the members of the Columbia Environmental Research Center Toxicology Branch and Chemistry Branch for technical support; G. Pond, M. Passmore, and A. Bergdale for input on experimental design; and D. Soucek and B. Williams for providing comments on an earlier draft of the paper. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US Government. Funding for J.M. Conley was provided by USEPA STAR Fellowship (GAD FP917322); the USEPA has not formally reviewed this publication, and the views expressed herein may not reflect the views of the USEPA.

REFERENCES

1. Williams WD. 2001. Anthropogenic salinization of inland waters. *Hydrobiologia* 466:329–337.
2. Society of Environmental Toxicology and Chemistry. 2004. Whole effluent toxicity Testing: Ion imbalance. SETAC Technical Issue Paper. SETAC Press, Pensacola, FL, USA.
3. Lindberg TT, Bernhardt ES, Bier R, Helton AM, Merola RB, Vengosh A, Di Giulio RT. 2012. Cumulative impacts of mountaintop mining on an Appalachian watershed. *Proc Natl Acad Sci U S A* 108:20929–20934.
4. Kennedy AJ, Cherry DS, Currie RJ. 2003. Field and laboratory assessment of a coal processing effluent in the Leading Creek Watershed, Meigs County, Ohio. *Arch Environ Contam Toxicol* 44:324–331.

5. Kennedy AJ, Cherry DS, Zipper CE. 2005. Evaluation of ionic contribution to the toxicity of coal-mine effluent using *Ceriodaphnia dubia*. *Arch Environ Contam Toxicol* 49:155–162.
6. Kaushal SS, Groffman PM, Likens GE, Belt KT, Stack WP, Kelly VR, Band LE, Fisher GT. 2005. Increased salinization of fresh water in the northeastern United States. *Proc Natl Acad Sci U S A* 102:13517–13520.
7. Mount DR, Gulley DD, Hockett JR, Garrison TD, Evans JM. 1997. Statistical models to predict the toxicity of major ions to *Ceriodaphnia dubia*, *Daphnia magna*, and fathead minnows (*Pimephales promelas*). *Environ Toxicol Chem* 16:2009–2019.
8. American Petroleum Institute. 1998. The toxicity of common ions to freshwater and marine organisms. Document 0300-029. Washington, DC.
9. Goodfellow WL, Ausley LW, Burton DT, Denton DL, Dorn PB, Grothe DR, Heber MA, Norberg-King TJ, Rodgers JH. 2000. Major ion toxicity in effluents: A review with permitting recommendations. *Environ Toxicol Chem* 19:176–182.
10. Pond GJ, Passmore ME, Borsuk FA, Reynolds L, Rose CJ. 2008. Downstream effects of mountaintop coal mining: Comparing biological conditions using family- and genus-level macroinvertebrate bioassessment tools. *J North Am Benthol Soc* 27:717–737.
11. Pond GJ. 2010. Patterns of Ephemeroptera taxa loss in Appalachian headwater streams (Kentucky, USA). *Hydrobiologia* 641:185–201.
12. US Environmental Protection Agency. 2005. Interagency Mountaintop Mining/Valley Fills in Appalachia: Final programmatic environmental impact statement. EPA 9/03/R-5002. Philadelphia, PA.
13. US Environmental Protection Agency. 2012. An evaluation of the *Ceriodaphnia dubia* 7-day chronic toxicity test as an indicator of in stream harm from alkaline coal mine discharges in Central Appalachia. Wheeling, WV.
14. Dwyer FJ, Burch SA, Ingersoll CG. 1992. Investigations on the combined toxicity of trace elements and salinity to aquatic organisms. *Environ Toxicol Chem* 11:513–520.
15. Ingersoll CG, Dwyer FJ, Burch SA, Nelson MK, Buckler DR, Hunn JB. 1992. The use of freshwater and saltwater animals to distinguish between the toxic effects of salinity and contaminants in irrigation drain water. *Environ Toxicol Chem* 11:503–511.
16. ASTM International. 2012. Standard guide for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. E729-96 (2007) In *Annual Book of ASTM Standards*, Vol 11.06. West Conshohocken, PA, USA.
17. Eaton AD, Clesceri LS, Rice EW, Greenberg AE, eds. 2005. *Standard Methods for the Examination of Water and Wastewater*, 21st ed. American Public Health Association, Water Environment Federation, American Water Works Association, Washington, DC.
18. Wang N, Consbrock R, Hardesty DK, Ingersoll CG. 2011. Memo to Ed Hammer, US EPA Region 5: Preliminary summary of acute and chronic water-only sodium sulfate toxicity testing with select freshwater organisms. Chicago, IL.
19. Besser JM, Brumbaugh WG, Kemble NE, Ivey CD, Kunz JL, Ingersoll CG, Rudel D. 2011. Toxicity of nickel-spiked freshwater sediments to benthic invertebrates. Spiking methodology, species sensitivity, and nickel bioavailability. Scientific Investigations Report 2011-5225. US Geological Survey, Reston, VA.
20. ASTM International. 2012. Standard guide for conducting laboratory toxicity tests with freshwater mussels. E2455-06. In *Annual Book of ASTM Standards*, Vol 11.06. West Conshohocken, PA, USA.
21. ASTM International. 2012. Standard test method for measuring the toxicity of sediment-associated contaminants with freshwater invertebrates. E1706-05. In *Annual Book of ASTM Standards*, Vol 11.06. West Conshohocken, PA, USA.
22. ASTM International. 2012. Standard guide for conducting three-brood, renewal toxicity tests with *Ceriodaphnia dubia*. E1295-01 (2006) In *Annual Book of ASTM Standards*, Vol 11.06. West Conshohocken, PA, USA.
23. Wang N, Ingersoll CG, Greer IE, Hardesty DK, Ivey CD, Kunz JL, Brumbaugh WG, Dwyer FJ, Roberts AD, Augspurger T, Kane CM, Neves RJ, Barnhart MC. 2007. Chronic toxicity of copper and ammonia to juvenile freshwater mussels (Unionidae). *Environ Toxicol Chem* 26:2048–2056.
24. Brunson EL, Canfield TJ, Dwyer FJ, Ingersoll CG, Kemble NE. 1998. Assessing the bioaccumulation of contamination with sediments from the Upper Mississippi River using field-collected oligochaetes and laboratory-exposed *Lumbriculus variegatus*. *Arch Environ Contam Toxicol* 35:191–201.
25. Ingersoll CG, Besser JM, Brumbaugh WG, Ivey CD, Kemble NE, Kunz JL, May TW, Wang N, MacDonald DD, Smorong DE. 2008. Sediment chemistry, toxicity, and bioaccumulation data report for the US Environmental Protection Agency—Department of the Interior sampling of metal-contaminated sediment in the Tri-state Mining District in Missouri, Oklahoma, and Kansas. Final Report CERC-8335-FY07- 20-12. US Geological Survey, Columbia, MO, USA and MacDonald Environmental Sciences, Nanaimo, BC, Canada.
26. Wang N, Ingersoll CG, Kunz JL, Brumbaugh WG, Kane CM, Evans RB, Alexander S, Walker C, Bakaletz S, Lott RC. 2013. Toxicity of sediments potentially contaminated by coal or natural gas mining-related activities to unionid mussels and commonly tested benthic invertebrates. *Environ Toxicol Chem* 32:207–221.
27. Sweeney BW, Funk DH, Standley JL. 1993. Use of the stream mayfly *Cloeon triangulifer* as a bioassay organism—Life-history response and body burden following exposure to technical chlordane. *Environ Toxicol Chem* 12:115–125.
28. Conley JM, Funk DH, Buchwalter DB. 2009. Selenium bioaccumulation and maternal transfer in the mayfly *Centroptilum triangulifer* in a life-cycle, periphyton-biofilm trophic assay. *Environ Sci Technol* 43:7952–7957.
29. Conley JM, Funk DH, Cariello NJ, Buchwalter DB. 2011. Food rationing affects dietary selenium bioaccumulation and life cycle performance in the mayfly *Centroptilum triangulifer*. *Ecotoxicology* 20:1840–1851.
30. Xie LT, Funk DH, Buchwalter DB. 2010. Trophic transfer of Cd from natural periphyton to the grazing mayfly *Centroptilum triangulifer* in a life cycle test. *Environ Pollut* 158:272–277.
31. US Environmental Protection Agency. 2002. *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms*, 5th ed. EPA 821/R-02/013. Washington, DC.
32. Erickson RJ. 2012. Toxicity Relationship Analysis Program (TRAP), Ver 1.21. EPA 600/C-11/002. Washington DC, U.S. Environmental Protection Agency.
33. Zalizniak L, Kefford BJ, Nugegoda D. 2006. Is all salinity the same? I. The effect of ionic compositions on the salinity tolerance of five species of freshwater invertebrates. *Mar Freshwater Res* 57:75–82.
34. van Dam RA, Hogan AC, McCullough CD, Houston MA, Humphrey CL, Harford AJ. 2010. Aquatic toxicity of magnesium sulfate, and the influence of calcium, in very low ionic concentration water. *Environ Toxicol Chem* 29:410–421.
35. US Environmental Protection Agency. 2011. A field-based aquatic life benchmark for conductivity in central Appalachian streams. EPA 600/R-10/023F. Cincinnati, OH.
36. Soucek DJ. 2007. Comparison of hardness- and chloride-regulated acute effects of sodium sulfate on two freshwater crustaceans. *Environ Toxicol Chem* 26:773–779.
37. Echols BS, Currie RJ, Cherry DS. 2010. Preliminary results of laboratory toxicity tests with the mayfly, *Isonychia bicolor* (Ephemeroptera: Isonychiidae) for development as a standard test organism for evaluating streams in the Appalachian coalfields of Virginia and West Virginia. *Environ Monitor Assess* 169:487–500.
38. Goetsch PA, Palmer CG. 1997. Salinity tolerances of selected macroinvertebrates of the Sabie River, Kruger National Park, South Africa. *Arch Environ Contam Toxicol* 32:32–41.
39. Lee CE, Kiergaard M, Gelembiuk GW, Eads BD, Posavi M. 2011. Pumping ions: Rapid parallel evolution of ionic regulation following habitat invasions. *Evolution* 65:2229–2244.
40. Soucek DJ. 2007. Bioenergetic effects of sodium sulfate on the freshwater crustacean, *Ceriodaphnia dubia*. *Ecotoxicology* 16: 317–325.

Acute Toxicity of Binary Mixtures of Five Cations (Cu^{2+}, Cd^{2+}, Zn^{2+}, Mg^{2+}, and K^+) to the Freshwater Amphipod *Gammarus lacustris* (Sars): Alternative Descriptive Models

B. G. E. de March

Department of Fisheries and Oceans, Freshwater Institute, 501 University Crescent, Winnipeg, Man. R3T 2N6

de March, B. G. E. 1988. Acute toxicity of binary mixtures of five cations (Cu^{2+}, Cd^{2+}, Zn^{2+}, Mg^{2+}, and K^+) to the freshwater amphipod *Gammarus lacustris* (Sars): alternative descriptive models. Can. J. Fish. Aquat. Sci. 45: 625–633.

The combined results of 10 acute toxicity experiments, each testing the joint toxicity of two of the ions Cu^{2+}, Cd^{2+}, Zn^{2+}, Mg^{2+}, and K^+, were examined in terms of different response surface models which could be used to make decisions about limiting toxic components in mixtures. The classical probit model for simple similar action described experimental results satisfactorily with a model R^2 of 0.282; equations in which probit(p) was described directly by a linear combination of toxicant concentrations fit data significantly better, with an R^2 of 0.527. Equations with more complex linear terms and appropriate weighting factors applied to the residual sums of squares yielded R^2 values up to 0.931. Predicted LC50 values were midrange compared with published values. Based on the linear description of the probit response, K or Mg in combination with either of Cu, Cd, or Zn had additive effects, the combinations Cu and Cd, Cu and Zn, and Cd and Zn had more-than-additive effects, and Mg and K had less-than-additive effects. The relationships between the response surfaces, other described modes of joint action, the toxic units model, and mixture toxicity indices are discussed.

L'auteur a analysé les résultats de dix essais de toxicité aiguë comportant chacun un test de la toxicité mixte de paire d'ions Cu^{+2}, Cd^{+2}, Zn^{+2}, Mg^{+2} ou K^+, dans le contexte des diverses réponses de modèles de surface pouvant servir à décider des composantes toxiques limitantes des mélanges. Le modèle classique par probit s'appliquant aux effets simples du même type permettait de décrire les résultats expérimentaux de façon satisfaisante avec une valeur de R^2 de 0,282. Les équations pour lesquelles le probit (p) était directement décrit par une combinaison linéaire des concentrations de toxiques présentaient un ajustement aux données significativement supérieur, la valeur de R^2 étant de 0,527. Des équations comportant des termes linéaires plus complexes et des facteurs de pondération appropriés appliqués aux sommes des carrés résiduelles permettaient d'obtenir des valeurs de R^2 pouvant atteindre 0,931. Les valeurs de CL50 prévues se situaient au centre de la gamme des valeurs publiées. Il est apparu, sur la base de la description linéaire de la réponse du probit, que les ions K ou Mg agissant avec les ions Cu, Cd ou Zn présentaient des effets additifs, que les combinaisons Cu et Cd, Cu et Zn et Cd et Zn avaient des effets plus qu'additifs, tandis que la combinaison Mg et K avait des effets inférieurs à des effets additifs. L'auteur traite aussi des relations entre les surfaces de réponse, d'autres modes d'action conjointe, du modèle des unités toxiques et des indices de toxicité des mélanges.

Received April 21, 1986
Accepted December 14, 1987
(J8763)

Reçu le 21 avril 1986
Accepté le 14 décembre 1987

The effects of mixtures of toxicants in aquatic systems is a topic which has received renewed attention because of numerous cases of aquatic systems stressed by toxicants from many sources. In spite of the high level of concern and discussion about approaches to mixtures (Anderson and Weber 1975; EIFAC 1980, 1987; International Joint Commission 1981; Stich et al. 1982; Vouk et al. 1987), there are few scientifically defensible methods for making decisions about limiting toxic components. Decisions could be based on the results of acute toxicity tests, for example with the use of "application factors" (Kenaga 1982) which convert acutely lethal levels to tolerable ones for certain groups of toxicants. The ability to make such decisions would then depend on the existence of a model that describes the effects of all toxicants in all possible concentration combinations.

This study was initially intended to show which one of several models, often discussed in the literature as if they were alternative hypotheses, would best describe mortality in experiments with mixtures of toxicants. For example, it was expected that "simple similar action" (Bliss 1939), also called the concentration–addition model (Anderson and Weber 1975; EIFAC 1987), might best describe the results of experiments with copper and cadmium, two metals expected to act similarly. It was expected that "independent joint action," also called the response–addition model (Anderson and Weber 1975; EIFAC 1987), "independent action," or a mode of joint action intermediate between the two (Bliss 1939), would best describe the results of experiments with copper and potassium, two ions expected to act differently. Then, it was hoped that the fusion of the two or expansion of one of two models might describe the results of more complex or combined experiments.

However, initial analyses of results from experiments with binary mixtures showed that simple similar action and independent joint action described the results of any given experiment equally well. At first this was assumed to be due to relatively large experimental errors that did not allow discrimination between models. However, closer scrutiny of the two models and their modifications (de March 1987b) showed that they were geometrically similar even if not algebraically identical. In practice the models were not alternative hypotheses, but alternative descriptive methods. Thus, it was decided that models which were based on the laws of probability, namely "independent joint action" and its extensions, would not be examined further. I believed that existing modifications of the basic definition were awkward. Also, I saw no reason why simple laws of probability should apply to whole organisms responding to stressors acting at several sites of action. Two other models, the classical probit model for simple similar action and its extensions, and various forms of linear models for describing the probit response, were examined further for their applicability to experimental results.

The extension of the classical probit response model (Bliss 1939) describing the noninteractive effects of several toxicants is as follows:

$$(1)\quad \text{Probit}(p) = k\cdot\ln(a_1\cdot C_1e_1 + a_2\cdot C_2e_2 + \ldots + a_n\cdot C_ne_n)$$

in which p is the percentage or probability of response, probit(p) is the probit transformation or normal equivalent deviate of p, a_1 to a_n are numerical coefficients, e_1 to e_n are exponents, and C_1 to C_n are concentrations of toxicants 1 to n. In the simple univariate model describing the effects of one toxicant, a lognormal distribution of the tolerances in relation to toxicant concentration is assumed; in a univariate but multivariable model such as the above, a lognormal distribution in response to a linear sum of concentrations is assumed. The chosen linear sum is partly based on kinetic and biochemical relationships between toxicants (Bliss 1939; Hewlett and Plackett 1952, 1959, 1964; Plackett and Hewlett 1952, 1963, 1967; Ashford and Smith 1964, 1965; Finney 1971). If all e_i's are equal to 1, then "simple similar action," the case in which the effects of any toxicant can be substituted by a multiple of the effects of any other, is described (Ashford and Smith 1964). If the e_i's are not equal, then nonparallel response curves describe the responses to individual toxicants.

Results of multivariable acute toxicity tests are also often described by statistical linear models such as regression analysis, mixed models, or by response surface methods (EIFAC 1987). The probability of response or a convenient transformation of it, such as probit(p), is described directly by linear combinations of functions of toxicant concentrations. This type of model is also examined in this paper because of its desirable statistical and mathematical properties.

The five ions used in these experiments, Cu^{2+}, Cd^{2+}, Zn^{2+}, Mg^{2+}, and K^+ were chosen because of sufficient knowledge of their physiological modes of action that different types of joint effects were expected. Balanced multifactorial designs for examining joint effects of toxicants are necessarily large and awkward, and some sort of experimental confounding or blocking is usually required to make experiments manageable. In this study, mixtures of only two toxicants were tested, with the assumption that third-order factor interactions would not be important or that the experimental error would be too large to examine higher order interactions. Experiments with only two toxicants were performed sequentially, with one toxicant from each experiment used in the next. This increased the probability of choosing an effective range of concentrations and also spread the use of each toxicant out over time. Also, toxicants were tested at different concentrations and at different ratios to ensure that response surface methodology could be used with the results.

TABLE 1. Summary information about the ten experiments performed.

Date (d/mo/yr)	Number of test cells	Toxicants and range of concentrations tested ($mg\cdot L^{-1}$)				
		Cu	Cd	Zn	K	Mg
02/09/83	21	0.013 0.081	0	0	4.24 35.2	0
02/10/83	22	0.027 0.127	0.005 0.068	0	0	0
12/10/83	23	0	0.010 0.054	0.024 0.433	0	0
22/11/83	24	0	0	0.043 1.1	0	2.63 17.8
03/01/84	21	0	0	0	2.53 10.8	1.84 12.2
16/01/84	22	0	0.002 0.406	0	4.46 39.0	0
25/01/84	23	0	0.017 0.110	0	0	4.27 19.0
03/02/84	24	0.054 0.392	0	0	0	4.65 43.2
10/02/84	24	0.155 0.388	0	0.410 1.70	0	0
23/02/84	23	0	0	0.066 0.233	12.93 36.91	0

Methods

Ten experiments, each examining the acute lethal response of the freshwater amphipod *Gammarus lacustris* (Sars) to mixtures with two of the five ions Cu^{2+}, Cd^{2+}, Zn^{2+}, Mg^{2+}, and K^+, were performed over 16 mo between October 1983 and March 1984 (Table 1). Each experiment consisted of 12–14 test cells with combinations of two toxicants in different ratios, 6 test cells with either of the two toxicants, and 2–4 control test cells with no toxicants. Experiments were done in a flow-through dispensing system using dechlorinated Winnipeg tap water, with the following average chemical mesurements: pH 7.8, hardness 80 mg $CaCO_3\cdot L^{-1}$, conductivity 200 $\mu S\cdot cm^{-1}$, 720 μmol dissolved inorganic $C\cdot L^{-1}$, 900 μmol dissolved organic $C\cdot L^{-1}$, 1.16 mg $K^+\cdot L^{-1}$, 5.84 mg $Mg^{2+}\cdot L^{-1}$, 6 mg $Cl^-\cdot L^{-1}$, and 7.7 mg $SO_4^{2-}\cdot L^{-1}$. Water was renewed completely every 2 h in each of the twenty-one to twenty-four 500-mL test cells, with a mean of 27 ± 8 animals per test cell. Most experiments lasted 6 d, one (February 23, 1984) lasted 3 d because complete mortality was achieved in all test cells by this time. Concentrations were calculated by measuring the dilution rate in each test cell before and after each experiment and occasionally confirmed analytically by the Metals Analysis group at this Institution. Additional acute toxicity tests with NaCl and $NaSO_4$ showed that the anion levels and salinities in these experiments were not at all stressful to *G. lacustris*.

Gammarus lacustris were obtained in the late fall from Nora Lake near Erickson, Manitoba. They were kept in the laboratory at cold temperatures near 5°C and at constant illumination so that reproduction would not be induced (de March 1982). Occasional accidental warm spells near 20°C aged animals rapidly, and consequently, control mortalities increased during 1984. In each experiment, dead animals were counted and removed at least every 8 h, and more often if rapid mortality occurred. Dead animals were easily distinguished in mixtures with metals because they quickly became turgid. Animals exposed to only K^+ or Mg^{2+} often had to be prodded for a reaction to distinguish signs of life.

In each test cell the acute mortality response over time was usually an S-shaped curve. The response in each test cell was described by the following curvilinear equation:

$$(2)\quad \text{Probit}(p) = a \cdot T^2 + b \cdot T + c + \epsilon$$

in which probit(p) is the probit of the cumulative percent mortality, T is the elapsed time in hours, a, b, and c are fitted coefficients, and ϵ is the residual error in the model. The fit of data to Equation (2) for each test cell was excellent. The error around these fitted equations were ignored because they constituted the subplot error in a repeated measures design (Cole and Grizzle 1966), which does not contribute to the evaluation of the joint effects of the toxicants. Further statistical analyses were performed with two summary statistics from these curves, the expected probit responses at 48 h (probit($p48$)) and/or at 96 h (probit($p96$)). The 96-h mortality at various toxicant concentrations in three of 10 experiments is shown in Fig. 1.

All statistical analyses, mainly curve fitting, were performed with the SAS Statistical Package (SAS Institute Inc. 1982). The procedures used to fit the data to the model equations were PROC NLIN (nonlinear regression procedure) and PROC GLM (general linear models procedure). PROC NLIN prints only R^2 values (multiple correlation coefficients) based on sums of squares not corrected for the mean. Corrected R^2 values were found by calculating 1 − (sums of squares for error)/(total sums of squares) so that the R^2 values for all models could be compared.

Results

In 9 of these 10 experiments analysed individually, either $p96$ or probit($p96$) was significantly correlated with levels of both toxicants ($P \leq 0.05$). The exception was the Cd–Mg experiment in which only Cd correlated with the response. The R^2 values obtained by fitting the equation probit($p96$) and/or probit($p48$) $= a \cdot C_1 + b \cdot C_1^2 + c \cdot C_2 + d \cdot C_2^2 + k$ for each experiment show that responses were moderately predictable within experiments (Table 2). The 96-h measurements were believed to be more reliable than those at 48 h, since toxicant concentrations were chosen to give a range of responses at 96 h.

A complete range of possible responses was not obtained in each experiment (Table 2). However, since each toxicant was used in four experiments, a reasonable range of responses was obtained for all toxicants except Mg. Correlation coefficients between probit responses and toxicant concentrations utilizing data from all 10 experiments combined were often significant, but expectedly low because each was calculated over a range of levels of the second toxicant in the mixture (Table 3).

Classical Probit Response Models

Fitting the 96-h probit responses (probit($p96$)) to Equation (1) yielded a response curve (not given) described with an R^2 of 0.310. Responses in control test cells (when all of C_1 to C_5 were zero) could not be used in fitting this curve; thus, this equation was fitted with only 170 of 204 points. Forcing a fit of simple similar action (that is, forcing all e_i's to equal 1) yielded an R^2 of 0.282. The overall fit of both models was significant ($P \leq 0.05$), but individual fitted parameters were not all so. The first model accounted for a significantly larger sum of squares than the latter at the $P \approx 0.20$ level using the generalized likelihood ratio test (Graybill 1976). Thus it is likely that some of the exponents were significantly different from 1 or from each other in this model, and simple similar action would not describe the joint effects of all pairs of toxicants. Even with this lack of significance, predicted LC50 values for all toxicants were within ranges reported in the literature, which often vary by an order of magnitude (Sprague 1985).

Textbooks on probit analysis suggest fitting probit curves with a weighting factor inversely proportional to the estimated variance applied to the residual sums of squares (Finney 1971; Hubert 1984). In fitting the above curves once more, such weighting was done, and the R^2 values increased to 0.795 and 0.715 for the above two equations, respectively. However, predicted LC50's and relative toxicities from the equations were orders of magnitude different from reported values. This may be due to the weighting factor emphasizing several high and slightly outlying responses, thus twisting the whole response

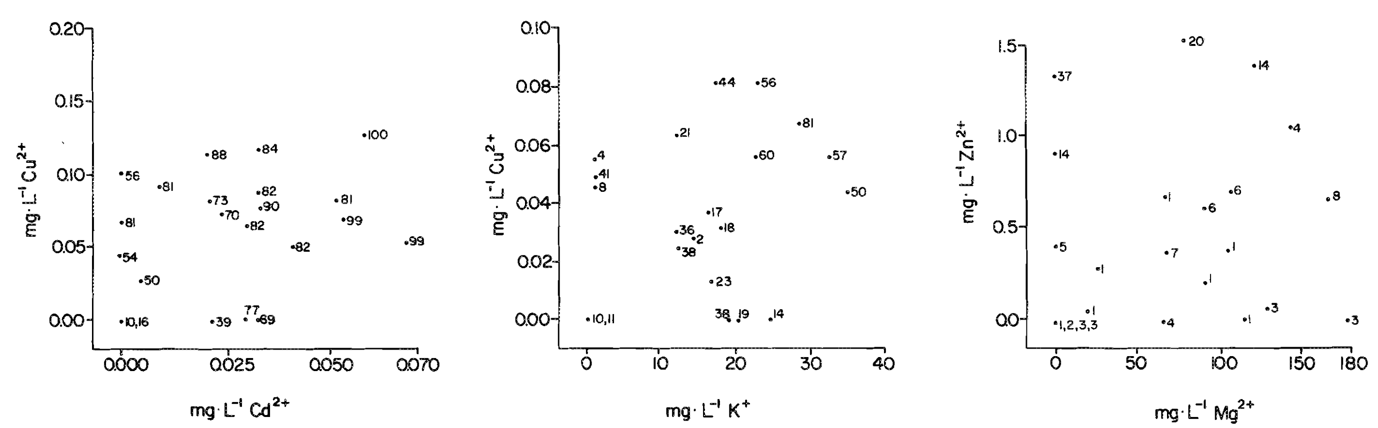


FIG. 1. Results of 3 of 10 experiments done with binary mixtures. The predicted mortality at 96 h, obtained from Equation (2), is plotted against the concentrations of the two toxicants.

TABLE 2. Predictability of mortality response in individual experiments. R^2 for individual experiments were obtained by regressing probit($p48$) and probit($p96$), from Equation (2), against linear and quadratic concentration terms of toxicant concentrations, that is probit(p) = $a \cdot C_1 + b \cdot C_2 + c \cdot C_1^2 + d \cdot C_2^2 + k$. Overall fit significant at $^*P \leq 0.10$ and $^{**}P \leq 0.05$.

Date (d/mo/yr)	Dependent variable	Toxicants	R^2	n	Response range (% mortality)
02/09/83	Probit($p48$)	Cu, K	0.363*	21	0–40
	Probit($p96$)		0.541**		2–81
02/10/83	Probit($p48$)	Cu, Cd	0.755**	22	6–94
	Probit($p96$)		0.824**		6–100
12/10/83	Probit($p48$)	Cd, Zn	0.460**	23	7–60
	Probit($p96$)		0.792**		7–87
22/11/83	Probit($p48$)	Zn, Mg	0.026	24	0–97
	Probit($p96$)		0.672**		1–97
03/01/84	Probit($p48$)	Mg, K	0.706**	21	0–22
	Probit($p96$)		0.682**		5–45
16/01/84	Probit($p48$)	K, Cd	0.281	22	0–16
	Probit($p96$)		0.511**		1–60
25/01/84	Probit($p48$)	Cd, Mg	0.282	23	0–7
	Probit($p96$)		0.337		2–19
03/02/84	Probit($p48$)	Mg, Cu	0.598**	24	0–22
	Probit($p96$)		0.712**		4–43
10/02/84	Probit($p48$)	Cu, Zn	0.460**	24	6–82
	Probit($p96$)		0.887**		21–94
23/02/84	Probit($p48$)	Zn, K	0.873**	23	20–99

TABLE 3. Simple correlation coefficients between probit ($p48$) or probit($p96$), from Equation (2), and concentrations (mg·L^{-1}) of individual toxicants in 10 experiments combined. The number of test cells of a possible 227 is in parentheses. Correlations significant at $^*P \leq 0.10$ and $^{**}P \leq 0.05$.

	Correlation with toxicant				
Dependent variable	Cu	Cd	Zn	K	Mg
Results from all test cells					
Probit($p96$)	0.452**	0.154**	0.255**	0.045	−0.219**
	(227)	(227)	(227)	(227)	(227)
Probit($p48$)	0.224**	−0.033	0.127	0.374**	−0.186**
	(204)	(204)	(204)	(204)	(204)
Test cells with two toxicants excluded					
Probit($p96$)	0.512**	0.521**	0.573**	0.311	0.409**
	(46)	(46)	(42)	(42)	(44)
Probit($p48$)	0.175	0.209	0.064	0.228	0.523**
	(50)	(50)	(49)	(47)	(51)
Test cells with no toxicant also excluded					
Probit($p96$)	0.263	0.339	0.710	0.651	0.285
	(12)	(12)	(8)	(9)	(10)
Probit($p48$)	0.143	0.309	0.760**	−0.581	0.313
	(12)	(12)	(11)	(9)	(12)

surface. Plots of observed versus predicted values in the next section will confirm that the probit transformation alone stabilized variances sufficiently such that the weighting factor was not required.

Linear Models

The fitting of the same 170 probit($p96$) values to simple linear combination of concentrations, that is, to an equation containing only the first six terms of the model

$$\text{(3)} \quad \text{Probit}(p96) = k + a \cdot C_1 + b \cdot C_2 + c \cdot C_3 + d \cdot C_4 + e \cdot C_5 + f \cdot C_1^2 + g \cdot C_2^2 + h \cdot C_3^2 + i \cdot C_4^2 + j \cdot C_5^2 + l \cdot C_1 \cdot C_2 + m \cdot C_1 \cdot C_3 + \ldots \text{all cross-products} \ldots + \epsilon,$$

yielded an R^2 of 0.373. All regression coefficients were significantly larger than zero ($P \leq 0.05$). Fitting the first 11 terms of Equation (3) yielded an R^2 of 0.527. Adding the cross-products increased the R^2 value to 0.709. The increase in the sums of squares accounted for was significant in both cases ($P \leq$

0.05, generalized likelihood ratio test; Graybill 1976). The fitting of all 204 data points, as opposed to the 170 noncontrol responses, yielded an R^2 of 0.700. In all cases, the resultant equations predicted midrange LC50's when compared with the literature. The weighting factor applied to the three linear equations above yielded R^2 values of 0.800, 0.874, and 0.931, respectively. This time, LC50 predictions were reasonable.

Reduced Descriptive Equations

The chosen linear models obviously described experimental results better than the probit model with the same number of terms. To reduce the unnecessary complexity of the fitted Equation (3), terms for which the coefficients which were not significant at the $P \leqslant 0.005$ level were dropped in a stepwise manner until only terms significant at that level remained. The final descriptive equation was

$$(4)\quad \text{Probit}(p96) = 5.731 \cdot \text{Cu} + 22.75 \cdot \text{Cd} + 221.7 \cdot \text{Cd}^2 + 0.5431 \cdot \text{Zn} + 0.02286 \cdot \text{K} - 0.02386 \cdot \text{Mg} + 494.2 \cdot \text{Cu} \cdot \text{Cd} + 101.2 \cdot \text{Cd} \cdot \text{Zn} + 0.01350 \cdot \text{K} \cdot \text{Mg} + 3.783 \qquad R^2 = 0.639,\ n = 204.$$

The mean control response predicted by the intercept is 11.18% = probit^{-1} (3.783), close to the actual control mortality of 12.2%. The 96-h LC50 concentrations for individual toxicants, calculated by sustituting probit(0.5) = 5.0 and by setting the concentrations values of other toxicants to zero, are 0.212 mg $\text{Cu}\cdot\text{L}^{-1}$, 0.0401 mg $\text{Cd}\cdot\text{L}^{-1}$, 2.24 mg $\text{Zn}\cdot\text{L}^{-1}$, and 53.2 mg $\text{K}\cdot\text{L}^{-1}$, respectively. These are midrange values in relation to published values (Clarke 1974). The binary combinations (Cu and Cd) and (Cd and Zn) were predicted to have more-than-additive effects on the probit response. Mg slightly decreased the toxicity of all toxicants except K. From the above equation, it is expected that 1 unit of Mg would reduce the LC50 of Cu by 0.004 unit, of Cd by 0.001 unit, and of Zn by 0.0435 unit. Mg is predicted to increase the toxicity of K. The standard error of the predicted response of the model was 0.621 probit unit, which would be approximately 23% near the 50% response and 20% near the 25 and 75% responses.

Plots of observed versus predicted probit values obtained from Equation (4) (Fig. 2A) suggest that the probit transformation was an appropriate variance-stabilizing one, with the residuals approximately normally distributed about the expected values.

It is possible to partly eliminate the experimental confounding by calculating an intercept for each experiment. This was done by using "Date" or "Experiment" as a class variable and concentrations as covariates in the SAS GLM procedure (SAS Institute Inc. 1982). The statistical analysis of the data is then an analysis of covariance, with the concentrations of the five toxicants as covariates. This analysis was done and, as before, terms not significant at the $P \leqslant 0.005$ level were dropped. The resultant descriptive equation was

$$(5)\quad \text{Probit}(p96) = \text{Correction factor by experiment} + 2.552 \cdot \text{Cu} + 7.910 \cdot \text{Cd} + 0.03501 \cdot \text{K} + 0.8289 \cdot \text{Zn} + 331.1 \cdot \text{Cu} \cdot \text{Cd} + 55.17 \cdot \text{Cd} \cdot \text{Zn} \qquad R^2 = 0.818,\ n = 204.$$

The predicted correction factor ranged from 2.880 = probit(1.7%) for the Zn–Mg experiment to 4.856 = probit(44.3%) for the Cu–Cd experiment. This correction factor is partly due to control mortality and partly to each experimental mean and is therefore not necessarily identical to the observed control mortality. The mean correction factor was 3.8736 = probit(9.4%). Predicted 96-h LC50 concentrations were 0.441 mg $\text{Cu}\cdot\text{L}^{-1}$, 0.126 mg $\text{Cd}\cdot\text{L}^{-1}$, 32.2 mg $\text{K}\cdot\text{L}^{-1}$, and 1.36 mg $\text{Zn}\cdot\text{L}^{-1}$. Mg was predicted to not have an effect. All four experiments with Mg (Table 1) had low control mortalities and low but not highly predictable response ranges which are largely accounted for by the correction factor. The standard error of the predicted response in this model was 0.448 probit unit, which is approximately 17% near the 50% response and 14% near the 25 and 75% responses. Observed versus predicted responses are shown in Fig. 2B and also show an acceptable distribution of error variances.

The response curve describing the 227 probit($p48$) values, obtained in the same manner, was

$$(6)\quad \text{Probit}(p48) = \text{Correction factor for date} + 0.04327 \cdot \text{K} + 0.02224 \cdot \text{Mg} + 329.3\% \text{Cu} \cdot \text{Cd} + 3.591 \cdot \text{Cu} \cdot \text{Zn} \qquad R^2 = 0.754,\ n = 227.$$

The mean correction factor was 3.5616 = probit(7.5%). The predicted 48-h LC50 of K was 33.2 mg $\text{K}\cdot\text{L}^{-1}$, not notably different from the 96-h LC50 of 32.2 mg $\text{K}\cdot\text{L}^{-1}$. This confirmed the visual observation that K exerted its effects quickly or not at all. Mg also had slight toxic effects during this short period of time. The extrapolated 48-h LC50 for Mg was 64.7 mg $\text{Mg}\cdot\text{L}^{-1}$, a value with little credibility. Cu, Cd, and Zn were effective only in the presence of other metal ions, as described by the cross-products. It is reasonable that in this short period of time, some toxicants were less effective, or perhaps not effective at all, unless another was present. The standard error of this model was 0.5394 probit unit, approximately 20% near the 50% response.

The responses at 96 and 48 h are obviously correlated, since both were derived from the same temporal response curves. The correlation between probit($p96$) and probit($p48$) was 0.839. Equation (6) does, however, describe several features expected of a short-term response.

Discussion

The unexplained variance in these data is high and is known to be due to variation in dilution rates. Nevertheless, examination of the literature suggests that the lack of predictability may not be unusual, but may be conspicuous due to the experimental design and data presentation. In experiments performed in a traditional dilutor, the probability of obtaining a smooth monotonic response curve is high, since errors in estimating concentrations in neighboring test cells might be in the same direction. In these experiments the errors in calculating the concentrations were independent of each other in all test cells, increasing the probability of scatter in both directions. Also, the presented experiments were done within small concentration ranges chosen after performing preliminary tests or after examining the results of the last experiment. Correlations between the response and concentrations cannot be expected to be as obvious as in experiments with complete response ranges and toxicants used in geometrically increasing concentrations. The standard errors of the probit response suggest that fiducial limits of all toxicants used would be reasonable considering variation possible in replicate tests (Sprague 1985).

The fact that the linear models had a considerably better predictive ability than the traditional probit equations was surpris-

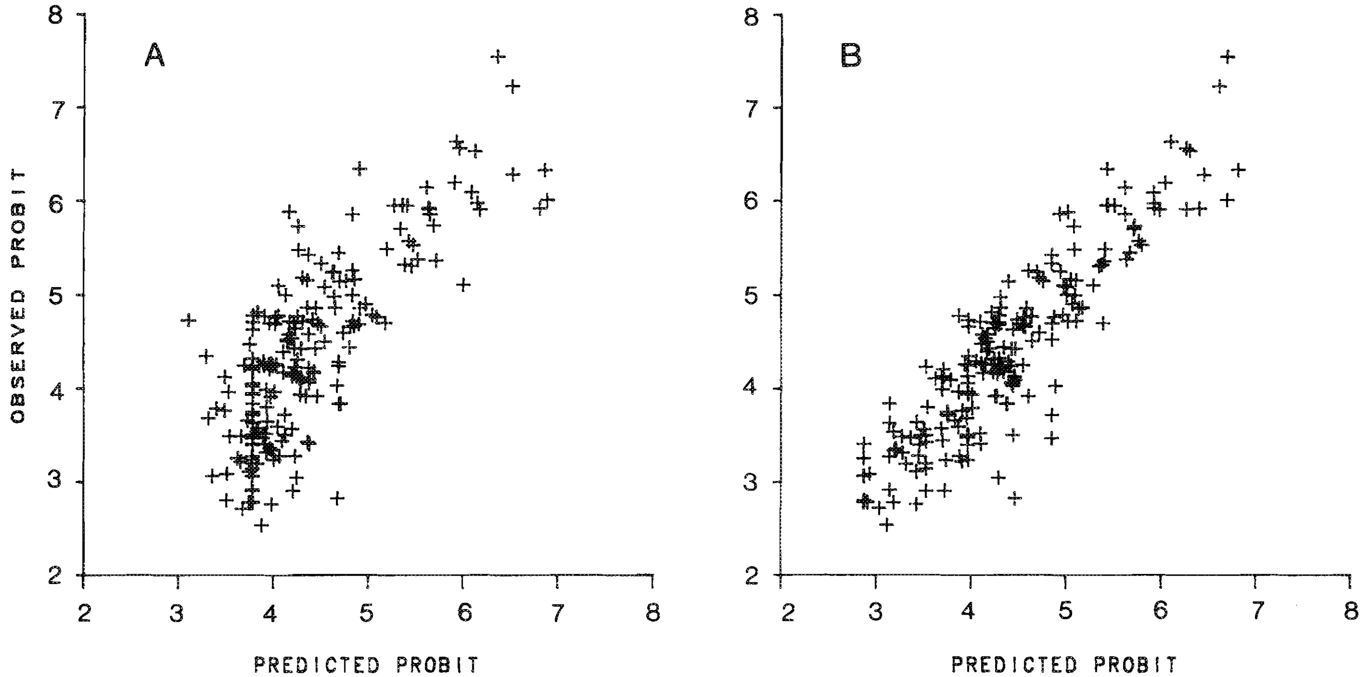


FIG. 2. Plots of observed versus expected probit values for data described by (A) Equation (4) and (B) Equation (5).

ing. The concept of the lognormal response to the linear combination of concentrations has seldom been questioned. The water used for these experiments did not have a strong buffering capacity; it may be that the responses were actually more normally than lognormally distributed over the toxicant concentrations used. Among the linear models, the ones with the correction factors by experiment are most likely the most desirable ones for both theoretical and biological reasons. Control mortalities varied between experiments; thus, a correction is appropriate. The response to each toxicant was still determined from four experiments spread out over time. It can be argued that the classical probit model also has a similar correction factor, since one of the main functions of the coefficients (a_i's in Equation (1) is to describe the asymptotic or control response.

There is an important conceptual difference between the linear and the classical probit models. In the linear model, a known and fixed form of the response, in this case probit(p), is partitioned into additive components. In the classical probit model, the response that is partitioned into additive components is EXP(probit(p)/k) (from Equation (1)), in which k can be determined only after the equation is fitted. The linear model can be easily extrapolated to include more toxicants by simply adding more terms obtained from different experiments; the nonlinear model is not as easily extrapolated, since the values of k and thus the independent variable described by the linear combination might differ between experiments. This difference is extremely important if some generalized method of predicting mixture toxicity based on additive components, such as the toxic units concept, is to be applied (more details later).

The experimental methodology used here was appropriate for testing hypotheses about the effects of mixtures with toxicants at different concentrations and ratios. In most experiments described in the literature, dilutions of only one mixture, with concentrations of toxicants proportional to their LC50's, were tested. Still, the hypotheses assumed to be confirmed were about the effects of toxicants at all possible ratios. In my experiments, only second-order interactions are examined and higher order interactions are confounded. In the usual design, all effects are described as one nth order interaction.

As mentioned previously, the toxicants in these experiments were chosen so that different types of physiological effects would occur, and hopefully, that different types of joint effects would be demonstrated. All three metal cations (Cu^{2+}, Cd^{2+}, and Zn^{2+}) are believed to act similarly at acutely lethal concentrations in that they cause precipitation and damage at the gill surface, ultimately leading to ionoregulatory problems. Cu^{2+} causes few structural changes but still impairs ionoregulation. Zn^{2+} does destroy gill architecture eventually leading to suffocation. Cd^{2+} additionally causes neurological dysfunction. Fish do acclimate to Cd^{2+}, and may acclimate better in the presence of sublethal levels of other metals due to enhanced metallothionein levels (Klaverkamp and Duncan 1987). The low concentrations of K^+ used in these experiments would upset the sodium–potassium balance in the nervous system. It is believed that the divalent ion Mg^{2+} competes with metals at active sites at cell surfaces and lessens toxicity. However, it is toxic in the hundreds of milligrams per litre range (Clarke 1974). LC50 concentrations for these five ions from various studies are given in Clarke (1974) and International Joint Commission (1981).

However, knowledge of physiological modes of action helps little in the interpretation of the results of experiments. There are no logical reasons why the effects of two toxicants acting differently or at different sites should not be described by equations for simple similar action or by equations with additive terms. Nor are there reasons why similar simple action should apply to any two of the above toxicants, since their physiological effects are similar but different. Also, mortality is an integrated and complex response; it does not seem reasonable that two toxicants would differ only in their strength of action. Moreover, although a certain combination of independent variables might describe one form of the response, a different description might be required for different transformations or descriptions of the response. Reporting joint lethal effects of two compounds is as new as describing the effects of a new unknown compound. Very little information is available to place the data in these experiments into perspective.

The classifications of Ashford and Smith (1964) are perhaps the only ones that are general enough to relate expected physiological modes of joint action to the response surfaces that were obtained. These classifications do not describe how a chosen response variable is described by a combination of terms of the independent variables; they describe the instantaneous rate of change of independent variables in relation to each other in the chosen description of the response. Ashford and Smith (1964) believed that there are only two main classifications of the effects of toxicants: interactive and noninteractive. Noninteractive action is demonstrated for two toxicants if the criterion $(d^2R/dC_idC_j)/((dR/dC_i)\cdot(dR/dC_j)) = f(R)$ (a function of the whole response surface equation) applies, or in other words, if the response surface can be expressed as modified response variable described by additive functions of each toxicant, namely as $f1(Y) = f2(C_i) + f3(C_j)$, in which $f1$, $f2$, and $f3$ are any functions. In the given response surfaces, noninteractive effects are described for all possible pairs of toxicants except for Cu and Cd in Equation (4). Equation (4) suggests a strongly enhanced toxic effect in the presence of both of these metals.

Simple similar action for two toxicants, a special case of noninteractive effects, can be demonstrated to occur if the response surface can be factored into the form $Y = f(a\cdot C_i + b\cdot C_j)$ or if dC_i/dC_j = a constant. Several possible cases of simple similar action are described by the given response surfaces. For example, in both Equations (4) and (5), simple similar action applies to Cu and Zn if Cd is held constant. Simple similar action applies to K and Zn and Cu and K, if other ions are held constant, in all three described response surfaces. Examination of the response surfaces show that simple similar action is described many times, often only at constant levels of other toxicants.

Simple similar action is the mode of joint action that has been examined and confirmed most often. It has usually been tested with toxicants expected to act similarly, based on knowledge of their modes of action and on the fact that individual dose–response curves were parallel. Eighteen such cases are listed in de March (1987b), the most well known among these being Lloyd and Jordan (1963, 1964), Herbert and Shurben (1964), Sprague (1964), Brown (1968), Anderson and Weber (1975), Wong et al. (1978), Koenemann (1981), and Hermens and Leenwaugh (1982). The model has been rejected only once by Anderson and Weber (1975) for mixtures of Cu and Zn (but confirmed for Cu and Ni). All reported experiments were done with mixtures of equitoxic toxicant concentrations, and all results were assumed to confirm simple similar action in general. There were other experimental inadequacies: in all cases, fewer than eight mixture levels were tested, and only 4–10 test organisms were used at every experimental value. Thus, fit to expected modes of action was biased by small sample sizes.

The only method of rejecting the hypothesis of simple similar action applying to two toxicants would be to demonstrate that the model with a forced simple linear combination did not describe experimental results adequately. In my case, I would have to show that the forced model probit$(p) = (a\cdot\text{Cu} + b\cdot\text{Cd} + c)^2 + k$ did not describe experimental results adequately. In fact, in the Cu–Cd experiment examined alone, the hypothesis of simple similar action tested by fitting the above equation could not be rejected (model ($R^2 = 0.81$, all coefficients significant). However, the simple linear model with linear and quadratic terms as in Equation (5) had a significantly better fit ($R^2 = 0.90$) (generalized likelihood ratio test, Graybill 1976).

Another appealing mode of joint action not easily distinguished by the linear model is that of independent joint action with completely correlated susceptibilities. This concept, here used in a more generalized sense than defined by Bliss (1934), describes the case in which the effects of a toxicant are dependent on the presence, but not the concentration, of another. This concept might have been appropriate for describing the 48-h response (Equation (6)). Specifically, terms such as $a\cdot C_1\cdot r$, in which r is the correlation in susceptibilities applicable only in the presence of the toxicant 2, might have described the results as well as the cross-product, $b\cdot C_1\cdot C_2$.

Quantifying the degree of additivity, similarity, or interactivity of the effects of two toxicants with indices derived from response surfaces is a possibility arising from these experiments. I have shown (de March 1987a) that any model with a linear combination of terms can be expressed as a tolerance model. The simplest of these, the toxic units model, has appealed to toxicologists since it can be used to predict the levels of toxicants that will jointly produce the tolerance response (Brown 1968; Sprague 1970; International Joint Commission 1981). In a tolerance model a fixed level of a chosen form of the response variable, the tolerance response, for example probit(0.50), is partitioned by linear terms containing critial concentration values such as LC50's, threshhold concentrations, and mixture toxicity indices (as opposed to regression parameters such as slopes and intercepts used in the original linear model). When extrapolating a tolerance model to untested combinations, some of the critical concentration values required are available in the literature, while others can be obtained from experiments with subsets of the toxicants of interest, or be assumed from tests with similar toxicants.

A tolerance model derived from the response surface of the form

$$(7)\quad \text{Probit}(p) = \sum_{i=1,n} a_i\cdot C_i + \sum_{\substack{i=1,n\, j=1,n \\ i\neq j}} b_{ij}\cdot C_i\cdot C_j + k$$

would be

$$1 = \sum_{i=1,n} \frac{M_i}{O_i} + \sum_{\substack{i=1,n\, j=1,n \\ i\neq j}} \left[\text{MTI}_{ij}\cdot \frac{M_i\cdot M_j}{O_i\cdot O_j} \right]$$

in which M_1 to M_n are the concentrations of toxicants 1 to n that will jointly produce the tolerance response p'. In the linear model (Equations (4) to (6)) the form of the response that is partitioned would be (probit(0.50) $- k) = R'$. O_1 to O_n are "objective concentrations," the concentrations of toxicants that when used individually produce the tolerance response. MTI is a mixture toxicity index suggested in de March (1987a). It is one of several that can be calculated from regression coefficients and/or LC50's. The MTI required in the above tolerance model would be calculated as $\text{MTI}_{ij} = (R'\cdot b_{ij})/(a_i\cdot a_j)$, the coefficients obtained from the response surface of the same form as Equation (7). The MTI is zero if the effects are simply additive and is larger when the effects are more-than-additive. When the MTI is -1 and one of the M_i/O_i values is 1, then a special case of less-than-additive effects, specifically independent effects, in which only one toxicant appears to be effective is described (de March 1987a).

The 96-h MTI's, based on Equation (5), and assuming 10% control mortality (probit(0.50) − probit(0.10) = 5 − 3.7183 = 1.2817), would be 21.0 = (1.2817·331.1)/(2.552·7.910) for Cu and Cd and 10.8 = (1.2817·55.17)/(7.910·0.8289) for Cd and Zn. The MTI's would be larger if a smaller control mortality is assumed.

Other mixture toxicity indices have been described, for example those of Colby (1967), Marking and Dawson (1975), Burrell and Corke (1980), and Koenemann (1981). These indices are defined as a combination of LC50's of individual toxicants and component concentrations at the interpolated 50% response from experiments with mixtures of equitoxic concentrations. Again, the indices are then assumed to be indicative of all possible combinations of concentrations which cause 50% response even though they are obtained from dilutions of one mixture.

Koenemann (1981) defined a mixture toxicity index which has the value 1 for simple similar action and zero for independent action. My suggested index takes on the values zero and −1 for the same two modes of action, respectively. The two indices can be roughly compared if mine is scaled by adding 1 and then taking the logarithm to the base 10. In this case my two mixture toxicity indices are 1.34 for Cu and Cd and 1.07 for Cd and Zn, classifying the joint effects as slightly (''supraadditive'' in Koenemann's classification system.

Marking and Dawson (1975) suggested a mixture toxicity index which is zero for additive toxicity and less than or greater than zero for less-than-additive or greater-than-additive toxicity. Rearrangement of his definitions of his index to one possible response surface, using methods similar to those in de March (1987a), shows that their index for more-than-additive toxicity is identical to my proposed index. Their index for less-than-additive toxicity is identical to an index calculated as $(c \cdot R')/(a \cdot b - c \cdot R')$ from Equation (7).

In general, my work has shown that resolving problems in the logic of describing joint effects of chemicals in mixtures may be more important than generating new data using dubious established methods. Practices such as the use of mixtures of toxicants at equitoxic concentrations to make generalizations about untested concentration ratios can easily be argued to be scientifically invalid. Similarly, the practice of defining mixture toxicity indices to summarize the results of a particular type of confounded experiment can be questioned. Confusing the many available definitions for ''joint'' or ''interactive'' effects, but derived from different models, from different possibilities within models, from relationships between either independent or dependent variables, or between specific or undefined response variables, seems to be characteristic of the field. Even if my experiments had smaller experimental errors, the choice of a multivariate model to describe data still would have been a serious decision which would not have been highly dependent on experimental results. The description of the degree of ''interactivity'' or ''nonadditivity,'' or classifications such as simple similar action, would still be model dependent and for that reason alone would not necessarily concur with other work that had been done.

Even if a consistent system of models, experimental designs, and definitions of summary statistics appropriate for mixture effects is eventually accepted, the methods of application of such information to real contamination problems will still be of interest. For example, it will be interesting to observe whether the problems in bodies of waters polluted from many sources, such as the Great Lakes, will eventually be solved by referring to quantitative models for the effects of mixtures, and by what methods these models will acquire scientific defensibility.

Acknowledgements

Many thanks to Dr. T. Dandy and Dr. L. Lockhart who made this study possible.

References

ANDERSON, P. D., AND L. J. WEBER. 1975. The toxicity to aquatic populations of mixtures containing certain heavy metals, p. 933–954. *In* International conference on heavy metals in the environment. Vol. 2. Part. 2. Toronto, Ont.

ASHFORD, J. R., AND C. S. SMITH. 1964. General model for quantal response to the joint action of mixtures of drugs. Biometrika 51: 413–428.

1965. An alternative system for the classification of mathematical models for quantal responses to mixtures of drugs in biological assay. Biometrics 21: 181–188.

BLISS, C. I. 1934. The method of probits. Science (Wash., DC) 79(2037): 38–39.

1939. The toxicity of poisons applied jointly. Ann. Appl. Biol. 26: 585–615.

BROWN, V. M. 1968. The calculation of the acute toxicity of mixtures of poisons to rainbow trout. Water Res. 2: 723–733.

BURRELL, R. E., AND C. T. CORKE. 1980. Interactions of the solvent acetone with the fungicides benomyl and captan in fungal assays. Toxicology 25: 554–561.

CLARKE, R. MCV. 1974. The effects of effluents from metal mines on aquatic ecosystems in Canada. A literature review. Fish. Mar. Serv. Dev. Tech. Rep. 488: 150 p.

COLBY, S. R. 1967. Calculating synergistic and antagonistic responses of herbicide concentrations. Weed Sci. 15: 20–22.

COLE, J. W. L., AND J. E. GRIZZLE. 1966. Applications of multivariate analysis of variance to repeated measures experiments. Biometrics 22: 810–828.

DE MARCH, B. G. E. 1982. Decreased daylength and light intensity as factors inducing reproduction in *Gammarus lacustris lacustris* Sars. Can. J. Zool. 60: 2962–2965.

1987a. Mixture toxicity indices in acute lethal toxicity tests. Arch. Environ. Contam. Toxicol. 16: 33–37.

1987b. Simple similar action and independent joint action — two very similar models for the joint effects of toxicants applied as mixtures. Aquat. Toxicol. 9: 291–304.

EIFAC (EUROPEAN INLAND FISHERIES ADVISORY COMMISSION). 1980. Report on combined effects on freshwater fish and other aquatic life of mixtures of toxicants in water. EIFAC (Eur. Inland Fish. Adv. Comm.) Tech. Pap. 37: 49 p.

1987. Revised report on combined effects of freshwater fish and other aquatic life of mixtures of toxicants in water. EIFAC/T (Eur. Inland Fish. Adv. Comm.) Tech. Pap. 37/T: 75 p.

FINNEY, D. J. 1971. Probit analysis. 3rd ed. Cambridge University Press, New York, NY. 333 p.

GRAYBILL, F. A. 1976. Theory and application of the linear model. Duxbury Press, North Scituat, MA. 704 p.

HERBERT, D. W. M., AND D. S. SHURBEN. 1964. The toxicity of fish to mixtures of poisons. I. Salts of ammonia and zinc. Ann. Appl. Biol. 53: 33–41.

HERMENS, J., AND P. LEENWAUGH. 1982. Joint toxicity of mixtures of 8 and 24 chemicals to the guppy *Poecilia reticulata* Ecotoxicol. Environ. Safety 6: 302–310.

HEWLETT, P. S., AND R. L. PLACKETT. 1952. Similar joint action of insecticides. Nature (Lond.) 169: 198–199.

1959. A unified theory for quantal responses to mixtures of drugs: non-interactive action. Biometrics 15: 591–610.

1964. A unified theory of quantal responses to mixtures of drugs: competitive action. Biometrics 20: 566–575.

HUBERT, J. J. 1984. Bioassay. 2nd ed. Kendall/Hunt Publishing Company, Dubuque, IA. 186 p.

INTERNATIONAL JOINT COMMISSION. 1981. Report of the Aquatic Ecosystems Objectives Committee. International Joint Commission, Windsor, Ont. 48 p.

KENAGA, E. E. 1982. Predictability of chronic toxicity from acute toxicity of chemicals in fish and aquatic invertebrates. Environ. Toxicol. Chem. 1: 347–358.

KLAVERKAMP, J. F., AND D. A. DUNCAN. 1987. Acclimation to cadmium toxicity by white suckers: cadmium binding capacity and metal distribution in gill and liver cytosol. Environ. Toxicol. Chem. 6: 275–289.

KOENEMANN, H. 1981. Fish toxicity tests with mixtures of more than two chemicals: a proposal for a quantitative approach and experimental results. Toxicology 19: 229–238.

LLOYD, R., AND D. H. M. JORDAN. 1963. Predicted and observed toxicities of several sewage effluents to rainbow trout. J. Proc. Inst. Sewage Purif. 1: 167–173.

1964. Predicted and observed toxicities of several sewage effluents to rainbow trout: a further study. J. Proc. Inst. Sewage Purif. 2: 183–186.

MARKING, L. L., AND V. K. DAWSON. 1975. Methods for assessment of toxicity or efficacy of mixtures of chemicals. U.S. Fish Wildl. Serv. Invest. Fish Control 67: 1–8.

PLACKETT, R. L., AND P. S. HEWLETT. 1952. Quantal responses to mixtures of poisons. J. R. Stat. Soc. B 14: 141–154.

1963. A unified theory for quantal responses to mixtures of drugs: the fitting to data of certain models for two non-interactive drugs with complete positive correlation of tolerances. Biometrics 19: 519–531.

1967. A comparison of two approaches to the construction of models for quantal responses to mixtures of drugs. Biometrics 23: 27–44.

SAS INSTITUTE INC. 1982. SAS user's guide: statistics. 1982 ed. SAS Institute Inc, Cary, NC. 584 p.

SPRAGUE, J. B. 1964. Lethal concentrations of copper and zinc for young Atlantic salmon. J. Fish. Res. Board Can. 21: 17–26.

1970. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. Water Res. 4: 3–32.

1985. Factors that modify toxicity, p. 124–163. *In* G. M. Rand and S. R. Petrocelli [ed.] Fundamentals in aquatic toxicology. Hemisphere Publishing Corporation, New York, NY.

STICH, H. F., H. W. LEUNG, AND J. R. ROBERTS [ED.] 1982. Workshop on the combined effects of xenobiotics. National Research Council of Canada Publ. No. NRCC/CNRC 18978, Ottawa, Ont. 254 p.

VOUK, V. B., G. C. BUTLER, A. C. UPTON, D. V. PARKE, AND S. C. ASHER [ED.] 1987. Methods for assessing the effects of mixtures of chemicals. John Wiley & Sons, New York, NY. 750 p.

WONG, P. T. S., Y. K. CHAU, AND P. L. LUXON. 1978. Toxicity of mixtures of metals on freshwater algae. J. Fish. Res. Board Can. 35: 479–481.

DETAILED STUDY OF IRRIGATION DRAINAGE IN AND NEAR WILDLIFE MANAGEMENT AREAS, WEST-CENTRAL NEVADA, 1987-90

Part B. Effect on Biota in Stillwater and Fernley Wildlife Management Areas and Other Nearby Wetlands

Robert J. Hallock *and* Linda L. Hallock, *Editors*
U.S. Fish and Wildlife Service

U.S. GEOLOGICAL SURVEY

Water-Resources Investigations Report 92-4024B

U.S. GEOLOGICAL SURVEY,
U.S. FISH AND WILDLIFE SERVICE,
U.S. BUREAU OF RECLAMATION, and
U.S. BUREAU OF INDIAN AFFAIRS

Carson City, Nevada
1993

U.S. DEPARTMENT OF THE INTERIOR
BRUCE BABBITT, Secretary

U.S. GEOLOGICAL SURVEY
DALLAS L. PECK, Director

For additional information write to:

District Chief
U.S. Geological Survey
333 West Nye Lane, Room 203
Carson City, NV 89706-0866

Copies of this report can be purchased from:

U.S. Geological Survey
Earth Science Information Center
Open-File Reports Section
Box 25286, MS 517
Denver Federal Center
Denver, CO 80225-0046

CONTENTS

Page

Figures

Tables

CONVERSION FACTORS, VERTICAL DATUM, DEFINITION, AND ABBREVIATED WATER-QUALITY UNITS

Multiply	By	To obtain
acre	4,047	square meter
acre-foot (acre-ft)	1,233	cubic meter
acre-foot per acre (acre-ft/acre)	0.3048	cubic meter per square meter
acre-foot per acre per year (acre-ft/acre/yr)	0.3048	cubic meter per square meter per year
acre-foot per year (acre-ft/yr)	0.001233	cubic hectometer per year
foot (ft)	0.3048	meter
foot per year (ft/yr)	0.3048	meter per year
gallon (gal)	3.785	liter
inch (in.)	25.4	millimeter
mile (mi)	1.609	kilometer
ounce (oz)	0.02957	liter
square foot per second (ft^2/s)	0.9072	square meter per second
square mile (mi^2)	2.59	square kilometer
ton per year (ton/yr)	0.9072	megagram per year
yard	0.9144	meter

Temperature: Degrees Celsius (°C) can be converted to degrees Fahrenheit (°F) by using the formula °F = [1.8(°C)]+32.

Sea level: In this report, "sea level" refers to the National Geodetic Vertical Datum of 1929 (NGVD of 1929, formerly called "Sea-Level Datum of 1929"), which is derived from a general adjustment of the first-order leveling networks of the United States and Canada.

Definition: The term "water year" refers to the 12-month period October 1 through September 30, during which a complete annual hydrologic cycle normally occurs. The water year is designated by the calendar year in which it ends. Thus, the year ending September 30, 1988, is called the "1988 water year."

Abbreviated water-quality units used in this report:

L (liter)
µg/g (microgram per gram)
mL (milliliter)
mg/L (milligram per liter)
ppt (part per thousand)
NTU (Nephelometric turbidity unit)
mg/kg (milligram per kilogram)
µm (micrometer)
µg/L (microgram per liter)
g (gram)
µS/cm (microsiemen per centimeter at 25°C)
g/L (gram per liter)
kg (kilogram)

DETAILED STUDY OF IRRIGATION DRAINAGE IN AND NEAR WILDLIFE MANAGEMENT AREAS, WEST-CENTRAL NEVADA, 1987-90

Part B. Effect on Biota in Stillwater and Fernley Wildlife Management Areas and Other Nearby Wetlands

Robert J. Hallock *and* Linda L. Hallock, *Editors*

Abstract

A water-quality reconnaissance investigation during 1986-87 found high concentrations of several potentially toxic elements in water, bottom sediment, and biota in and near Stillwater Wildlife Management Area (WMA). These results prompted the U.S. Department of the Interior to initiate a more detailed study in 1988 to determine the hydrogeochemical processes that control water quality in the Stillwater WMA and other nearby wetlands, and the resulting effects on biota, especially migratory birds.

The average historical size of the natural wetlands at Carson Lake and Stillwater Marsh, the water quantity, and the average dissolved-solids concentration and load in the water in these wetlands were estimated. Present wetland size is about 10 percent of historical size; the dissolved-solids load in these now-isolated wetlands has increased only moderately, but the concentration has increased more than seven-fold. Wetland vegetation has diminished and species composition has shifted to predominantly salt-tolerant species in many areas. Decreased vegetative cover for nesting is implicated in declining waterfowl production. Decreases in numbers or virtual absence of several wildlife species are attributed to degraded water quality.

Toxicity tests established that water in some drains and wetland areas was acutely toxic to some fish and invertebrates. Toxicity is attributed to the combined presence of arsenic, boron, lithium, and molybdenum. Rapid fluctuations in specific conductance and atypical ionic composition, which may increase acute toxicity, were observed in some drainwater. A strong relation was found between trace elements and both daily and average specific conductance.

Biological pathways are involved in the transport of mercury and selenium. Concentrations of selenium and mercury in drainwater were very low to below analytical reporting limits, but these elements had bioaccumulated in plants, and selenium had biomagnified in one trophic level (invertebrates) up to 10,000-fold. Selenium and mercury accumulated in plants, detritus, and invertebrates had been transported through irrigation drains to large wetland areas frequented by waterfowl and other wildlife, but no evidence of expected long-term selenium build-up since development was found in the wetlands. Several source areas of selenium and mercury were identified.

Hatch success of both artificially incubated and field-reared duck eggs was 90 percent or greater; no teratogenesis was observed. Boron and selenium concentrations in eggs were generally low—all below adverse effect levels. Mercury concentrations were also low except in eggs from Stillwater WMA, where about 30 percent of the eggs contained concentrations above the adverse effect level. Boron, mercury, and selenium concentrations in pre-flight juvenile ducks ranged from insignificant to levels associated with impaired survival. Boron concentrations were at or below effect levels in various duck species, but above effect level in coots (*Fulica americana*). Mercury concentrations in birds from Stillwater WMA, Carson Lake, and Carson Valley were above effect levels. Mean concentrations of selenium ranged from 30 to 77 micrograms per gram (µg/g) in birds from Fernley WMA and Massie and Mahala Sloughs, all above effect level. Survival of juvenile birds that had accumulated selenium, however, was not reduced. Field nest success was 26 percent and estimated overall production was about 2,400 ducklings from 6,800 breeding pairs. This poor production was attributed primarily to drought conditions and dissolved-solids accumulation that caused vegetative loss and exposed the nests to predators.

Maximum concentrations of mercury in muscle and liver tissue of waterfowl harvested from Carson Lake and Fernley WMA were 15.5 and 38.9 µg/g, wet weight, respectively, which exceeded the established human health criterion of 1.0 µg/g. Selenium concentrations in livers of juvenile waterfowl from Fernley WMA and Massie Slough were four times the established criterion for human health.

INTRODUCTION

In the last several years, concern has been increasing about the quality of irrigation drainage, and its potential adverse effects on human health, fish, and wildlife. Recent studies at several National Wildlife Refuges and Wildlife Management Areas (WMA) throughout the western United States have identified elevated concentrations of selenium and other trace elements that are a potential threat to biota within the management areas (Knapton and others, 1988; Lambing and others, 1988; Peterson and others, 1988; Radtke and others, 1988; Schroeder and others, 1988; Stephens and others, 1988; Wells and others, 1988; Hoffman and others, 1990; and Setmire and others, 1990). In 1988, the U.S. Department of the Interior (DOI) directed the U.S. Geological Survey, U.S. Fish and Wildlife Service, U.S. Bureau of Reclamation, and the U.S. Bureau of Indian Affairs to implement detailed studies that would provide information useful for mitigating the negative effects of irrigation-drainage water. The detailed studies were to provide information on (A) toxic constituents, (B) their effects on biota, and (C) a summary of significant findings.

The Stillwater WMA (and other nearby wetlands) in west-central Nevada (fig. 1) is one of several areas in the western United States selected for detailed examination of the mobilization, transport, and fate of potentially toxic constituents involving the biotic and abiotic environment.

Background

The changes that have occurred in the wetlands associated with the Newlands Irrigation Project in the Carson Desert are widely recognized and are partly summarized in a report by the U.S. Department of the Interior (1988). An overview of the increment of change that may be ascribed to irrigation drainage was needed to place the related DOI irrigation-drainage studies in perspective.

Prior to these investigations, few studies had documented the effects of the toxic components of irrigation drainage on biota in and near Stillwater WMA. The first comprehensive study of irrigation-induced water-quality problems was made during 1986-87 by Hoffman and others (1990). Henny and Herron (1989) studied irrigation-drainage-related contaminants in white-faced ibis (*Plegadis chihi*) at Carson Lake.

Several contaminants found in irrigation drainage are known to directly affect fish and invertebrates, and thus indirectly, birds; both fish and invertebrates are important in the diets of many migratory birds at Stillwater WMA. Aquatic invertebrates are a frequent food source for birds during reproduction because ingested invertebrates provide high energy food for rapid growth of young birds in preparation for migration. Salinity is often high at Stillwater WMA, and both water and biota contain elevated levels of

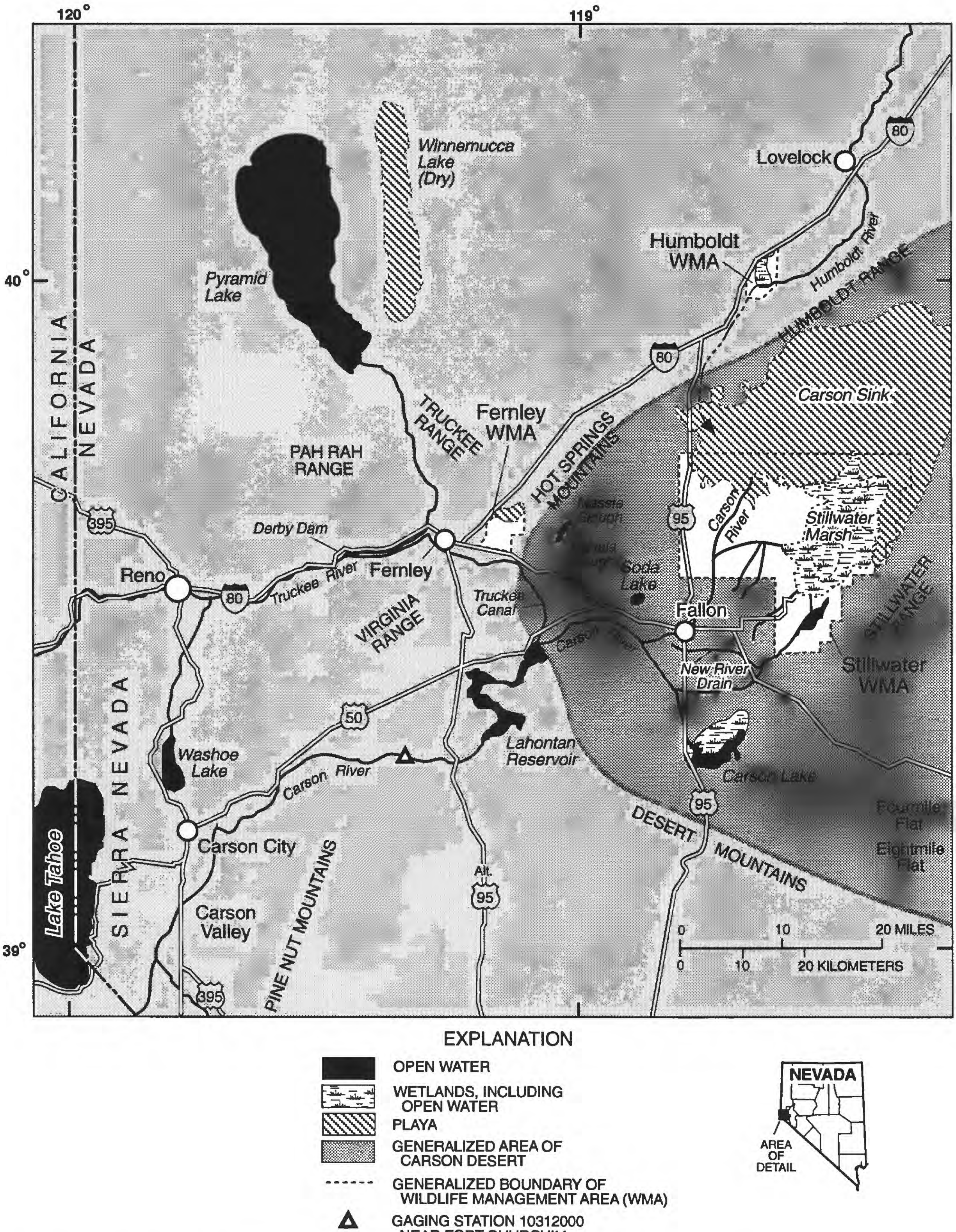


Figure 1. General physiographic features in the study area and western Nevada (Modified from Lico, 1992).

potentially toxic trace elements (Hoffman and others, 1990, p. 76-77). At Stillwater WMA, Largemouth bass (*Micropterus salmoides*), which once supported a popular sport fishery, are gone and the diverse fish forage base supporting American white pelicans (*Pelecanus erythrorhynchos*) is greatly reduced (U.S Fish and Wildlife Service, 1988, p. 132).

Some water-quality components associated with irrigation drainage may directly affect migratory birds. Both selenium and mercury have bioaccumulated and biomagnified in migratory bird tissues from the study area to the extent that reproduction may fail (Hoffman and others, 1990, p. 60, 67, 72, 77; Eisler, 1985, p. 38). High concentrations of selenium were found in juvenile migratory birds confined to wetlands that commonly contained less than the analytical reporting limit (<1.0 µg/L) of dissolved selenium concentrations in water. In these same wetlands, selenium concentrations in sediment (≤ 1.2 µg/g) were well below the level of concern (4.0 µg/g) recommended by Lemly and Smith (1987, p. 9). Selenium was found in shallow ground water affected by irrigated agriculture in and near the headwaters of TJ Drain (U.S. Bureau of Reclamation, 1987, p. B14). Most of the mercury in the study area originated from 19th century mining and milling practices in the middle Carson River basin (Smith, 1943, p. 247). The distribution of mercury throughout the study area is associated with floodways and channels of the Carson River that existed before construction of the Newlands Irrigation Project. Low concentrations of mercury were found in filtered sample water from the study area. The highest concentration of mercury in water, reported by Hoffman and others (1990, p. 36), was 1.1 µg/L in Lead Lake in Stillwater WMA.

Reproductive life phases are typically sensitive and vulnerable to the effects of contaminants. Trace-element toxicity is a possible contributing factor to the steady decline in waterfowl production in wetlands maintained by drainage from the Newlands Irrigation Project (U.S. Department of the Interior, 1988, p. E-7-E-10). In addition to the white-faced ibis found to be accumulating selenium and mercury in the breeding grounds at Carson Lake (Henny and Herron, 1989, p. 1032), deformed ibis chicks have been observed at Stillwater WMA (U.S. Fish and Wildlife Service, Fallon, Nev., unpublished data, 1987). Dead and dying waterfowl found in Carson Sink and in various parts of this study area (Stillwater WMA, Humboldt WMA, and Carson Lake) had selenium levels in liver tissue sufficiently high to cause toxicosis (> 30 µg/g), although necropsy reports did not identify selenium toxicosis as the immediate cause of death (Rowe and Hoffman, 1990, p. 39; Hoffman and others, 1990, p. 74). The primary effect of excessive dietary selenium on mallards (*Anas platyrhynchos*) is reproductive—reduced reproductive efforts, hatch rates, and duckling survival (Lemly and Smith, 1987, p. 5). Chronic sublethal mercury concentrations are also related to reduced reproductive success (Heinz, 1979, p. 398; Finley and Stendell, 1978, p. 54). Laboratory studies of boron as a dietary supplement showed reduced hatch rates, hatch weights, growth rates, and duckling survival (Smith and Anders, 1989, p. 943). Selenium, mercury, and boron were found in migratory birds, food-chain organisms, and sediment in the study area during the reconnaissance study by Hoffman and others (1990, p. 58, 62).

Wildlife from study-area wetlands have been used as food by humans for more than 4,000 years (Kelly, 1988, p. 11). Although the wetlands no longer support a popular fishery, harvest and consumption of waterfowl continues. When potential toxic elements, such as mercury and selenium, accumulate in waterfowl tissues, a concentration may be reached at which human consumption is inadvisable. During 1986-87, Hoffman and others (1990, p. 77) found indications that juvenile migratory birds, including waterfowl, in and near Stillwater WMA and Carson Lake were accumulating mercury and selenium in liver and muscle tissue. Some of the concentrations exceeded established criteria for human health. Cooper and others (1985, p. 56) identified a possible threat to human health from mercury in fish fillets taken from various wetlands in the Newlands Irrigation Project area, including Stillwater WMA.

Purpose and Scope

This report presents findings of the five detailed study elements that evaluate general and specific effects of irrigation drainage on biota and mechanisms or linkages related to these effects in wetlands in and near Stillwater WMA. The primary purpose in studying these wetlands was to assist remediation efforts in support of migratory waterfowl, a Federal trust responsibility; the common theme of these five elements is the possible adverse effects of irrigation drainage on migratory waterfowl. The five study elements, in order of presentation in this report, examine the historical conditions of the wetlands compared to the present, determine which potentially toxic trace elements are present in the area, explore pathways by which the trace elements move through the wetlands, determine the effects of the contaminants on waterfowl production, and consider human-health implications.

Because of the length and scope of this report, those primarily interested in the collective findings of all the studies may wish to pass the detailed sections and proceed directly to the summary section at the back of this report. The more technically oriented reader may wish to examine the individual sections for more detail.

This investigation was made and the report authored by U.S. Fish and Wildlife Service scientists. The report is published by the U.S. Geological Survey to maintain continuity with the other detailed-study reports for the area (Rowe and others, 1991; Lico, 1992; and Hoffman, in press). While the period of study was 1988-90, some biological data collected in 1987 were used to assess the concentrations of mercury and selenium in waterfowl.

Study Area

The Carson Desert hydrographic area (Rush, 1968, pl. 1) occupies a mostly flat area of about 2,020 mi^2 in the lower Carson River drainage basin. The area is about 70 mi east of Reno, and is one of the largest basin-fill valleys in northern Nevada. The Carson Desert includes natural wetlands at Carson Lake and—within the artificial boundaries of Stillwater WMA—Stillwater Marsh and the southern part of the Carson Sink (fig. 1). The Carson Sink is a nearly barren, flat, salt-encrusted playa occupying an area of about 400 mi^2 on the northern boundary of Stillwater WMA. In abnormally high precipitation years, wetlands may emerge in the Sink, supported by flow of the Carson River and, on occasion, overflow from the Humboldt River.

Historically, these wetlands were supported primarily by the Carson River, but since completion of the Newlands Irrigation Project, additional water has been imported from the Truckee River drainage basin to the Carson River by way of the Truckee Canal. The Carson River is a major tributary draining the eastern slopes of the central Sierra Nevada. As with most rivers associated with the Great Basin, its annual discharge patterns are highly variable.

Biological samples were collected from additional wildlife areas because of their proximity to Stillwater WMA, and because they are maintained by a combination of ground water and drainage from DOI irrigation projects. These other areas include the largely Project-created wetlands at Fernley WMA near Fernley, Nev., about 30 mi west of Stillwater WMA; Massie and Mahala Sloughs, about 25 mi west of Stillwater WMA; and the natural wetlands at Humboldt WMA (fig. 1).

Summary descriptions of climate, geology, soils, water use, hydrologic setting, wetland areas, and wildlife use were reported by Hoffman and others (1990) and Lico (1992).

Acknowledgments

The authors of this report gratefully acknowledge the following individuals who provided valuable assistance during the course of these investigations: Ron Crunkilton, Missouri Department of Conservation, for providing bluegills for the study; Rod Parrish, U.S. Environmental Protection Agency Laboratory, Gulf Breeze, Fla., for providing saltwater species; and Eugene Greer and Glenn Wylie, National Fisheries Contaminant Research Center, Columbia, Mo., for providing fathead minnow larvae and daphnids, respectively. The maintenance staff at the Stillwater National Wildlife Refuge provided valuable assistance in setting up the mobile toxicity testing facility. Mark Kaiser assisted in the analysis of data and provided valuable suggestions on parts of this report.

The following people also assisted in the study by providing data, information, or technical reviews of parts of this report: Roy Leach and Norman A. Saake, Nevada Department of Wildlife; James J. Cooper, Nevada Division of Environmental Protection; Theodore G. Roefs and Thomas Strekal, U.S. Bureau of Reclamation; Ronald M. Anglin, Timothy Bowman, C. Clifford Creger, Gerald Deutscher, Leslie Dubec, Eugene E. Duffney, Charles J. Henny, Earnest Lantos, Delven D. Lee, A. Dennis Lemly, Kenneth Merritt, Craig Stockwell, and Steven P. Thompson, all with the U.S. Fish and Wildlife Service; and Lyman F. McConnell and Willis Hyde of the Truckee-Carson Irrigation District.

And, finally, we thank Ray J. Hoffman, Study Team Leader, and Carol A. Myers, Technical Editor, of the U.S. Geological Survey for their substantial efforts in making this a more concise and cohesive document.

(p. 7 follows)

ESTIMATED HISTORICAL CONDITIONS OF THE LOWER CARSON RIVER WETLANDS

To evaluate the effect of irrigation drainage on the wetlands of the lower Carson River, Carson Lake, Stillwater Marsh, and associated intermittent wetlands in the Carson Sink (fig. 1), an estimate was needed of the conditions prevailing before the onset of irrigation and development. These estimated historical wetland conditions were then compared to present and projected wetland conditions. To do this, it was necessary to distinguish between effects resulting from irrigation and those resulting from wetland management practices. Climatic changes, extreme variability of the streamflow of the Carson River, the cyclic nature of this variation, and the presence of lands irrigated with Carson River water upstream of the Newlands Irrigation Project are important considerations for the determination of historical conditions.

The historical flow, seasonal variation, average wetland size, and water quality were estimated on the basis of extrapolations from data in existing records, reports by early explorers, and archaeological findings. Early reports and evidence from archaeological sites were used to estimate the types and abundance of vegetation and wildlife historically present. Present conditions were determined from data collected for this study and from other recent State and Federal reports and data, particularly data analysis compiled in April 1988 by the U.S. Fish and Wildlife Service at Stillwater Wildlife Management Area for OCAP (Operating Criteria and Procedures) of the Newlands Project (U.S. Fish and Wildlife Service, 1988), which are summarized in Appendix E of the Final Record of Decision (U.S. Department of the Interior, 1988).

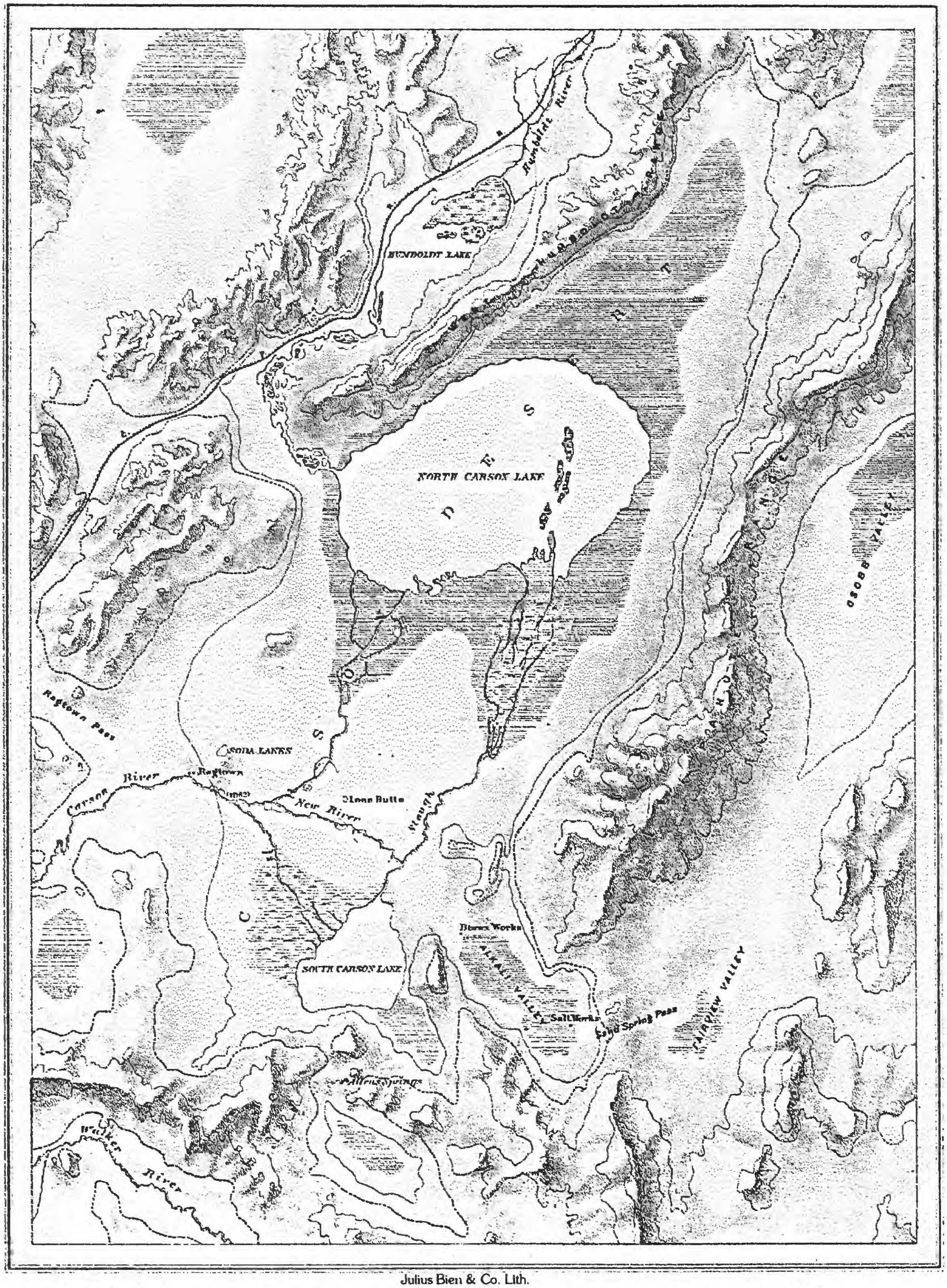
ESTIMATED HISTORICAL CARSON RIVER FLOW AND WETLAND SIZES

Commonly, the flow path of the unregulated Carson River was to Carson Lake, out through Stillwater Slough into Stillwater Marsh, then terminated in Carson Sink, as discussed by Morrison (1964, p. 104) and Russell (1885, p. 44-45) and shown in figure 2. That flow pattern will be followed in this discussion, with alternating consideration of inflow quantities, wetland sizes, and probable water losses along the downgradient flow path.

Unregulated Flow of the Carson River

Systematic records of streamflow and water quality of the Carson River and associated wetlands have been kept only during this century. Data extrapolated from the existing streamflow records were used to estimate a representative annual average[1] volume of water inflow to the wetlands before the initiation of irrigation (pre-1860) in the Carson Desert. This predevelopment inflow was then used to estimate an average wetland acreage that would have been maintained primarily by inflow of the Carson River. Because the annual streamflow of the Carson River is highly variable, the extrapolation included the following information, estimates, and assumptions:

[1]The term "average" is used here to indicate a representative situation—sometimes stable, sometimes subject to extreme fluctuation; it does not mean a mathematical average or a usual or "normal" situation.



Julius Bien & Co. Lith.

Lahontan Beach

Playas

CARSON DESERT, NEVADA.

Scale of Miles

5 4 3 2 1 0 5 10 15 20

Interval between Contours 1000 feet.

Figure 2. Reproduction of early map showing configuration of the lower Carson River basin before development (1880's). Note differences in name designations: in particular, "North Carson Lake," which is now part of Stillwater Marsh and Carson Sink, and "South Carson Lake," now designated simply Carson Lake. (Illustration was published in 1885 as Plate VII by I.C. Russell in U.S. Geological Survey Monograph 11, a report on ancient Lake Lahontan.)

(1) The average annual streamflow of the Carson River near Fort Churchill (U.S. Geological Survey station 10312000, approximately 50 river miles upstream from Carson Lake; fig. 1) was about 270,000 acre-ft (rounded) for the period of record, 1911-88 (Pupacko and others, 1989, p. 117).

(2) The average annual streamflow of the Carson River to Carson Lake and the connected Stillwater Marsh before agricultural development (pre-1860) was estimated to be equal to the present average annual streamflow near Fort Churchill **plus** the average annual flow of all water now consumed by agriculture upstream of that point. Other historical water losses, primarily seepage and evapotranspiration from the formerly more extensive wetlands associated with the upper river, were probably equal to other modern losses. These losses include seepage and evapotranspiration from reservoirs and canals, municipal uses, and agricultural uses in excess of the Alpine Decree[1] (Garry Stone, Federal Water Master, oral communication, 1990).

[1]The Alpine Decree is the adjudication of the Carson River water rights (California Department of Water Resources, 1991).

(3) About 56,000 acres are irrigated along the Carson River above Fort Churchill, with an annual evapotranspiration rate of 2.5 acre-ft/acre, as calculated from the Alpine Decree (California Department of Water Resources, 1991, p. 126), which results in an estimated 140,000 acre-ft of water being diverted from the Carson River and consumed annually. This amount is in agreement with the findings of Brown and others (1986, p. 30) that about 137,000 acre-ft of water may be consumed annually from the area above Lahontan Reservoir.

Thus, adding the estimate of agricultural consumptive use (about 140,000 acre-ft) to the Carson River discharge at Fort Churchill (about 270,000 acre-ft), the historical, unregulated annual average discharge of the Carson River at Fort Churchill was approximately 410,000 acre-ft. Based on the 1911-1988 period of record, the flow ranged from about 90,000 acre-ft/yr (sum of Carson River inflows above Fort Churchill [U.S. Geological Survey, 1978]) to about 940,000 acre-ft/yr (800,000, rounded [Frisbie and others, 1984, p. 127], plus 140,000). Seasonal variations in Carson River flows for the period of record (1919-1969) are discussed by Glancy and Katzer (1976, p. 34-42) and shown for the Fort Churchill station in figure 3 of this report.

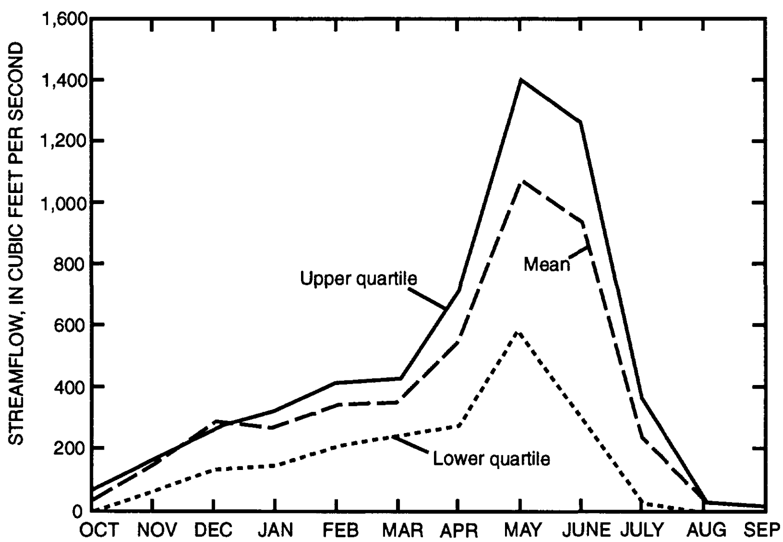


Figure 3. Mean monthly flow and quartile statistics, Carson River gage near Fort Churchill, USGS station 10312000, water years 1919-69. Modified from Glancy and Katzer (1976, p. 35).

Average Historical Size of Carson Lake

The areal extent of wetlands at Carson Lake when maintained by the entire average annual flow of the Carson River was dependant primarily on the annual evapotranspiration rates, wetland morphology, and magnitude of seasonal inflow. Natural variation in the course of the river where it entered the Carson Desert is discussed by Morrison (1964, p. 104). During recent geologic time, the Carson River usually flowed southeastward toward Carson Lake. As observed by Russell (1885, p. 44-45) in the mid 1800's, the Carson River also flowed northward to Carson Sink (North Carson Lake in fig. 2). The frequency of such flow-path changes over geologic time is unknown.

From his camp on Stillwater Slough in 1859, Simpson (1876, p. 85) observed a distinct line of cottonwood trees along the Carson River where it entered Carson Lake. E.M. Kern, a topographer with the Fremont Expedition of 1845, also reported seeing trees, probably cottonwoods, at the mouth of the Carson River from across the lake (Spence and Jackson, 1973, p. 51). These trees, large enough to be visible from a distance of at least 5 mi, as calculated from the map in figure 2, are an indication that a relatively stable geographic configuration of the lower Carson River had existed for 20-50 years prior to Kern's visit. For purposes of the present analysis, Carson Lake is assumed to have been a permanent wetland feature.

The maximum depth of Carson Lake at full pool is 10 ft, with the lake-bottom altitude at 3,909 ft above sea level (Hart and Bixby, 1922, p. 13) and the rim altitude at 3,919 ft (Morrison, 1964, p. 104). The maximum surface area of Carson Lake at the rim is about 34,000 acres. Russell (1885, p. 44-45; 1895, p. 108) observed the Carson Desert in the early 1880's, after the onset of early irrigation and after the Carson River had split with one part bypassing Carson Lake, thus reducing inflow to the lake. He described Carson Lake as varying in size and depth with the alternation of the seasons, generally outflowing through Stillwater Slough (1885, p. 68-69). The lake was discharging through the slough in June 1881, had an open water area of about 26,000 acres in 1882, and by September 1883, much of the lake appeared to be a swamp (Russell, 1885, p. 45, 69). Because emergent vegetation in this region is typically found where water depths are less than 3 ft, by September 1883 the surface of Carson Lake probably was at least 7 ft below its natural rim. This inferred difference in water level is greater than the evapotranspiration rate of 5 ft/yr (U.S. Department of the Interior, 1988, p. A-31). Outflow from Carson Lake in 1882 was nonexistent, as noted by Russell (1885, p. 45, 69). Apparently, the lake, at the time of his estimate of about 26,000 acres, was smaller than its maximum of 34,000 acres. About one fourth of the adjacent marsh, which was above the normal maximum lake-surface altitude, was probably intermittently flooded and may have covered about 4,000 additional acres (fig. 2). For purposes of this discussion, then, the maximum wetland acreage of Carson Lake and the adjacent marsh land is estimated to have been about 38,000 acres.

Large variations in the Carson River streamflow, both seasonal and annual, would have affected the size of Carson Lake seasonally. Typically, the seasonal flow patterns vary from low flow from August through November, to a slow increase through March, and finally to a spring peak late in April that falls off by July (fig. 3). Seasonally diminishing streamflows of the Carson River usually limit agriculture in the basin above Fort Churchill by July 15 (Garry Stone, Federal Water Master, oral commun., 1990).

The summer and fall streamflow in the Carson River may regularly have been insufficient to maintain Carson Lake, and the lake size may have peaked at the rim in early summer, then decreased. The estimated 4,000 acres of adjacent marsh is assumed, for purposes of this discussion, not to change in size. The seasonal variation noted by Russell (1885, p. 68-69) may have been usual. Richard Burton, in 1860 (1963, p. 551), found the water to be about a mile away from a Pony Express station (at the southwestern edge of the lake), but the following summer DeQuille (1963, p. 28) observed that the water was again up to the dock of the station. A summer-fall evaporative loss of 3 ft in depth would have reduced the surface of Carson Lake from a possible maximum of about 34,000 acres to about 29,000 acres. The lake size also has been periodically reduced by drought (Morrison, 1964, p. 104; Russell, 1895, p. 108). Thus, on the basis of the discussion above, the historical average wetted surface area of both open water and adjacent marsh at Carson Lake was less than the historical maximum, 38,000 acres, and is conservatively estimated to be 27,000 acres (table 1). This lesser acreage amount is used in the subsequent water-consumption estimate for Carson Lake.

Table 1. Estimated historical (predevelopment) wetland acreage compared to projected wetland acreage at Carson Lake and the combined wetlands of Stillwater Marsh and Carson Sink

{All values have been rounded]

Wetland area	Wetland Acreage: Predevelopment estimate (1845-60) Range	Wetland Acreage: Predevelopment estimate (1845-60) Average	Wetland Acreage: Projected average[1]
Carson Lake (South Carson Lake in figure 2)	[2]25,000-[3]38,000	27,000	5,000
Stillwater Marsh and Carson Sink (North Carson Lake and adjacent marsh lands in figure 2)	[2]0-200,000	120,000	[4]11,000
TOTAL (rounded)		150,000	16,000

[1] U.S. Fish and Wildlife Service, 1988, p. 19, fig. 21.
[2] Estimated minimum size associated with the 1911-88 period of Carson River flow records.
[3] Includes about 4,000 acres of adjacent marsh land.
[4] Includes 3,000 acres of historical Stillwater Marsh located within the Canvasback Gun Club (C. Clifford Creger, U.S. Fish and Wildlife Service, written commun., 1992).

Flow Through Stillwater Slough

In 1861, DeQuille described Stillwater Slough as 60 ft wide, with vertical banks 8-10 ft high, having no grass along the stream, and cutting through the barren sandy plain with only scattered clumps of sage and greasewood, which is typical of an intermittent waterway in this area (DeQuille, 1963, p. 36-37). The absence of hydrophytic vegetation in this channel also supports the contention that summer-fall flow in the Carson River was regularly insufficient to maintain flow through Stillwater Slough. Evapotranspiration losses at Carson Lake reduced the flow of the Carson River before it discharged through Stillwater Slough to Stillwater Marsh. These losses also decreased the duration of flow in the Carson River system below Carson Lake. With 27,000 acres average surface area and an evapotranspiration rate of 5 ft/yr, Carson Lake would have consumed about 140,000 acre-ft of water per year. Subtracting this from the unregulated average annual flow in the Carson River, about 410,000 acre-ft, leaves an annual average flow of 270,000 acre-ft to pass through Stillwater Slough into the wetlands—Stillwater Marsh plus Carson Sink.

Average Historical Size of Stillwater Marsh

The wetted area of Stillwater Marsh and the downgradient playa wetlands (much of Carson Sink) were more variable in size than Carson Lake because of the cumulative evapotranspiration losses in these shallow, sequentially distributed wetlands (Morrison, 1964, p. 104). Because there are no early quantitative estimates of the size of Stillwater Marsh—a series of interconnecting wetlands and ponds flowing generally from south to north, the average size of the Marsh must be estimated using other approaches. Archaeological evidence and pollen cores indicate that a relatively permanent marsh existed in the Stillwater area for the last 4,000 years (Kelly, 1988; Warburton and others, 1990, p. 74), an area known to have been regularly inhabited by man, and by other water-dependent species.

Stillwater Marsh was called Stillwater Lakes by Morrison (1964, p. 104). That observation implies enough water depth (at least 3 ft) to possibly preclude the growth of emergent vegetation and maintain open water areas. Minor variations in topographic elevations (5-15 ft) may be responsible for the formation of these "lakes" at Stillwater Marsh (Raven and Elston, 1989, p. 33). These depressions, by their proximity to flow from Stillwater Slough and by retaining water, gave a degree of permanence to these wetlands compared to the relatively flat areas of Carson Sink.

Raven and Elston (1989, p. 64, 69) have defined two broad categories of historical wetlands at Stillwater, namely "marsh" and "playa," based on soil types and hydrologic characteristics. Both wetland types supported human occupation and water-dependant biota. "Marshes" were those areas which characteristically had enough differences in topography to maintain a combination of open water, emergent vegetation, and upland sites suitable for human habitation. In the absence of detailed land surveys prior to development of Lahontan Valley, the historical amount of each wetland type is uncertain. Elevations determined from U.S. Geological Survey 7.5-minute quadrangle maps indicate that up to 55,000 acres of wetlands were possible in what is now (1993) the Stillwater National Wildlife Refuge. Therefore, wetland acreage could have ranged from 0 to 55,000 acres. Classification of wetland types on current U.S. Geological Survey 7.5-minute quadrangle maps indicates that wetlands at full pool consisted of an average of approximately 15,000 acres of marsh and 40,000 acres of playa (C. Clifford Creger, U.S. Fish and Wildlife Service, Fallon, Nev., written commun., 1992). These areas are consistent with a predictive model based largely on soil types, hydrology, and topography developed by Raven and Elston (1990, p. 123-34).

Carson River inflow through Stillwater Slough was seasonally intermittent. Parts of Stillwater Marsh were subjected to seasonal drying, as were the margins of Carson Lake. Although a graded change in evaporation losses would be predicted as the marsh diminished, it is conservatively estimated that 75 percent of this wetland, about 11,000 acres, would remain wetted and lose water at a rate of 5 acre-ft/yr. This lesser acreage is used **only** in the subsequent water consumption estimate for Stillwater Marsh.

Stillwater Marsh would have been dry only once during the period of record for the Carson River (1911-1988), resulting from the drought of water-years 1976-77.

Estimated Flow into Carson Sink Wetlands

Average water loss by evapotranspiration from the estimated 15,000 (full-pool) acres of Stillwater Marsh would have been 56,000 acre-ft, assuming that 25 percent of the area was seasonally dry. Thus, the 270,000 acre-ft of Carson River water entering the marsh by way of Stillwater Slough each year would be further reduced to an average of about 210,000 acre-ft before entering wetlands in the Carson Sink.

Estimated Size of Carson Sink Wetlands

Wetland acreage in the Carson Sink maintained by Carson River water was, as it is now, generally ephemeral. Ephemeral wetlands are typically shallow, receiving only a few inches of water at the distal edges, and tend to evaporate completely—or nearly so—before the next inflow season. Under unregulated conditions, average Carson River flow would have refilled Carson Lake and Stillwater Marsh and begun to flow into the Carson Sink during winter. Most flow would have reached the sink following spring snow melt or occasional rain-on-snow events. During the spring, wetlands in the southeastern portion of the Carson Sink may have been characterized by having large volumes of flowing water relatively low in dissolved solids. This portion of Carson Sink wetlands may have been wetted for as long as 6 months in most years. In response to decreasing Carson River inflow and increasing evapotranspiration rates in the summer, the palustrine regime would have ceased and the wetlands would have rapidly receded in size and depth to zero or some variable minimum size. Wetlands at the advancing edge of water during peak flow events would have been the most ephemeral, persisting only a few weeks.

Approximately 40,000 acres of playa wetlands on the Carson Sink within the confines of the present (1993) Stillwater National Wildlife Refuge also contain human occupation sites and evidence of water-dependent biota (C. Clifford Creger, U.S. Fish and Wildlife Service, Fallon, Nev., written commun., 1992).

Two accounts of water on the Carson Sink provide a basis from which to estimate an average wetland depth. This, combined with the average Carson River inflow, is used to estimate the average size of wetlands in the Carson Sink.

During September 1929, Sperry (1929, p. 1-3) surveyed the wetlands on the Carson Sink at the mouth of the Carson River. At that time, the river emptied into the Sink in the vicinity of what is now Fallon NWR, adjacent to Stillwater and topographically similar—mostly flat with shallow sloughs and low rises. Sperry considered the water supply to be deficient that year and below normal, and reported the water to be mostly less than 1 ft deep, with a few ponds 2.5-3 ft deep. In addition to alkali bulrush (*Scirpus maritimus*) beds, he saw blackened basal stalks that "coat long reaches of the higher ground and indicate an enormous extension of big beds during wet seasons." His observation of "carpets" of sago pondweed (*Potamogeton pectinatus*), some in mature fruit, left exposed by the receding waters, indicates that the water receded rapidly and suggests fairly rapid decreases in wetland size in the fall.

During the 1980's, the Carson Sink was inundated by flood flows from both the Carson and Humboldt Rivers. This is considered an anomalous event; flooding on that scale last occurred in the 1860's. The 1980's occurrence created unusual physical and water-quality conditions, and is of interest here primarily because of observations of water depth made at various times during the inundation.

Between July 1984 and February 1985, flood water inundating Carson Sink and Stillwater Marsh covered about 212,000 acres of surface area to a maximum depth of nearly 12 ft (Rowe and Hoffman, 1990, p. 37). At that size, the average depth would have been 8 ft and the volume about 1,700,000 acre-ft. In mid-January of 1987, water in the Carson Sink had receded to less than 180,000 surface acres, with an average depth of 2 ft and a maximum of 6 ft (Rowe and Hoffman, 1990, p. 37). The volume of water in the Carson Sink would then have been less than 360,000 acre-ft.

Although it is recognized that wetlands on the Carson Sink were dynamic, an average depth is needed to estimate the extent of wetlands which may typically have been maintained by the Carson River. Based on the observations by Sperry (1929) and Rowe and Hoffman (1990), 2 ft is a reasonable estimate for an average depth. Using this 2-ft depth, the 210,000 acre-ft inflow from the Carson River could have seasonally flooded an average of 105,000 surface acres of wetlands on Carson Sink. This area represents an average of maximum wetland sizes that could be anticipated from the flows of the 1911-1988 period of record for the Carson River.

The Carson Sink wetlands may have varied in size from 0 to 190,000 surface acres, based on fluctuations of the Carson River alone during the 78-year period of record. The low point, zero, would have occurred with flow similar to that of water-year 1977 (the second year of the 1976-77 drought) when the Carson River flow would not have reached the Carson Sink. The maximum, 190,000 acres, is based on the flow of the Carson River during water-year 1983. The adjusted flow of the Carson River at Fort Churchill in water-year 1983 was about 940,000 acre-ft (800,000 acre-ft, rounded [Frisbie and others, 1984, p. 127], plus 140,000 acre-ft from assumption no. 3 in the subsection "Estimated Historical Carson River Flow and Wetland Sizes"), and the inflow to the sink from the Carson River through Stillwater Marsh is estimated to have been about 750,000 acre-ft. Based upon a linear relationship of surface areas and water volumes observed by Rowe and Hoffman (1990), this 750,000 acre-ft would have inundated about 190,000 surface acres on the Carson Sink, with an average depth of nearly 4 ft and a maximum of 8 ft. Some of this water would be expected to remain on the sink into the following year. Under these extreme hydrologic conditions, parts of Stillwater Marsh also would be flooded.

ESTIMATED HISTORICAL WATER QUALITY

Little information about the historical water quality of the lower Carson River system exists, but explorers and local residents of the mid to late 1800's reported good water (presumably potable, in the historical sense), plentiful vegetation, and abundant wildlife at various locations in the lower Carson River basin and its wetlands.

In June 1859, Simpson (1876, p. 85) visited Carson Lake and wrote the following:

> We are encamped at the head of the outlet from Carson Lake into the sink of Carson, where our only fuel is dry rush. This outlet is about 50 feet wide and 3 or 4 feet deep, and voids the lake rapidly into its sink, which is some 10 or 15 miles to the northeast of us. The water is of a rather whitish, milky cast, and though not very lively, is yet quite good. The Carson River to the northwest, where it empties into the lake, can be seen quite distinctly, marked out by its line of green cottonwoods.
>
> The name of the river and lake was given by Colonel Fremont, in compliment to Kit Carson, one of his celebrated guides.
>
> The alluvial bottom about Carson Lake is quite extensive and rich, as the luxuriant growth of rushes shows, and could, I think, be easily irrigated. The only drawback to its being unexceptionable for cultivation in every part is its being somewhat alkaline in places, particularly toward its southern portion. Curlew, pelican, and ducks, and other aquatic birds frequent the locality, and the lake is filled with fish.

Wuzzie George, a Native American residing in Lahontan Valley in the early 1900's and a keen observer of natural things, referred to the abundance of submergent vegetation in the Stillwater marshes (U.S. Fish and Wildlife Service, 1952, p. 19), an indication of water clarity. Hart and Bixby (1922, p. 15) found Carson Lake in 1922 to be slightly alkaline, with very little accumulation of salts on the edges, and with "vegetation [that] ... indicates the absence of an excess of harmful salts." At Stillwater Marsh, the remains of freshwater clams (*Anadonta sp.*), fish, mink (*Mustela sp.*), and river otter (*Lutra sp.*) in archaeological sites indicate the marsh had higher water quality than at present. The wetland ecosystem in the southern inflow area of Stillwater Marsh would have flourished with natural flushing, but water in the ephemeral wetlands on the Carson Sink would have accumulated salts. The fluctuating north and west margins of this ephemeral marsh probably resembled the barren areas associated with current managed wetlands, where water with dissolved solids concentrated beyond the tolerance level of aquatic plants is disposed of through evaporation.

Historical water-quality conditions were estimated using period-of-record data on streamflow and dissolved solids for the Carson River at the Fort Churchill gage. The average measured dissolved-solids concentration at the Fort Churchill gage for the period 1970-88, the only data available, was 218 mg/L and ranged from 70 to 454 mg/L (Ray J. Hoffman, U.S. Geological Survey, written commun., 1990). Although the average Carson River streamflow measured at Fort Churchill has decreased because of upstream diversions, the recent average dissolved-solids load at Fort Churchill (about 90,000 ton/yr) is assumed, for the purpose of the present analysis, to be about equal to the average historical dissolved-solids load. Although predevelopment loads did not include post-development contributions of dissolved solids from irrigation returns and sewage disposal, this assumption should result in a reasonable estimate of an upper limit for predevelopment dissolved-solids loads and concentrations. The average historical (1845-1860) concentration of dissolved solids entering Carson Lake is estimated to be about 170 mg/L. This concentration was determined by computing the ratio of the average volume of water for the period 1970-88 to the historical volume of water, then multiplying by the average dissolved-solids concentration for the period of record and adding an estimate for natural accretion (see footnotes 7 and 8 in table 2):

(300,000 acre-ft / 410,000 acre-ft) 218 mg/L
+ 10 mg/L = 170 mg/L (rounded).[1]

Historically, an estimated annual average of about 410,000 acre-ft of water reached Carson Lake by way of the Carson River; most of it overflowed to Stillwater Marsh. The water entering Carson Lake, with an estimated dissolved-solids concentration of 170 mg/L, carried a probable dissolved-solids load of about 95,000 ton/yr to the wetlands (table 2). This estimated load to the wetlands provides a benchmark for understanding baseline water-quality conditions that existed in Carson Lake, Stillwater Marsh, and the Carson Sink.

---

[1]The estimated historical dissolved-solids concentrations presented in this section are intended solely as a historical baseline for comparison with existing water quality of wetlands in the lower Carson River basin receiving irrigation drainage. Because of variation of flow and the high evaporation rate, the average conditions described herein may have been greatly exceeded at times; thus, the estimated predevelopment concentrations should not be used to set downstream water-quality standards.

Table 2. Estimated historical (predevelopment) and recent or projected water quantity and dissolved-solids concentrations and loads for Carson River, Carson Lake, and Stillwater Marsh. (Estimated historical dissolved-solids concentrations should not be used to set water-quality standards for wetlands in the study area)

[Abbreviations: acre-ft, acre-feet; ft/yr, foot per year; mg/L, milligrams per liter; ton/yr, tons per year]

	Carson River at Fort Churchill gaging station	Carson Lake	Stillwater Marsh
Water quantity (acre-ft)			
Historical (1845-60)	[1]410,000	[2]410,000	[3]270,000
Recent (1970-88) or projected by OCAP[4]	[5]300,000	[6]25,000	[6]55,000
Dissolved-solids concentration (mg/L)			
Historical (1845-60)	[7]160	[7,8]170	[7,8]270
Recent (1970-88) or projected by OCAP[4]	[7]220	[9]1,170	[9]1,170
Dissolved-solids load (ton/yr)[10]			
Historical (1845-60)	89,000	95,000	99,000
Recent (1970-88) or projected by OCAP[4]	90,000	40,000	88,000

[1] Estimated historical unregulated discharge at Fort Churchill gage (average annual streamflow for period of record plus upstream consumptive use; see text for details).

[2] All of Carson River flow estimated to enter Carson Lake.

[3] Water entering Stillwater Marsh estimated by using total inflow to Carson Lake (410,000 acre-ft), minus evaporation rate (5 ft/yr) multiplied by average wetland acreage of Carson Lake (27,000, rounded; table 1).

[4] OCAP (Operating criteria and procedures for Newlands Project) projection for 1992 and beyond (U.S. Fish and Wildlife Service, 1988).

[5] Recent (1970-88) annual average (R.J. Hoffman, U.S. Geological Survey, written commun., 1990).

[6] Estimated acreage projected for OCAP (table 1) multiplied by evaporation rate (5 ft/yr).

[7] Calculated from quantity and loads: $C = \frac{L}{Qf}$

where

C is concentration, in mg/L;
L is load, in ton/yr;
Q is quantity (streamflow), in acre-ft; and
f is factor for converting mg/L to tons (0.00136).

[8] Estimate includes calculated natural accretion of dissolved solids along the stream channel (R.J. Hoffman, U.S. Geological Survey, written commun., 1990).

[9] U.S. Fish and Wildlife Service, 1988, p. 51.

[10] All values calculated from estimated discharge and concentration $(L = C \times Q \times f)$.

The average wetted surface area of Carson Lake (27,000 acres) would have lost, through evapotranspiration, about 140,000 acre-ft of water annually. This loss of water from Carson Lake would have increased the dissolved-solids concentration in the remaining 270,000 acre-ft of water to about 260 mg/L as it discharged through Stillwater Slough. Because of its shallow depth and shape, Carson Lake would have exchanged water easily with the inflow. The average flow of the Carson River was about 2.5 times greater than the lake's maximum volume, thus long-term concentration of dissolved solids in Carson Lake would not have occurred, and the dissolved-solids load leaving the lake would have been similar to the inflow, about 95,000 ton/yr. Because the average volume of the outflow from Carson Lake is about 10 times the volume of Stillwater Marsh, essentially the same dissolved-solids load would have passed through the Marsh into Carson Sink. The dissolved-solids concentrations, however, would have been higher and more variable in these lower wetlands than in Carson Lake.

Several early reports are available that describe water quality in Stillwater Slough and Carson Lake. Kern (Fremont Expedition of 1845) said of the water at the outlet of Carson Lake that it was "indifferently good" (Spence and Jackson, 1973, p. 52). Stillwater Slough water was described as "quite good" by Simpson (1876, p. 85). DeQuille, in the summer of 1861, described the water at the south end of Carson Lake as having the taste of decayed tules, but found the water in the upper slough to have a "touch of alkali" and at the lower end—at the mouth of the sink, "a strong alkali twang"; he sent an Indian several hundred yards into the marsh for drinking water (1963, p. 28, 38, 42). After 60 years of agricultural activities (1862-1922), the dissolved-solids concentration of the water in Carson Lake was still only about 1,000 mg/L (Hart and Bixby, 1922, p. 15). For comparison, the maximum permissible dissolved-solids concentration in Nevada public water supplies is 1,000 mg/L. Dissolved-solids concentrations would have fluctuated seasonally when the lake surface regularly dropped below its natural rim in the fall and during droughts. As an example, Professor F.W. Clarke found a dissolved-solids concentration of about 1,500 mg/L in a water sample collected from Carson Lake in October 1863, a year after the Carson River was diverted from the lake during the floods of 1862 (Russell, 1885, p. 44, 69).

Approximately half the streamflow of the Carson River would have come during peak snowmelt runoff from April through mid-July, then in most years would have diminished abruptly. This pattern would have resulted in dissolved-solids concentrations that were more variable in Stillwater Marsh than in Carson Lake. In most instances, dissolved-solids concentrations in Carson Lake and much of Stillwater Marsh would not have been a limiting factor to the biota known to have existed in the system. From examination of pollen in a core of Lead Lake sediment, scientists of the Desert Research Institute found that the wetland water had been alternately brackish and fresh (Warburton and others, 1990, p. 73-74). Because of the irregular topography of Stillwater Marsh, some wetland areas may have been poorly flushed and occasionally bypassed by the flow of the Carson River.

Although Carson Lake and Stillwater Marsh currently are hydrologically isolated, the dissolved-solids loads projected under OCAP were summed (128,000 ton/yr) and compared to historical load (99,000 ton/yr) to assess the extent of change. While the projected load is estimated to increase somewhat—about 30 percent—over the historical load, the projected concentration would increase greatly—about 400 percent. Such a large increase in concentration is significant because living organisms respond physiologically to concentration rather than to load. Although the historical dissolved-solids concentration in Carson Lake would have increased as a result of evapotranspiration, the concentration in the water as it was flushed into Stillwater Marsh may have averaged 270 mg/L (see footnotes 7 and 8 in table 2) because of the higher river flows at that time. This concentration would have been representative of conditions throughout most of Stillwater Marsh following the spring flow peak.

The extrapolated estimates and existing reports indicate that the predevelopment water supply was probably adequate and of suitable quality to support healthy wetlands and their associated plants, fish, and wildlife. In the absence of agricultural irrigation, which has been shown to mobilize salts and trace elements such as arsenic, boron, selenium, molybdenum, and lithium (Hoffman and others, 1990, p. 31-38), and a corresponding increase in dissolved-solids concentrations, the water reaching Carson Lake and Stillwater Marsh in predevelopment time (pre-1860) was of better quality than water reaching the wetlands today.

Historical water quality in the ephemeral wetlands of Carson Sink, however, would have varied greatly. Under a representative average condition, 210,000 acre-ft of fresh water (about 270 mg/L, dissolved solids) would have passed into these ephemeral wetlands, flushing the area near Stillwater Marsh. Regular flushing in the spring would have created conditions favorable for aquatic vegetation and many forms of wildlife. Sperry (1929, p. 1-2) reported a wide variety of both submergent and emergent vegetation and wildlife in the sink, including extensive beds of alkali bulrush and sago pondweed. Alkali bulrush tolerates brackish water ranging from a specific conductance of 995 μS/cm (about 650 mg/L, dissolved solids) to 25,800 μS/cm (about 16,800 mg/L, dissolved solids). Sago pondweed grows in water containing as much as 16,800 mg/L, dissolved solids (25,800 μS/cm; Stewart and Kantrud, 1972, p. D5, D21, and D25). Archaeological sites that were occupied by humans are found in the playa habitat within the Carson Sink. These sites are similar to those in Stillwater Marsh, indicating the presence of wetland plants and animals (C. Clifford Creger, U.S. Fish and Wildlife Service, Fallon, Nev., written commun., 1992). Thus, these sites probably had potable water, in the historical sense, during periods of human use.

Water-quality characteristics are different when the Carson Sink is filled. Such an event, anomalous under current managed conditions, occurred between 1982 and 1988 when large quantities of relatively dilute flood water from both the Carson and Humboldt Rivers filled Carson Sink to a depth of nearly 12 ft (average 8 ft). In July 1983, specific conductance of flood water in the Carson Sink near Humboldt Slough was 4,700 μS/cm (about 3,100 mg/L, dissolved solids), but by January 1987 water in the Carson Sink had reached a dissolved-solids concentration of 20,000 mg/L (Rowe and Hoffman, 1990, p. 37). This concentration cannot be accounted for by evaporative water loss alone; much of the dissolved solids probably resulted from redissolving salts previously deposited in the sink. During this event, no vascular aquatic plants grew in the sink. Fish flourished during the first years, then perished when the dissolved-solids concentrations of water became intolerably high as the water evaporated. A similar cycle probably occurred in the 1860's; the water in Carson Sink was 20 ft deep in 1863, according to Morrison (1964, p. 104).

In summary, the historical condition of the Carson River-Carson Sink system was a flush-through pattern with most of the runoff occurring in the spring. Runoff flowed into Carson Lake, overflowed into Stillwater Marsh, and then created extensive ephemeral wetlands in the Carson Sink. Almost the entire 410,000 acre-ft (average annual flow) of Carson River with a probable low average (<300 mg/L) concentration of dissolved solids, entered the wetlands. The wetlands would have been largest in the spring, decreasing by evapotranspiration through the summer and fall. Salts deposited primarily in the sink by evaporating water would have been redissolved by the flushing action of subsequent spring peak flows and carried farther out into the sink. The distal edges of the wetlands would normally have been brackish, and the farthest reaches, at times, even more saline.

VEGETATION

Early reports of the study area describe a biologically productive marsh with a great diversity of vegetation. In 1845, when Kern reported timber at the mouth of the Carson River, he also described Carson Lake as bordered by "a thick growth" of bulrush about 30-40 yards wide at the mouth of the river (Spence and Jackson, 1973, p. 51-52). Simpson (1876, p. 85-86) called the alluvial bottom around the lake in 1859 "extensive and rich, as the luxuriant growth of rushes shows." Bailey (1898, p. 3) described Stillwater Marsh as "... half shallow lake, half tule swamp [which] extends for 20 miles along the valley bottom and furnishes enough salt grass, sedges, and tules to winter many thousand head of stock and a breeding ground for great numbers of water and shore birds."

Early in this century, Sperry (1929, p. 1-3) described the vegetation in the southwestern part of Carson Sink. At that time, alkali bulrush dominated the marsh, and cattail *(Typha sp.)* was common. Spike rush *(Eleocharis acicularis)* was abundant. Open water areas contained abundant stands of sago pondweed, horned pondweed *(Zannichellia palustris)*, and algae. The old high-water line was marked by iodine brush *(Allenrofea sp.)*, below that was a bank of pickle weed *(Salicornia sp.)*, and all around, even out in the mudflats, were patches of salt grass *(Distichlis spicata)*.

Wuzzie George described Stillwater Marsh of the early 1900's as abundant in both submergent and emergent vegetation; alkali bulrush was the most common emergent, followed by hardstem bulrush (*Scirpus acutus*) and cattails (U.S. Fish and Wildlife Service, 1952, p. 19).

At Stillwater Marsh, inflow water was sufficient to maintain luxurient wetland vegetation until 1967 when regular winter water releases from Lahontan Reservoir for power generation were discontinued. During the early 1950's, cattails were the dominant emergent species in the marsh, followed by alkali bulrush, then hardstem bulrush, at a ratio of 4.5:1.5:1 (U.S. Fish and Wildlife Service, Fallon, Nev., 1952, written commun.). A quantitative survey in Stillwater Marsh in 1959 found a diversity of vegetation. Emergent vegetation was then dominated by alkali and hardstem bulrushes and submergent plants by horned pondweed, sago pondweed, and western pondweed (*Potamogeton filiformis*). Other submergent plants observed by the U.S. Fish and Wildlife Service included coontail (*Ceratophyllum demersum*), muskgrass (*Chara sp.*), widgeon grass (*Ruppia maritima*), and curly-leaf pondweed (*Potamogeton crispus*). Some submergent plants, such as horned and sago pondweeds and widgeon grass, are relatively insensitive to high concentrations of dissolved solids (Stewart and Kantrud, 1972, p. D25; Steward and others, 1963, p. 50). The abundance of these submergent plants, as well as more sensitive species in Stillwater Marsh in 1959, indicates the presence of a mixture of fresh and brackish water at that time (U.S. Fish and Wildlife Service, 1969).

In the Stillwater WMA, coontail and other less salt-tolerant pondweeds that were abundant in the 1959 survey have decreased, not only in total abundance but also relative to the more tolerant species, such as widgeon grass and sago pondweed. The abundance of some plants, such as western pondweed, is correlated with freshwater inflow. Cattails are extremely sensitive to increased dissolved-solids concentrations, whereas alkali and hardstem bulrushes tolerate higher concentrations (Stewart and Kantrud, 1972, p. D21); cattails in Stillwater WMA are now found only in scattered patches.

Information from U.S. Geological Survey topographic maps (Carson Lake and Fallon quadrangles) published in 1951 indicates that vegetation has also diminished at Carson Lake by about 50 percent in the last 30 years. Today, much of Carson Lake is a pasture for livestock. Much of the remaining water area is entirely devoid of vascular aquatic vegetation, and emergent vegetation is reduced to relatively small stands of bulrush near the inflow from drains.

WILDLIFE

Historically, the wetlands of the Carson Lake-Stillwater area supported a large and diverse assemblage of animals as well as plants. Archaeological studies have determined that the wetland areas were used extensively by native people for a period exceeding 4,000 years (U.S. Department of the Interior, 1988, p. F1; Kelly, 1988, p. 11), an indication that the early (pre-1860) wetlands were a more productive and reliable habitat than those existing today. Little information is available to quantify the abundance of wildlife in the study area prior to irrigation diversion, but reports from early explorers and archeologists indicate that populations and diversity of wildlife were much greater than exist today.

According to Simpson (1876, p. 85), the marsh supported abundant fish populations. Simpson wrote, "... the lake is filled with fish...[the Indians] have piles of fish lying about drying, principally chubs and mullet." DeQuille (1963, p. 33) saw Indians with "several fine strings of fish" in 1861 and the Indians fishing nearby were having "first-rate luck"; one had caught four nice fish in a few minutes. Fish bones are commonly found in archaeological sites, suggesting that fish were a significant food source for the people; skeletal remains of tui chub (*Gila bicolor*) and tahoe sucker (*Catostomus tahoensis*) are abundant at archaeological sites in Stillwater Marsh (Greenspan, 1988, p. 315-326). Both fish species are tolerant of wide ranges of dissolved solids. Lahontan cutthroat trout (*Onchorhynchus clarki henshawi*), although not widely distributed, were found in some Stillwater archaeological sites (Smith, 1985, p. 177). Lahontan cutthroat trout are tolerant of dissolved solids up to approximately 12,000 mg/L (Taylor, 1972, p. 7), but are sensitive to high water temperature. Historical water quality in Carson Lake and the southern part of Stillwater Marsh should not have been a limiting factor in the survival and distribution of cutthroat trout at most times, but changing habitat and water temperature resulting from water-level fluctuations may have been.

River otter and mink, both fish-eating animals, were present and used by the native people (Schmitt, 1988, p. 272). Pelicans (*Pelecanus erythrorhynchos*), also fish eaters, were "characteristic" of the area (Simpson, 1876, p. 86).

Freshwater clams and aquatic snails (gastropods) were once abundant throughout the wetlands. Simpson (1876, p. 86) observed in 1859 that the shores of Carson Lake were covered with clam shells, and Russell (1885, p. 69) reported various species of freshwater clams and snails in Carson Lake. Drews (1988, p. 329-333) found evidence that clams were a food item at various sites in Stillwater Marsh. Sperry (1929, p. 3), surveying the adjoining wetland in Carson Sink in 1929 (now Fallon NWR), found that clams, frogs (*Rana sp.*), and snails (*Physa sp.)* were common, and that muskrats (*Ondatra sp.*) were reported as abundant throughout Carson Sink.

Mink and otters are absent from the wetlands today, as are frogs and turtles. Muskrats are no longer abundant. Fish populations are greatly reduced, and the species composition has changed since historical times. Most of the native fishes are now absent from most areas; Lahontan cutthroat trout are entirely absent. Several species of non-native fishes, primarily sport fish, were introduced early in the century, but most have decreased to remnant numbers. Introduced Largemouth bass (*Micropterus salmoides)* was a major fishery until the 1970's, when the population virtually disappeared. Today, only remnant populations of freshwater clams remain in Stillwater Point Reservoir and in the D-line Canal, the areas with the lowest dissolved-solids concentration.

Historical references record the abundance of pelicans, curlews (*Numenius americanus*), other shore birds, ducks, geese, and other aquatic birds (Simpson, 1876, p. 85-86; Bailey, 1898, p. 3; DeQuille, 1963, p. 28-32). Shore birds, including curlew, are largely insectivorous and tend to frequent shallow, sparsely vegetated ephemeral water areas and mud flats—wetland types that historically covered thousands of acres on the Carson Sink in the spring and are now scarce. None of the birds are entirely absent today, but none could be termed abundant and some, such as the curlew, are uncommon.

PRESENT/PROJECTED WETLAND CONDITIONS

Court-ordered Operating Criteria and Procedures (OCAP) for the Newlands Project, to be fully in effect in 1992, were evaluated in detail by the U.S. Department of the Interior (1988). An estimate of projected wetland conditions was made, based on data for inflow of water from all sources available for wetland maintenance between 1967 and 1986 and on surveys of varying reliability, made in September for many years, of all major wetlands in the area. The managed wetlands at Carson Lake and Stillwater Marsh (Stillwater WMA and Canvasback Gun Club) under the 1992 institutional (OCAP) constraints, are used here to represent present/projected wetland conditions. It is important to note that the Carson River no longer flows through Carson Lake and thence to Stillwater Marsh. Both wetlands are isolated; are maintained with drainwater, operational releases, and occasional flood flows; and are manipulated with man-made water-control structures.

Recent (1967-1986) average wetland sizes for Carson Lake and Stillwater Marsh were 10,000 and 14,000 acres, respectively. Under OCAP, flow to these primary wetlands, and their average size, are reduced about 50 percent (U.S. Department of the Interior, 1988, p. E5; U.S. Fish and Wildlife Service, 1988, fig. 5, p. 52). Under projected managed conditions, an average 25,000 acre-ft of water would maintain about 5,000 wetland acres at Carson Lake, and an average 55,000 acre-ft of water in Stillwater WMA Marsh (including the Canvasback Gun Club) would maintain about 11,000 acres of wetlands. This projected total of average wetland sizes in Carson Lake and Stillwater Marsh (16,000 acres) is about 11 percent of the estimated historical sum of 150,000 average wetland acres in and near Carson Lake, Stillwater Marsh and Carson Sink. Present wetland conditions are not directly comparable to historical conditions because most of the water now available is used for maintenance of permanent wetlands. In contrast to the natural pattern of the unregulated, historical Carson River (fig. 3), with an abrupt spring runoff peak, the water to Stillwater WMA, dictated by agricultural practice, comes in a reduced, protracted flow from March through November, without a substantial flushing flow in the spring (U.S. Fish and Wildlife Service, 1988, fig. 8). In addition, much of the remaining flow is managed by artificial structures to create permanent wetlands consuming about 5 acre-ft/acre of water per year. Because of this management, present wetlands are smaller but more permanent than historical wetlands supported by a given amount of water. To allow comparison, under OCAP the average 16,000 acres projected to remain in Carson Lake and Stillwater Marsh would require at least 80,000 acre-ft/yr of water. However, the dissolved-solids concentrations in the inflow water of Stillwater Marsh and Carson Lake are now, respectively, about four to seven times greater than historical concentrations; wetland flushing is restricted only to years with exceptionally high streamflow.

Water quality projected for the wetlands was described by the U.S. Fish and Wildlife Service (1988, p. 50). Dissolved-solids concentrations in water entering primary wetlands would average 1,170 mg/L, as projected for OCAP. The dissolved-solids concentration of the Carson Lake inflow is about seven times the estimated historical concentration of 170 mg/L of solids for the Carson River, and the Stillwater Marsh inflow is about four times more concentrated than the estimated 270 mg/L historical Stillwater inflow by way of Stillwater Slough. Again, the reader is cautioned that the historical estimates are just that—estimates—and should not be used beyond that intended for this report.

In Stillwater WMA, dissolved solids are progressively concentrated through evapotranspiration as inflow water is stored for waterfowl use then moved through sequentially arranged ponds (table 3). These are historical ponds with water-control structures added. Under past management practices, the initial wetland would concentrate inflowing dissolved solids by nearly four times, to about 4,600 mg/L, and the next sequential wetland would further concentrate dissolved solids by six times, to about 28,000 mg/L (U.S. Fish and Wildlife Service, 1988, table 7).

Present concentrations of dissolved solids would rarely have occurred in the historical flow-through wetlands at Carson Lake and Stillwater Marsh, but they may have routinely occurred in parts of the more ephemeral wetlands in the Carson Sink. For example, high dissolved-solids concentrations were measured in the Carson sink during the 1980's (Rowe and Hoffman, 1990, p. 37). Present water-quality conditions in parts of Carson Lake and Stillwater Marsh are limiting to some aquatic plants and animals. In addition, trace elements toxic to fish and invertebrates are being released from Carson Desert soils by agricultural drainage and are entering these wetlands, as discussed in the next section on toxicity.

In summary, the pattern of water flow, quality of the water, and the size and quality of the resultant wetlands, as well as vegetation and wildlife, have all changed dramatically since the onset of irrigation in the late 1800's.

Table 3. Concentration factors between wetland units and recent and projected concentrations of dissolved solids, Stillwater Wildlife Management Area

[Values are based on data collected by the U.S. Fish and Wildlife Service (1988)]

Wetland unit[1]	Recent average dissolved solids (mg/L) (1967-1986)	Approximate concentration factor	Projected average dissolved solids (mg/L)
Wetland inlet flow	600	[2]2	1,170
Primary unit	2,360	4	4,610
Secondary unit	14,180	6	27,600
Tertiary unit	28,400	2	55,200

[1] Primary, secondary, and tertiary units refer to a series of shallow ponds that receive water progressively more concentrated with dissolved solids.

[2] Initial change in dissolved-solids concentrations resulting from irrigation practices.

TOXICITY OF IRRIGATION DRAINAGE AND ITS EFFECT ON AQUATIC ORGANISMS

The wetlands in Stillwater Wildlife Management Area (WMA) are managed primarily to support migratory waterfowl. Many water-dependent migratory birds require a variety of fish and invertebrates in their diets. As a result of irrigation drainage, water in Stillwater WMA has an unnaturally high salinity, and both water and biota contain elevated concentrations of potentially toxic trace elements that can affect fish and invertebrates (Hoffman and others, 1990, p. 76-77). For example, Stillwater WMA can no longer sustain a Largemouth bass (*Micropterus salmoides*) population (U.S. Fish and Wildlife Service, 1988, p. 132), and invertebrate density is low. Toxicity tests were conducted to assess the suitability of water from various drains and wetland areas to support fish and invertebrates.

METHODS

Sample Collection

Water samples were collected at sites in Stillwater WMA on Paiute Diversion Drain, D-Line Canal, Hunter Drain, Lead Lake, Stillwater Point Diversion Drain, Stillwater Point Reservoir, and TJ Drain (including one USGS well near TJ Drain). Sampling sites are shown in figure 4.

Composite water samples were collected daily from each drain location during August 9-18, 1988. Each sample consisted of water collected at 15-minute intervals during a 24-hour period by an ISCO model 2710 automatic composite sampler. Water was pumped from the drain through Teflon tubing into 19-L, acid-washed, glass collection containers. The containers were held in ice baths during the collection period to keep the samples at approximately 4°C during the day. Water samples from Lead Lake and Stillwater Point Reservoir were collected daily as "instantaneous" samples and, as such, represent the quality of the water at the time of sampling. Onsite toxicity tests were made with portions of these samples collected daily from each location. Principal constituents and properties of each water sample (temperature, pH, dissolved oxygen, hardness, alkalinity, turbidity, specific conductance, salinity, and calcium, sulfate, and chloride) were measured on-site daily. Ammonia and nitrate were measured near the beginning, middle, and end of the toxicity tests.

A portion of the composite water collected daily was used for determination of additional inorganic constituents (magnesium, sodium, potassium, silica, phosphorus, arsenic, barium, boron, lithium, mercury, molybdenum, selenium, strontium, vanadium, and zinc). These subsamples were collected in 1-L, linear polyethylene bottles that had been previously washed with soap and water, rinsed with tap water, soaked in concentrated nitric acid, and rinsed twice with deionized water. Samples were filtered through a 0.4-µm polycarbonate filter and preserved with 2 mL of ultrapure nitric acid for in the laboratory analysis. Chemical analyses of the water were made by Environmental Trace Substances Research Center (Columbia, Mo.). Spiked samples, blanks, and replicates prepared onsite were included as quality-assurance samples (Peden, 1986).

A one-time collection of water from an observation well near TJ Drain was made by U.S. Geological Survey personnel using a bailer and according to standard methods (Claasen, 1982). The well water, which represented potential seepage to the drain, was tested for acute toxicity and analyzed for trace-element concentrations.

A one-time collection of water from all drains also was made for analysis of potentially toxic man-made organic constituents. Analysis was made by the U.S. Geological Survey National Water Quality Laboratory, Denver, Colo. The data are on file at the U.S. Geological Survey, Nevada District Office, Carson City.

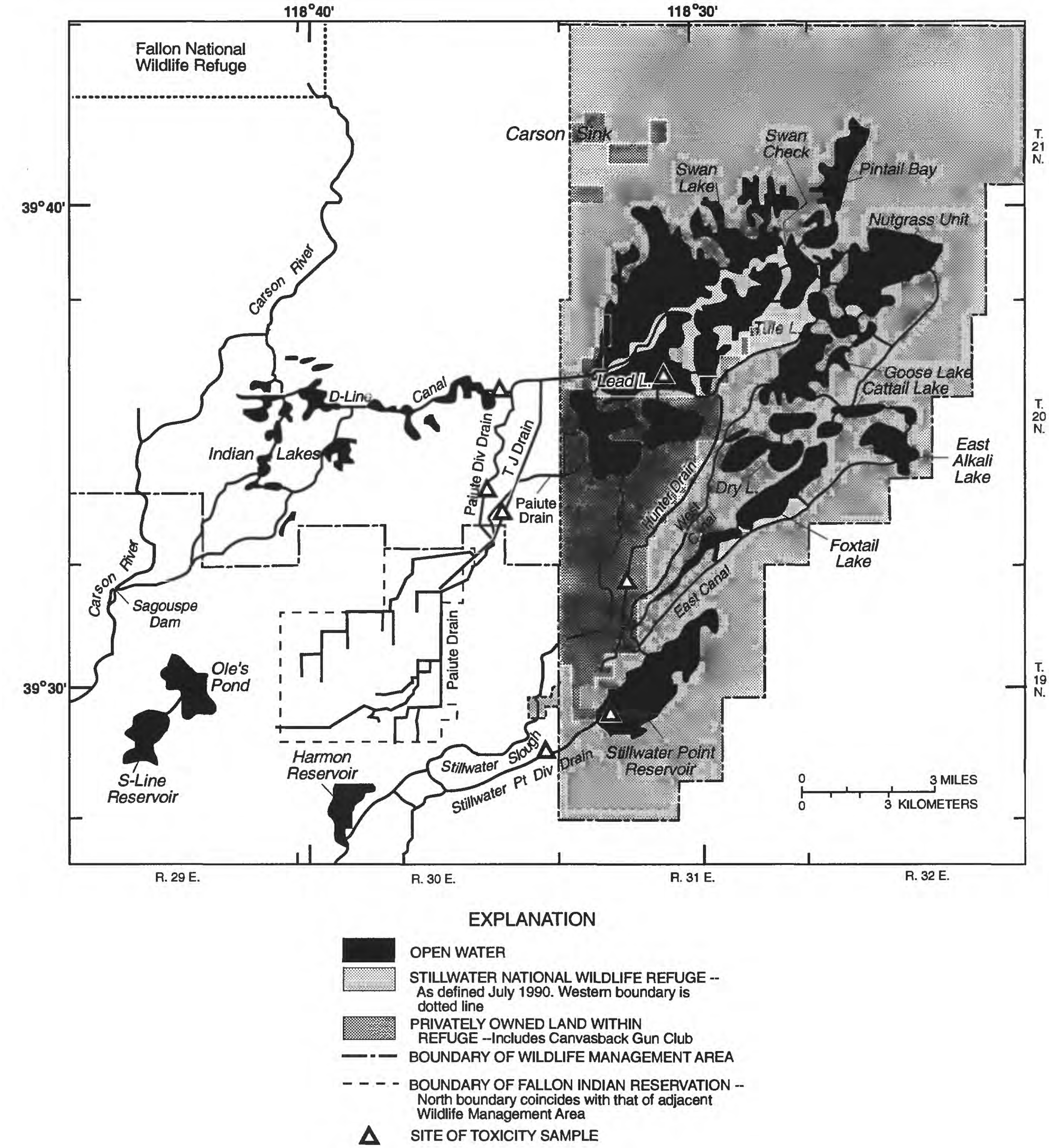


Figure 4. Locations of toxicity sampling sites and important hydrologic features in and near Stillwater Wildlife Management Area. (Map modified from Lico, 1992.)

Toxicity Assessments

Toxicity tests were made using a portion of the daily composite water sample from each location to renew the test solution daily throughout the test period. The species tested were bluegills (*Lepomis macrochirus*), larval fathead minnows (*Pimephales promelas*), and daphnids (*Daphnia magna*). Daphnids and fathead minnow larvae were obtained from cultures at the National Fisheries Contaminant Research Center (NFCRC), Columbia, Mo., and were maintained onsite in well water (hardness, 283 mg/L as $CaCO_3$; alkalinity, 255 mg/L as $CaCO_3$; pH, 7.8) that had been transported from NFCRC. Bluegills ($\leq$0.1 g) were obtained from the Missouri Department of Conservation, shipped to Stillwater WMA, and transferred into the NFCRC well water for 48 hours of observation prior to use. Using Hunter Drain water, in which salinity exceeded 15 ppt, additional tests were made with salt-tolerant species—sheephead minnow (*Cyprinodon variegatus*) larvae, and mysid shrimp (*Mysidopsis bahia*). These organisms were obtained from the U.S. Environmental Protection Agency (USEPA) Laboratory in Gulf Breeze, Fla.

Tests were made as static renewals under appropriate 10-day test procedures (USEPA, 1985; American Society for Testing Materials, 1988). All organisms were fed freshly hatched brine shrimp (*Artemia*) twice daily. Tests with daphnids and fathead minnow larvae included 2 replicates per treatment and 10 organisms per replicate. Tests with bluegills consisted of 2 replicates per treatment and 5 organisms per replicate. Treatments included full-strength water and dilutions of 50, 25, and 12.5 percent. Dilution water for each test was reconstituted to the appropriate hardness, alkalinity, specific conductance, and pH for that test-location water by the addition of major cations and anions using Instant Ocean and deionized water. By reconstituting dilution water to the same ionic composition as the drainwater, consistent concentrations of constituents such as calcium, magnesium, sodium, potassium, chloride, and sulfate were maintained for all treatments for each sampling site. All sampling sites were evaluated using this reconstituted water as a diluent and as a control. In addition, Paiute, TJ, and Stillwater Point Diversion Drains were also assessed by using water into which they discharged (Paiute and TJ into D-Line, and Stillwater Point Diversion Drain into Stillwater Point Reservoir) as diluent and control. The NFCRC well water provided a second control to evaluate the condition of test organisms throughout the tests. Dissolved-oxygen concentrations exceeded 40 percent saturation in all tests. Acceptability of a test required that control mortality not exceed 10 percent.

Evaluation of potential toxicity problems in the Stillwater area included a total of 35 concurrent tests on water samples:

Sample site	Dilution source	Number of tests
Paiute Diversion Drain	Reconstituted water	3
Paiute Diversion Drain	D-Line Canal water	3
TJ Drain	Reconstituted water	3
TJ Drain	D-Line Canal water	3
Stillwater Point Diversion Drain	Reconstituted water	3
Stillwater Point Diversion Drain	Stillwater Point Reservoir water	3
Stillwater Point Reservoir	Reconstituted water	3
Lead Lake	Reconstituted water	3
Hunter Drain	Reconstituted water	5
D-Line Canal	Reconstituted water	3
TJ Drain ground water	Undiluted water	3

During the tests, mortality was recorded daily for each species. Bluegills were weighed and measured to assess growth at the initiation and termination of the test. A subsample of daphnids was preserved on the first day of the test and the surviving adults were also preserved on the final day of the test for later examination. For daphnids, time to first brood production, total number of broods, and number of young per brood were recorded as measures of reproductive success. Temperature, pH, and dissolved oxygen were measured daily in each beaker prior to renewal of test solutions.

Chemical Analyses

Principal constituents and properties, along with ammonia and nitrate, were determined using standard methods (magnesium, sodium, potassium, silica, phosphorus, arsenic, barium, boron, lithium, mercury, molybdenum, selenium, strontium, vanadium, and zinc).

Filtered water samples to be analyzed for inorganic constituents were digested in 100-mL borosilicate glass beakers that had been cleansed by a concentrated nitric acid, 30-minute, reflux cycle. About 40 mL of the digested sample was combined with 15 mL of concentrated nitric acid and 2.5 mL of concentrated perchloric acid, then evaporated over low heat until only 1 mL remained. This digestate was diluted to 50 mL with ultrapure water and analyzed for selenium and arsenic by hydride generation atomic absorption. The remaining digestate was analyzed for additional elements with an inductively coupled plasma spectrophotometer. Mercury was determined by cold vapor atomic absorption. Analytical procedures are described in detail by the U.S. Fish and Wildlife Service (1985).

All results were within 20 percent of confidence intervals for certified or recommended concentrations for the reference materials. Of the samples analyzed, 10 percent were blanks and 20 percent were blind replicates and spiked samples. No values for blanks exceeded analytical reporting limits for any element (table 4).

Daily water-quality measurements for the seven surface-water sites are presented in the supplemental data tables at the back of this report. A summary of ranges is shown in table 6.

Water samples for analysis of manmade organic constituents were extracted three times in the laboratory at both acidic and basic pH with methylene chloride. The extracts were concentrated to about 1 mL, combined with an internal standard, and analyzed by gas-chromatography, electron-impact, mass spectrometry. Surrogate compounds were added to each sample prior to extraction to verify method recoveries (Wershaw and others, 1987). The analytical results are discussed in the subsection titled "Stillwater Point Diversion Drain and Stillwater Point Reservoir."

Table 4. Analytical reporting limits for selected inorganic constituents in water (from Environmental Trace Substances Research Center, Columbia, Mo.)

[All units in micrograms per liter, except where indicated; mg/L milligrams per liter]

Element	Concentration	Element	Concentration
Calcium (mg/L)	0.002	Copper	2
Magnesium (mg/L)	.002	Iron	5
Sodium (mg/L)	.02	Lead	40
Potassium (mg/L)	1	Lithium	.5
Sulfate (mg/L)	1	Manganese	5
Silica (mg/L)	.01	Mercury	.3
Chloride (mg/L)	.15	Molybdenum	20
Phosphorus (mg/L)	.10	Nickel	100
Aluminum	30	Selenium	.3
Antimony	40	Silver	20
Arsenic	.5	Strontium	4
Barium	1	Thallium	3
Beryllium	1	Tin	10
Bismuth	60	Titanium	4
Boron	20	Tungsten	2
Cadmium	2	Vanadium	2
Chromium	10	Zinc	2
Cobalt	10		

RESULTS OF TOXICITY TESTS

Paiute Diversion Drain and D-Line Canal

Water from Paiute Diversion Drain diluted with D-Line Canal water was not acutely toxic to bluegills, fathead minnow larvae, or daphnids (table 5). No substantial mortality occurred in tests with water from Paiute Diversion Drain regardless of dilution water, and no mortality was recorded for any species exposed to water from D-Line Canal. No sublethal responses were identified in daphnids exposed to water from either location. Daphnids produced equal numbers of young in all treatments, with one brood produced in every treatment on day 8. For all treatments, mean number of young per brood ranged from 8.1 to 9.4. Microscopic examination of adults at the end of the test revealed production of eggs for a second brood and substantiated the continued reproductive development that would be expected in healthy organisms. Concentrations of constituents in Paiute Diversion Drain and D-Line Canal water were similar, and daily fluctuations in water quality were minimal during the study period (table 6). Neither mercury nor selenium was detected in any water sample. In addition, aluminum, antimony, beryllium, bismuth, chromium, cobalt, copper, iron, nickel, silver, tin, titanium, thallium, and tungsten did not exceed reporting limits.

TJ Drain

Water from TJ Drain was acutely toxic to bluegills, fathead minnow larvae, and daphnids (tables 7 and 8). Cumulative mortality in 100-percent TJ Drain water was similar for fathead minnows and bluegills, and ranged from 85- to 90-percent mortality after 9 days of exposure. No mortality was observed in well-water controls, reconstituted-water controls, or 100 percent D-Line Canal water also used as control. Daphnids were more sensitive than were fish species to the two highest drain-water concentrations. Total mortality of daphnids occurred in full-strength drain-water after 6 days and in the 50-percent dilution by the end of the test; no substantial mortality occurred in the 12.5-percent dilution. No daphnid reproduction occurred in the TJ Drain water regardless of dilution water. Control daphnids in reconstituted water reproduced on day 8, with a mean brood size of 9.6; a first brood (9.3 young) was also produced on day 8 in the D-Line Canal control water.

Table 5. Cumulative mortality of bluegills, fathead minnow larvae, and daphnids after exposure to water from Paiute Diversion Drain diluted with (A) reconstituted water[1] and (B) water from D-Line Canal

Species (and number sampled)	Cumulative mortality of species after tests (percent)					
	Proportion of drain water in sample					NFCRC well-water control[2]
	100 percent	50 percent	25 percent	12.5 percent	0 percent	
A. Drainwater, diluted with reconstituted water						
Bluegills (10)	0	0	10	0	10	0
Fathead minnow larvae (20)	0	0	0	0	0	0
Daphnids (20)	0	0	10	0	5	0
B. Drainwater, diluted with D-Line Canal water						
Bluegills (10)	0	10	10	0	0	0
Fathead minnow larvae (20)	0	0	0	0	0	0
Daphnids (20)	0	10	0	0	0	0

[1] Deionized water reconstituted to the same hardness, alkalinity, specific conductance, and pH as the Paiute Diversion Drain water.

[2] Well water from National Fisheries Contaminant Research Center, Columbia, Mo.

Table 6. Ranges of water-quality measurements in surface-water samples collected daily, August 10-18, 1988, for aquatic toxicity tests[1]

[Abbreviations: NTU, nephelometric turbidity units; ppt, parts per thousand; µS/cm, microsiemens per centimeter at 25°C; µg/L, micrograms per liter; mg/L, milligrams per liter.]

	Paiute Diversion Drain	D-Line Canal	TJ Drain	Hunter Drain	Lead Lake	Stillwater Point Diversion Drain	Stillwater Point Reservoir
Specific Conductance (µS/m)	370-480	350-610	6,100-14,900	410-27,500	5,100-6,100	470-720	1,590-2,510
pH, field (units)	7.5-8.6	8.6-9.4	8.4-8.6	8.0-8.8	8.6-8.9	8.2-8.6	9.0-9.2
Turbidity (NTU)	15-35	3-9	2-8	7-37	37-96	21-54	170-580
Dissolved oxygen (mg/L)	6.8-9.7	9.0-10.3	7.4-10.9	5.6-8.8	5.6-9.2	8.3-9.6	8.2-10
Calcium (mg/L)	31-36	26-32	140-310	32-390	64-74	42-51	22-36
Magnesium (mg/L)	10-13	9-11	160-410	10-250	90-103	12-18	16-21
Hardness (mg/L as $CaCO_3$)	110-140	94-130	1,100-2,500	110-2,300	570-680	140-200	130-170
Sodium (mg/L)	62-84	83-110	1,900-4,700	98-8,600	980-1,100	120-270	310-480
Potassium (mg/L)	2.8-5.4	5.4-9.4	29-57	4.0-210	29-33	7.3-13	15-21
Alkalinity (mg/L as $CaCO_3$)	111-158	113-174	217-319	102-276	227-272	114-312	235-300
Sulfate (mg/L)	52-85	47-130	880-3,600	52-2,900	360-680	110-150	100-240
Chloride (mg/L)	50-70	12-50	2,700-6,200	84-13,000	1,200-1,800	43-63	300-550
Silica (mg/L)	5.8-7.2	5.9-7.6	0.1-3.8	5.0-13	5.1-7.5	11-14	10-12
Salinity (ppt)	0.1-0.3	0-0.5	4.1-13.0	0.0-28	3.1-4.2	0.2-0.8	0.9-1.9
Phosphorus (mg/L)	<0.1-0.2	<0.1	0.4-1.0	<0.1-0.9	0.20-0.37	0.20-0.30	<0.1
Arsenic (µg/L)	10-20	40-50	110-170	20-190	100-130	40-50	80-110
Barium (µg/L)	70-90	40-60	50-110	50-100	110-140	60-70	90-120
Boron (µg/L)	520-760	620-810	6,800-17,000	810-49,000	5,000-6,000	860-1,800	2,000-3,000
Lithium (µg/L)	50-60	50-60	340-760	60-2,300	340-390	60-100	100-130
Mercury (µg/L)	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
Molybdenum (µg/L)	<20-20	<20-20	270-670	20-1,300	110-130	30-50	50-70
Selenium (µg/L)	<0.3	<0.3	0.9-1.6	<0.3-3.6	<0.3	0.4-0.6	0.4-0.6
Strontium (µg/L)	350-410	340-370	3,200-7,900	390-10,400	1,800-2,000	450-670	520-570
Vanadium (µg/L)	10	10-20	10-20	10-30	20	20	20-30
Zinc (µg/L)	20-70	10-40	10-90	10-90	10-40	40-100	10-50

[1] Analyzed by Environmental Trace Substances Research Center, Columbia, Mo. These water-quality data do not necessarily conform to U.S. Geological Survey guidelines for reporting significant figures.

Table 7. Cumulative mortality of bluegills, fathead minnow larvae, and daphnids exposed to water from TJ Drain diluted with reconstituted water[1]

Species (and number sampled)	Exposure (days)	Cumulative mortality of species in tests (percent)					
		Proportion of drainwater in sample					NFCRC well-water control[2]
		100 percent	50 percent	25 percent	12.5 percent	0 percent	
Bluegills (10)	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	20	20	0	0	0	0
	4	40	30	10	0	0	0
	5	50	30	10	0	0	0
	6	70	30	10	10	0	0
	7	90	50	30	10	0	0
	8	90	50	30	10	0	0
	9	90	70	30	20	0	0
Fathead minnow larvae (20)	1	20	0	0	0	0	0
	2	20	0	0	0	0	0
	3	20	10	0	0	0	0
	4	20	20	10	0	0	0
	5	45	20	10	0	0	0
	6	45	30	20	10	0	0
	7	60	30	20	10	0	0
	8	60	40	20	10	0	0
	9	85	45	20	10	0	0
Daphnids (20)	1	10	0	0	0	0	0
	2	25	0	0	0	0	0
	3	25	20	0	0	0	0
	4	60	30	10	0	0	0
	5	95	30	15	0	0	0
	6	100	40	20	10	0	0
	7		75	25	10	0	0
	8		80	25	10	0	0
	9		100	25	10	0	0

[1] Deionized water reconstituted to the same hardness, alkalinity, specific conductance, and pH as the TJ Drain water.
[2] Well water from National Fisheries Contaminant Research Center, Columbia, Mo.

A dose-response pattern was evident for all species regardless of dilution water. Although the fluctuating salinity was undoubtedly stressful to test organisms, survival of organisms in controls with identical salinity fluctuations strongly suggests that mortalities in drainwater did not result from exposure to salinity alone.

Concentrations of trace elements fluctuated throughout the exposure period, but concentrations of arsenic, boron, lithium, and molybdenum were consistently higher in TJ Drain water than in Paiute Diversion Drain or D-Line Canal water, where no mortality was observed. Levels of arsenic in TJ Drain consistently exceeded 100 µg/L and boron concentrations reached a maximum level of 16,700 µg/L. Concentrations of dissolved selenium ranged from 0.9 to 1.6 µg/L; the highest concentrations were on day 4 when salinity was highest, and the lowest concentrations were on the day of lowest salinity. No mercury was detected in any sample. In addition, aluminum, antimony, beryllium, bismuth, chromium, cobalt, copper, iron, nickel, silver, tin, titanium, thallium, and tungsten did not exceed reporting limits. Ranges of concentrations of other constituents are reported in table 6.

Table 8. Cumulative mortality of bluegills, fathead minnow larvae, and daphnids exposed to water from TJ Drain diluted with water from D-Line Canal

Species (and number sampled)	Exposure (days)	Cumulative mortality of species in tests (percent) — Proportion of drainwater in sample: 100 percent	50 percent	25 percent	12.5 percent	0 percent	NFCRC well-water control[1]
Bluegills (10)	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	20	20	0	0	0	0
	4	40	30	10	0	0	0
	5	50	30	10	0	0	0
	6	70	30	10	10	0	0
	7	90	50	30	10	0	0
	8	90	50	30	10	0	0
	9	90	60	30	20	0	0
Fathead minnow larvae (20)	1	0	0	0	0	0	0
	2	10	0	0	0	0	0
	3	25	10	0	0	0	0
	4	40	15	0	0	0	0
	5	60	20	5	0	0	0
	6	75	40	10	10	0	0
	7	80	40	10	10	0	0
	8	80	40	10	10	0	0
	9	85	40	10	10	0	0
Daphnids (20)	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	35	20	0	0	0	0
	4	60	45	10	0	0	0
	5	100	50	15	0	0	0
	6		70	30	10	0	0
	7		70	30	10	0	0
	8		100	30	10	0	0
	9			30	10	0	0

[1] Well water from National Fisheries Contaminant Research Center, Columbia, Mo.

Well Near TJ Drain

Ground water from the USGS well DH-102B, near TJ Drain (Hoffman and others, table 3), was the most toxic of the water tested. The test was conducted as a static nonrenewal with the one 5-gal sample. Salinity of the ground water was 7.9 ppt and specific conductance was 12,000 µS/cm; the water was tested with bluegills, larval fathead minnows, and daphnids. All organisms exposed to 100-, 50-, 25-, and 12.5-percent ground water died within 24 hours of exposure. No mortality occurred in any controls. The ionic composition and water quality of TJ Drain ground water was different from that of TJ Drain surface water (table 6). The ground water was harder; the calcium level was about two-fold higher than that of the drainwater and was more highly buffered (higher alkalinity). In addition, the pH of the ground water (7.0) was lower than that of the drainwater (8.4-8.6).

Concentrations of arsenic in the ground water (table 9) were higher than in any drainwater tested. Concentrations of lithium and molybdenum were higher in ground water than those measured in TJ Drain surface water. No selenium or mercury was detected in the ground-water sample, but concentrations of boron were high. Survival of freshwater control organisms in the reconstituted water with a salinity of 7.9 ppt indicates that the observed mortality was probably induced by the presence of trace elements in the ground water.

Table 9. Concentrations of inorganic constituents in ground water collected from U.S. Geological Survey well DH-102B in the vicinity of TJ Drain, August 17, 1988[1]

[Except where indicated, all values are in micrograms per liter; mg/L, milligrams per liter.]

Element	Concentration	Element	Concentration
Calcium (mg/L)	544	Iron	<5
Magnesium (mg/L)	268	Lead	<40
Sodium (mg/L)	2,390	Lithium	1,640
Potassium (mg/L)	87	Manganese	1,440
Silica (mg/L)	36.1	Mercury	<0.3
Phosphorus (mg/L)	0.80	Molybdenum	1,150
Aluminum	<30	Nickel	<100
Antimony	<40	Selenium	<0.3
Arsenic	560	Silver	<20
Barium	48	Strontium	13,300
Beryllium	<1	Thallium	<3
Bismuth	<60	Tin	<10
Boron	24,400	Titanium	<20
Cadmium	<2	Tungsten	<2
Chromium	<10	Vanadium	<2
Cobalt	<10	Zinc	57
Copper	<2		

[1] These water-quality data do not necessarily conform to U.S. Geological Survey guidelines for reporting significant figures

Hunter Drain

Hunter Drain water was acutely toxic to all species tested. This was the only water with salinity high enough to allow tests with saltwater species. Neither freshwater nor saltwater organisms survived in treatments of 100-percent or 50-percent drainwater. All species died quickly; 90 percent of the mysids, daphnids, and sheephead minnows died after 48 hours of exposure. After 9 days, at least 40 percent of every species had died in all dilutions (tables 10, 11).

The water quality in Hunter Drain was highly variable (table 6). Salinity ranged from 0 to 28 ppt. The highest salinity was measured on days 3, 4, and 5 under conditions of low flow when there was no visible discharge of irrigation water to the drain. During this 3-day period, the seepage of ground water into Hunter Drain was evident along the banks of the drain. The addition of operational spill water to the drain on day 6 was apparently responsible for the reduction in salinity and conductivity. All the bluegills died on day 4, after 24 hours of exposure to Hunter Drain water when salinity was 28 ppt, which was more than twice as high as on the previous day. Only 10 percent of the fish died on day 4 in other treatments exposed to this level of salinity, but the cumulative mortality of 20 percent in the controls on day 5 is probably related to the high salinity.

Survival, even for one day, of any bluegill at a salinity of 28 ppt was unexpected, based on accepted salt tolerances of the species. The ionic content of water in Hunter Drain and other Stillwater locations differed from that of seawater, which is typically used to estimate salinity tolerances of freshwater organisms. Although the test organisms were apparently more tolerant of salinity with these ionic ratios than of a similar salinity in seawater, it is probable that continued exposure to elevated salinity would have reduced survival in control water. It is unlikely that daily fluctuations in water quality of the magnitude experienced here would be tolerated by the tested species for an extended period.

Table 10. Cumulative mortality of bluegills, fathead minnow larvae, and daphnids exposed to water from Hunter Drain diluted with reconstituted water[1]

Species (and number sampled)	Exposure (days)	Cumulative mortality of species in tests (percent)					
		Proportion of drainwater in sample					NFCRC well-water control[2]
		100 percent	50 percent	25 percent	12.5 percent	0 percent	
Bluegills (10)	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	100	10	10	0	0	0
	4		60	10	10	10	0
	5		80	30	10	20	0
	6		80	30	20	20	0
	7		100	50	20	20	0
	8			60	30	20	0
	9			60	40	20	0
Fathead minnow larvae (20)	1	25	10	0	0	0	0
	2	40	50	0	0	0	0
	3	65	85	0	0	0	0
	4	70	90	20	10	0	0
	5	100	90	30	10	0	0
	6		100	45	25	0	0
	7			60	25	0	0
	8			60	25	0	0
	9			60	40	0	0
Daphnids (20)	1	25	20	0	0	0	0
	2	90	50	0	0	0	0
	3	100	85	0	0	0	0
	4		90	15	10	0	0
	5		90	30	10	10	0
	6		100	50	25	10	0
	7			50	25	10	0
	8			60	25	10	0
	9			75	50	10	0

[1] Deionized water reconstituted to the same hardness, alkalinity, specific conductance, and pH as the Hunter Drain water.
[2] Well water from National Fisheries Contaminant Research Center, Columbia, Mo.

Tests with saltwater species—sheephead minnow larvae and mysid shrimp—were initiated when salinity first exceeded 15 ppt on day 3. Total mortality of both species occurred in the 100-, 50-, and 25-percent dilutions after 7 days of exposure (table 11). The saltwater organisms were exposed under conditions of static renewal during the first 72 hours, but because the salinity of the drainwater decreased to 7 ppt on day 6, testing continued without renewal for the remainder of the 7-day exposure period. Dissolved-oxygen concentrations of the test water exceeded 6.4 mg/L throughout the study and pH ranged from 8.4 to 8.5. Mortality of both species of at least 55 percent occurred in all drain-water dilutions; no mortality occurred in the well-water controls.

Higher levels of trace-element concentrations corresponded with the higher levels of salinity. During days with elevated salinity, concentrations of arsenic in Hunter Drain were similar to those measured in TJ Drain; levels of boron and lithium in Hunter Drain were higher than at any other location, and molybdenum nearly so (table 6). Concentrations of dissolved selenium ranged from below detection limits to 3.6 µg/L and were highest during periods of highest salinity. No mercury was detected in samples from Hunter Drain. In addition, aluminum, antimony, beryllium, bismuth, chromium, cobalt, copper, iron, nickel, silver, tin, titanium, thallium, and tungsten did not exceed reporting limits.

Table 11. Cumulative mortality of saltwater species (sheephead minnow larvae and mysid shrimp) exposed to water from Hunter Drain diluted with reconstituted water[1]

Species (and number sampled)	Exposure (days)	Cumulative mortality of species in tests (percent)					
		Proportion of drainwater in sample					NFCRC well-water control[2]
		100 percent	50 percent	25 percent	12.5 percent	0 percent	
Sheephead minnow larvae (20)	4	60	45	25	20	0	0
	5	90	65	40	35	0	0
	6	100	75	55	40	0	0
	7		100	80	50	0	0
	8			100	55	0	0
	9				55	0	0
Mysid shrimp (20)	4	70	50	20	20	0	0
	5	90	65	35	15	0	0
	6	100	90	50	30	0	0
	7		100	70	55	5	0
	8			100	60	5	0
	9				60	10	0

[1] Deionized water reconstituted to the same hardness, alkalinity, specific conductance, and pH as the Hunter Drain water.
[2] Well water from National Fisheries Contaminant Research Center, Columbia, Mo.

Lead Lake

Water from Lead Lake was moderately toxic to all species tested; the death rate accelerated after 4 days (table 12). Survival rates of bluegill and fathead minnow larvae were similar; the lowest level of effect occurred in the 25-percent dilution. Daphnids were more sensitive to the toxic components in the water than were the fish, and died sooner. No species died in the 12.5-percent dilution or in the controls. Total survival of all organisms in the reconstituted control water, where salinity was similar to that in the Lead Lake water treatments, suggests that salinity alone did not account for the observed mortality.

Reproduction of daphnids was delayed in all treatments. No young were produced by daphnids in any Lead Lake water treatment and there was no evidence of development of a first brood in any individuals examined at the termination of the exposure. Young were produced in both the reconstituted and well-water controls on day 9. Mean number of young per brood in these controls was 8.9 and 9.2, respectively.

Levels of trace elements did not fluctuate markedly throughout the exposure period. Concentrations of selenium and mercury were below analytical reporting levels. In addition, aluminum, antimony, beryllium, bismuth, chromium, cobalt, copper, iron, nickel, silver, tin, titanium, thallium, and tungsten did not exceed reporting limits. Concentrations of arsenic, boron, lithium, and molybdenum were consistently higher in Lead Lake water than in either Paiute Diversion Drain or D-Line Canal, where no mortality was observed. The concentrations of these four trace elements, which were associated with water hardness and specific conductance, appear to be strongly influenced by inflow from TJ Drain. Concentrations of salts in Lead Lake may be naturally increased by evaporative loss, but the addition of water from TJ Drain does not enhance the water quality of Lead Lake.

Table 12. Cumulative mortality of bluegills, fathead minnow larvae, and daphnids exposed to water from Lead Lake diluted with reconstituted water[1]

Species (and number sampled)	Exposure (days)	Cumulative mortality of species in tests (percent)					
		Proportion of drainwater in sample					NFCRC well-water control[2]
		100 percent	50 percent	25 percent	12.5 percent	0 percent	
Bluegills (10)	1	0	0	0	0	0	0
	2	20	0	0	0	0	0
	3	20	0	0	0	0	0
	4	30	0	0	0	0	0
	5	30	20	0	0	0	0
	6	30	20	10	0	0	0
	7	40	20	10	0	0	0
	8	40	30	10	0	0	0
	9	60	30	10	0	0	0
Fathead minnow larvae (20)	1	0	0	0	0	0	0
	2	20	0	0	0	0	0
	3	20	5	5	0	0	0
	4	25	5	5	0	0	0
	5	25	5	10	0	0	0
	6	30	15	10	0	0	0
	7	40	20	10	0	0	0
	8	60	25	15	0	0	0
	9	60	30	15	0	0	0
Daphnids (20)	1	0	0	0	0	0	0
	2	20	0	0	0	0	0
	3	20	5	5	0	0	0
	4	25	5	5	0	0	0
	5	30	15	10	0	0	0
	6	30	15	10	0	0	0
	7	45	30	15	0	0	0
	8	60	35	20	0	0	0
	9	80	45	20	0	0	0

[1] Deionized water reconstituted to the same hardness, alkalinity, specific conductance, and pH as the Lead Lake water.
[2] Well water from National Fisheries Contaminant Research Center, Columbia, Mo.

Stillwater Point Diversion Drain and Stillwater Point Reservoir

Test organisms were differentially sensitive to water from Stillwater Point Diversion Drain. The water was toxic to bluegills and marginally toxic to fathead minnow larvae and daphnids (tables 13 and 14). Mortality occurred after an extended exposure to drainwater; no mortality of any species occurred during the first 3 days of exposure. Bluegill mortality reached 80 percent in undiluted water from Stillwater Point Diversion Drain. In the tests where drainwater was diluted with reconstituted water (table 13), no appreciable mortality occurred in the 12.5-percent dilution, but in tests using water from Stillwater Point Reservoir as a diluent, 20 percent of the fish died in the 12.5 percent dilution (table 14). In controls, no bluegills died in well water, 10 percent died in reconstituted water (table 13), and 20 percent died in Stillwater Point Reservoir water (table 14). Cumulative mortality of fathead minnow larvae and daphnids exposed to Stillwater Point Diversion Drain water diluted with reconstituted water (table 13) was lower than that of bluegills and ranged from 5 to 30 percent. Mortality of fathead minnows and daphnids exposed to Stillwater Point Diversion Drain water diluted with Stillwater

Point Reservoir water (table 14) ranged from 10 to 30 percent and was much lower than bluegill mortality, but generally higher than in tests using reconstituted water (table 13) as a diluent (5-30 percent), but with no clear pattern of dose response. No fathead minnow larvae or daphnids died in the reconstituted or the well-water controls, but in Stillwater Point Reservoir water controls, mortality was 25 percent (table 14).

The range in concentrations of the constituents in Stillwater Point Diversion Drain and Stillwater Point Reservoir remained less variable during the study than those in TJ and Hunter Drains (table 6). The ambient water was well oxygenated and of moderately low specific conductance. Turbidity in Stillwater Point Diversion Drain was similar to Paiute Diversion Drain. Concentrations of constituents also were similar to those found in Paiute Diversion Drain and in D-Line Canal (table 6). Mercury and selenium concentrations in Stillwater Point Diversion Drain were at or below analytical reporting levels. In addition, aluminum, antimony, beryllium, bismuth, chromium, cobalt, copper, iron, nickel, silver, tin, titanium, thallium, and tungsten did not exceed reporting limits.

Table 13. Cumulative mortality of bluegills, fathead minnow larvae, and daphnids exposed to water from Stillwater Point Diversion Drain diluted with reconstituted water[1]

Species (and number sampled)	Exposure (days)	Cumulative mortality of species in tests (percent)					
		Proportion of drainwater in sample					NFCRC well-water control[2]
		100 percent	50 percent	25 percent	12.5 percent	0 percent	
Bluegills (10)	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	20	20	10	0	0	0
	5	40	30	20	0	0	0
	6	40	30	20	10	0	0
	7	40	30	20	10	10	0
	8	60	30	20	10	10	0
	9	80	40	20	10	10	0
Fathead minnow larvae (20)	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	5	0	10	0	0	0
	5	5	5	10	0	0	0
	6	10	10	10	0	0	0
	7	10	10	10	0	0	0
	8	15	10	10	5	0	0
	9	30	10	10	5	0	0
Daphnids (20)	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	0	0	10	0	0	0
	5	10	10	10	0	0	0
	6	10	10	10	0	0	0
	7	15	10	10	0	0	0
	8	15	10	10	5	0	0
	9	25	10	10	5	0	0

[1] Deionized water reconstituted to the same hardness, alkalinity, specific conductance, and pH as the Stillwater Point Diversion Drain water.

[2] Well water from National Fisheries Contaminant Research Center, Columbia, Mo.

Table 14. Cumulative mortality of bluegills, fathead minnow larvae, and daphnids exposed to water from Stillwater Point Diversion Drain diluted with water from Stillwater Point Reservoir

Species (and number sampled)	Exposure (days)	Cumulative mortality of species in tests (percent)					
		Proportion of drainwater in sample					NFCRC well-water control[1]
		100 percent	50 percent	25 percent	12.5 percent	0 percent	
Bluegills (10)	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	30	20	10	0	0	0
	5	60	20	10	0	0	0
	6	60	20	10	0	0	0
	7	60	20	10	10	0	0
	8	70	30	20	10	10	0
	9	80	40	20	20	20	0
Fathead minnow larvae (20)	1	0	0	0	0	0	0
	2	0	0	0	0	5	0
	3	0	0	0	0	10	0
	4	10	15	10	0	10	0
	5	10	15	15	10	10	0
	6	15	20	15	10	15	0
	7	20	20	15	10	20	0
	8	25	20	15	15	20	0
	9	30	25	20	25	25	0
Daphnids (20)	1	0	0	0	0	0	0
	2	0	0	0	0	5	0
	3	0	0	0	0	5	0
	4	10	20	10	0	10	0
	5	10	20	10	0	10	0
	6	15	20	10	0	20	0
	7	25	20	10	0	20	0
	8	25	20	10	0	25	0
	9	25	25	20	20	25	0

[1] Well water from National Fisheries Contaminant Research Center, Columbia, Mo.

Because mortality in water from Stillwater Point Diversion Drain was higher than in apparently similar water from Paiute Diversion Drain and D-Line Canal, water samples from all drain sites were collected August 17, 1988, for chemical analysis of man-made organic constituents. No organic constituent was found at concentrations that might be expected to cause adverse effects on the test organisms. Consistent levels of phenols (2.6-5.4 µg/L) were detected in every sample, which suggests incidental contamination during sample collection or chemical analysis. Results from these analyses did not explain the mortality observed in Stillwater Point Diversion Drain water.

Mortality in undiluted water from Stillwater Point Reservoir was low, but consistent (20-25 percent). No substantial mortality of any species occurred in dilutions of Stillwater Point Reservoir water. Daphnids reproduced equally in all treatments on day 9, with the mean number of young per brood ranging from 8.0 to 9.5. The reservoir water was turbid, ranging from 168 to 580 NTU's, which may have stressed the test organisms in the 100-percent treatment. Concentrations of boron, lithium, and molybdenum in the reservoir (table 6) were generally similar to those in Stillwater Point Diversion Drain water. Concentrations of arsenic were only slightly lower than those in Lead Lake and TJ Drain and may have contributed to the observed mortality. Arsenic concentrations in the reservoir were approximately twice those in Stillwater Point Diversion Drain.

EFFECT OF IRRIGATION DRAINAGE ON AQUATIC ORGANISMS

Water from Hunter and TJ Drains and ground water from the well near TJ Drain was acutely toxic to all species tested (fig. 5). On the basis of effects of the drainwater on cumulative mortality and daphnid reproduction, the "No Observed Effect Concentration" (NOEC)[1] for both Hunter and TJ Drains was less than 12.5 percent drainwater. Lead Lake water was moderately toxic to all species tested, with an NOEC of 25 percent drainwater for both fish species and an NOEC of less than 12.5 percent for daphnids. In tests with Stillwater Point Diversion Drain water diluted with Stillwater Point Reservoir water, where mortality occurred in every treatment, an NOEC was not established, but in tests with Stillwater Point Diversion Drain water diluted with reconstituted water, the NOEC was 25 percent for daphnids and fathead minnows and 12.5 percent for bluegill. Water samples from Paiute Diversion Drain and D-Line Canal were not toxic to the species tested.

[1]A time-independent measure that describes the threshold concentration below which predefined effects are not observed.

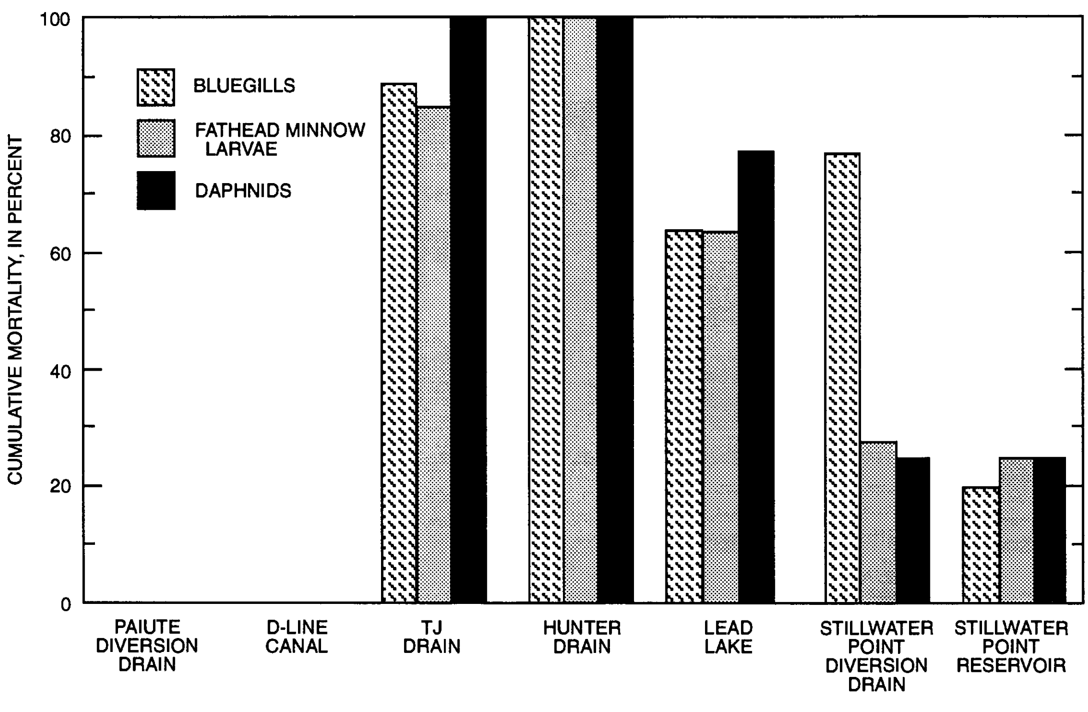


Figure 5. Cumulative mortality of freshwater organisms in undiluted surface-water samples collected in and near Stillwater Wildlife Management Area, August 1988. Data for Paiute Diversion Drain and D-Line Canal showed no mortality.

In both Hunter and TJ drains, dramatic daily fluctuations in water quality were measured, and specific conductance ranged from 410 to 27,500 µS/cm (about 270 to 17,900 mg/L dissolved solids) in Hunter Drain and from 6,100 to 14,900 µS/cm (about 4,000 to 9,700 mg/L dissolved solids) in TJ Drain (fig. 6). Control organisms in reconstituted water, where conductance was similar to that of the drainwater, survived exposure to daily fluctuations of similar magnitudes. Saltwater organisms, sheephead minnow larvae and mysids, did not survive in Hunter Drain water although the salinity was within their range of tolerance.

A dose-response relationship was evident in TJ drainwater when diluted with reconstituted water of a similar salinity and when salinity was decreased by dilution with water from D-Line Canal. From 85 to 100 percent of the organisms died in Lead Lake water; controls with similar specific conductance (that is, salinity) showed no mortality. Although the elevated salinity in tests with drainwater from both locations undoubtedly stressed the organisms, the results suggest that salinity alone does not account for the mortality observed.

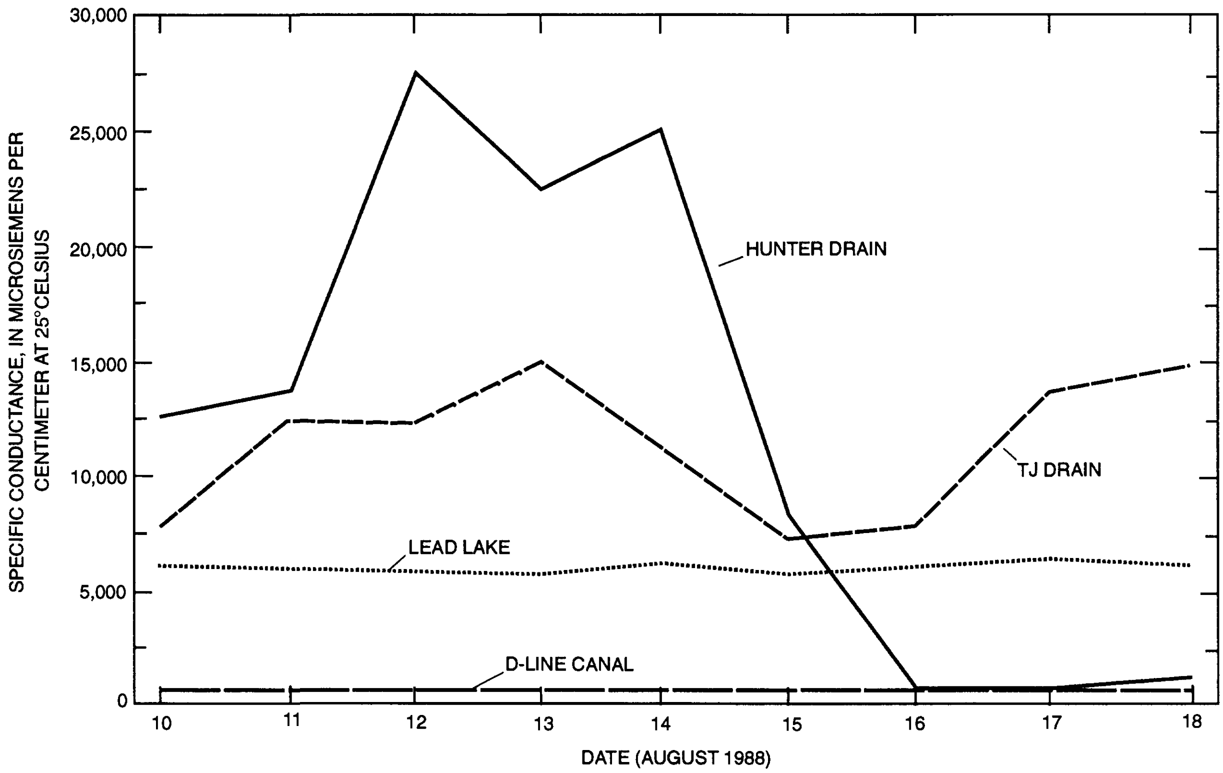


Figure 6. Changes in specific conductance in Lead lake and selected drains during toxicity study, August 10-18, 1988.

In general, higher concentrations of trace elements corresponded to higher levels of specific conductance (fig. 7). In addition, subjective comparison of cumulative mortalities and concentrations of trace elements at each location suggested that the concentrations or arsenic, boron, lithium, and molybdenum were higher at locations where appreciable mortality occurred. No single trace element was present in concentrations that were acutely toxic to the species tested (U.S. Environmental Protection Agency, 1986); therefore, the toxicity is attributed to the interactive effects of the aggregate trace elements present. This conclusion is supported by the results of Dwyer and others (1990, p. 18) who exposed striped bass (*Morone saxatilis*) to these elements and also to copper and strontium, both by single element and in combination. Individually, these six elements were not acutely toxic to the bass in the concentrations found at Stillwater WMA, but in aggregate were acutely toxic.

Ionic Composition

In assessing the hazard to aquatic organisms, the ionic composition of the water should also be considered. For example, in a study subsequent to this one that used striped bass in water reconstituted to resemble Pintail Bay in Stillwater WMA, Dwyer and others (1990, 1992) concluded that the unusually low hardness of this saline water did not diminish toxicity of the combined trace elements—arsenic, boron, copper, lithium, molybdenum, and strontium.

In the present study, water from the shallow well near TJ Drain was acutely toxic to bluegills, larval fathead minnows, and daphnids. This ground water was classified as hard, with the calcium concentration about two-fold higher than the nearby TJ Drain water. Mortality is attributed primarily to a combination of toxic trace elements regardless of the benefit of increased hardness. In follow-up work by Dwyer and others (1990, p. 26), using water from five additional wells in and near Stillwater WMA, acute toxicity to striped bass—a salt tolerant species—was demonstrated.

Thus, salinity alone may not explain the mortality observed in certain drainwater, but rather a mixture of trace elements and atypical ion ratios (compared to seawater; Ingersoll and others, 1992).

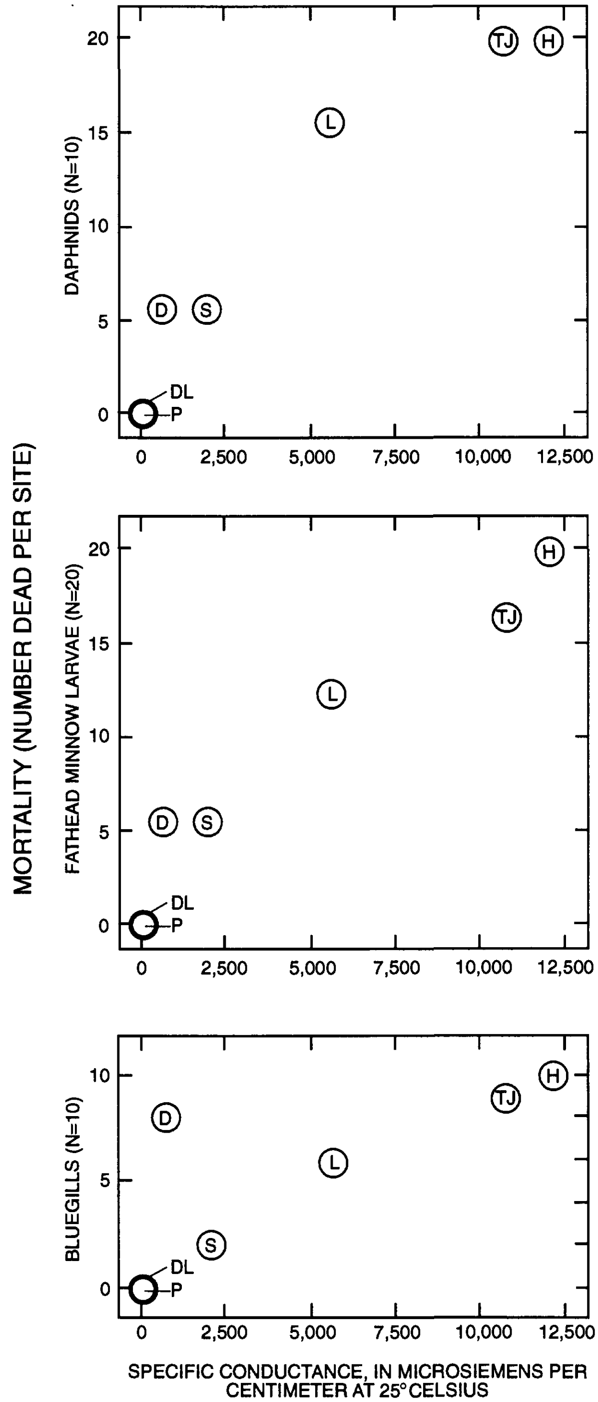


Figure 7. Relations between specific conductance and mortality of bluegills (r=0.88), fathead minnow larvae (r=0.93), and daphnids (r=0.97) for water from Paiute Diversion Drain (P), D-Line Canal (DL), TJ Drain (TJ), Hunter Drain (H), Lead Lake (L), Stillwater Point Diversion Drain (D), and Stillwater Point Reservoir (S).

(p. 39 follows)

BIOLOGICAL PATHWAYS: MOVEMENT OF SELENIUM AND MERCURY

Some potentially toxic trace elements in irrigation drainage are known to biomagnify up the foot chain, thus affecting wildlife of an area, particularly waterfowl. Selenium and mercury are important trace elements in the study area and both are known to biomagnify.

STUDY AREAS

Three areas were selected for in-depth study of biological pathways: TJ Drain/Lead Lake and Hunter Drain/Goose Lake systems in Stillwater WMA (fig. 8), and A-Drain and ponds of the Fernley WMA (fig. 9). These lakes and ponds include emergent wetlands and are managed with water-control structures. Additional samples were collected from other wetlands and from irrigation drains throughout the Fallon agricultural area of the Newlands Irrigation Project area (table 15, fig. 10) to provide a broader scope of the extent of high selenium and mercury concentrations.

Lead Lake, which has three major water sources, is the most hydrologically complex of the areas. It is a 1,000-acre, initial wetland unit that receives irrigation drainage directly before much of the water evaporates and constituents become concentrated; therefore, its water quality is commonly better than that in the connecting wetlands downgradient. However, Lead Lake has a history of avian botulism and unexplained fish and migratory bird deaths, and most of the emergent vegetation has perished in the last 30 years. Selenium was found in the headwaters of the recently constructed (1982-1983) TJ Drain, which ultimately discharges into Lead Lake (U.S. Bureau of Reclamation, 1987, p. B14). Selenium accumulation in juvenile migratory birds from Lead Lake was documented by Hoffman and others (1990, p. 67).

Hunter Drain was included in the pathways study because refuge biologists had noted exceptionally high specific conductance (Steven P. Thompson, U.S. Fish and Wildlife Service, oral commun., 1988) and selenium concentrations above effect level in some juvenile birds from Goose Lake, at the drain terminus (Hoffman and others, 1990, p. 66). This 9-mile drain, which serves only about 180 acres of irrigated land, is a relatively simple flow system. Discharge is typically less than 1 ft^3/s, but operational releases occasionally cause flow peaks of relatively fresh water.

Fernley WMA, near Stillwater WMA, was studied because selenium in livers of juvenile migratory birds exceeded effect levels (Hoffman and others, 1990, p. 67). About 40 percent of the inflow is ground water from Truckee Canal seepage (Van Denburgh and Arteaga, 1985, p. 6), which passes through the same ancient lake-bed sediments as does the drainage from the Stillwater Wildlife Management Area. Most of the water enters the wetlands through A-Drain (from irrigation drainage) and discharges into South Pond, then into a series of connecting ponds (fig. 9).

Most of the additional samples for selenium and mercury were collected principally from drains throughout the irrigation area—about 60,300 acres served by about 350 mi of drainage ditches. Sites were selected to represent, approximately, each 1- to 2-mi^2 area of irrigated land in the study area. These sites are shown in fig. 10 and listed in table 15.

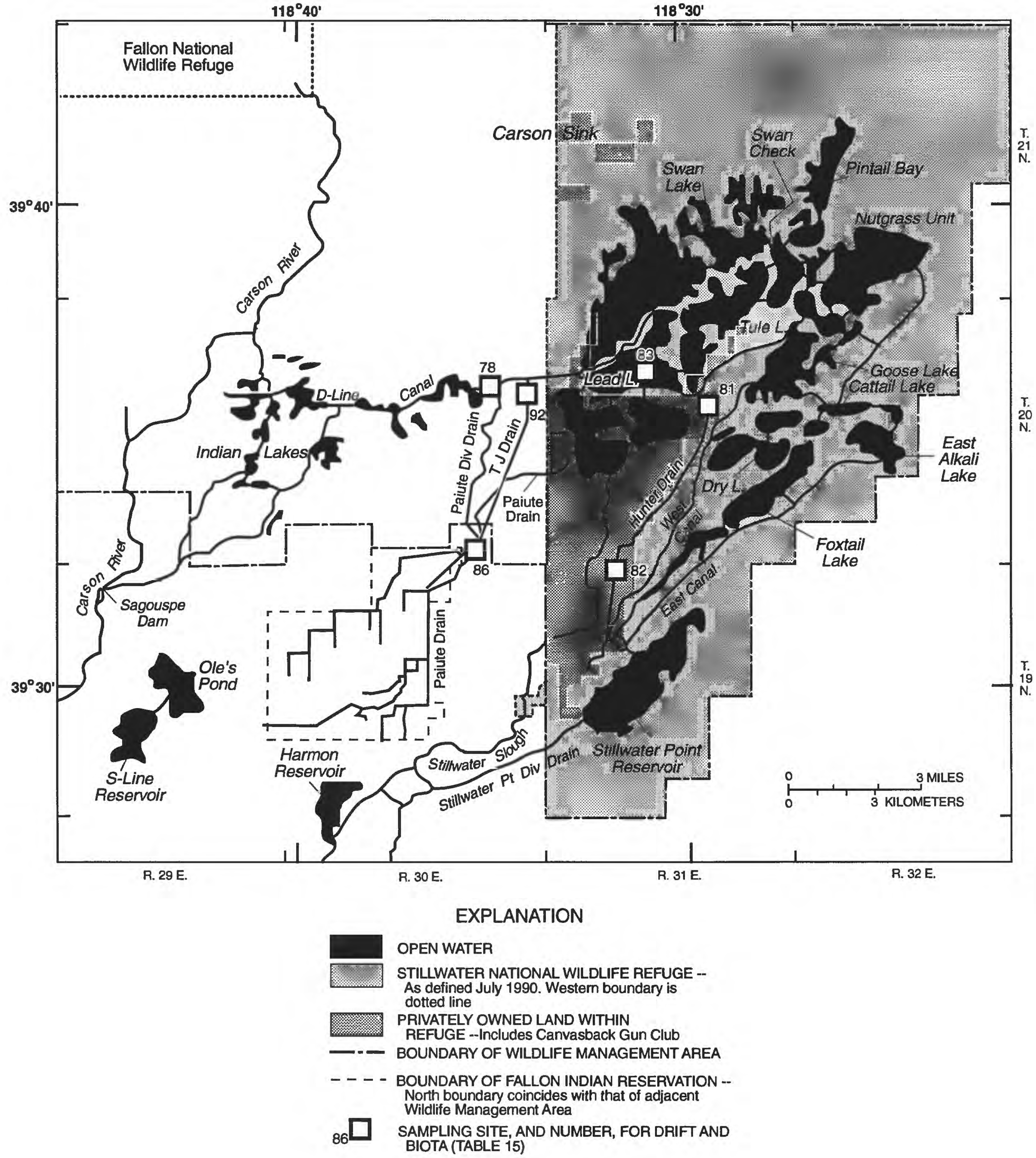


Figure 8. Location of sampling sites for drift and biota in and near Stillwater Wildlife Management Area. Sites are listed in table 15. (Map modified from Lico, 1992.)

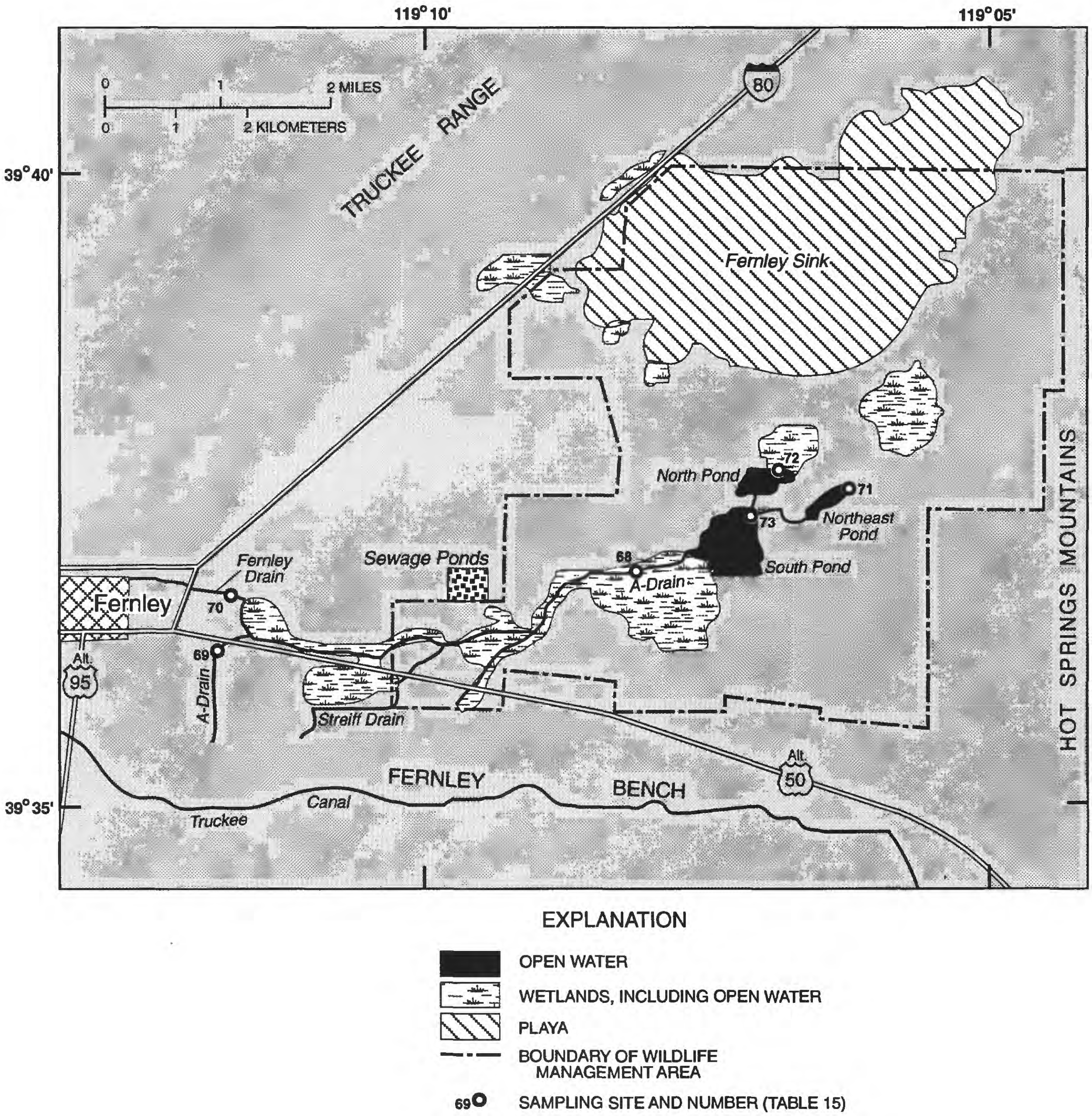


Figure 9. Location of sampling sites for drift and biota in and near Fernley Wildlife Management Area. Sites are listed in table 15. (Map modified from Lico, 1992.)

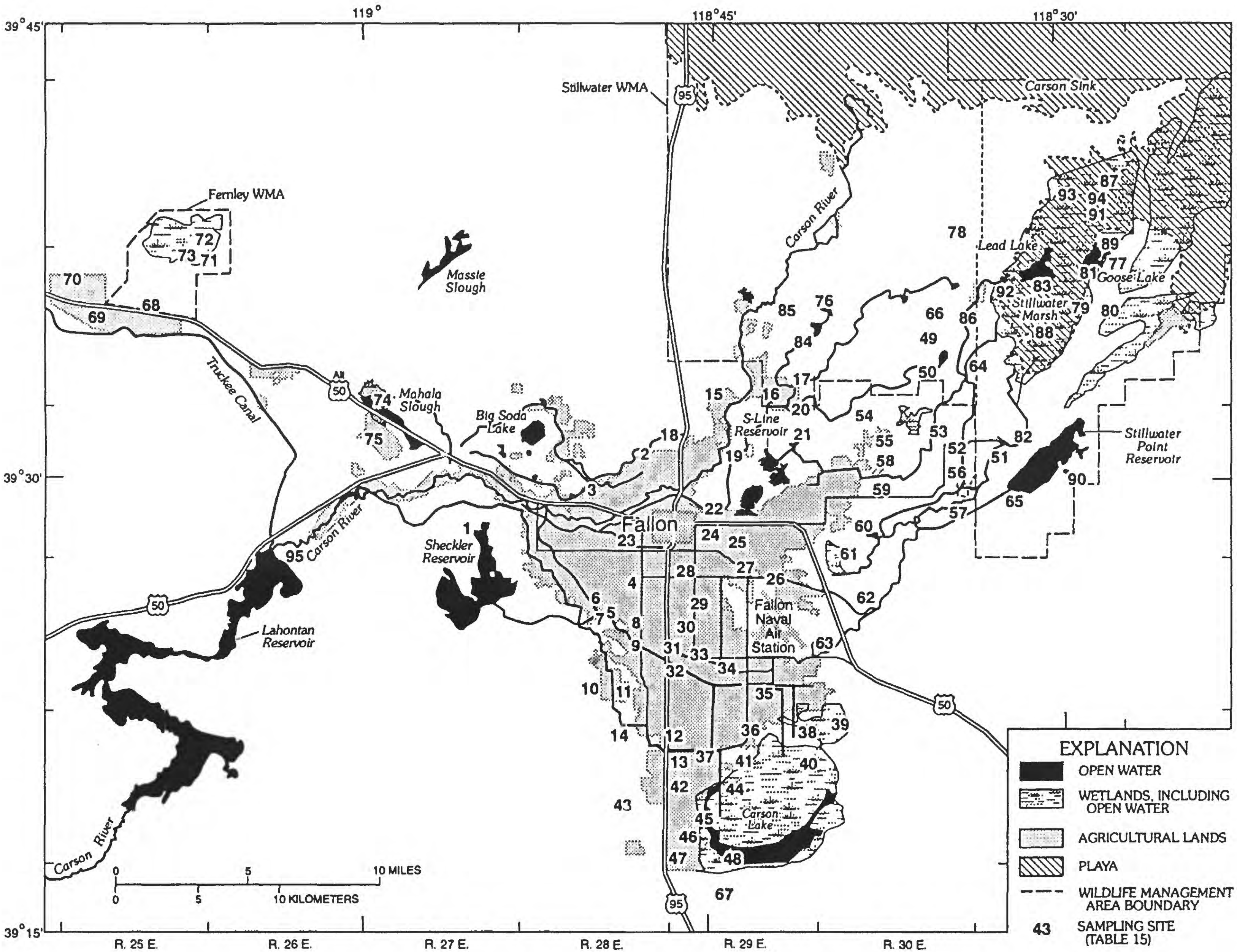


Figure 10. Location of additional sampling sites for detritus and algae in and near Stillwater and Fernley Wildlife Management Areas and Carson Lake. Site names are listed in table 15. (Map modified from Seiler and Allander, in press.)

Table 15. Sampling sites for drift, detritus, and algae

[Locations are shown in figures 8, 9, and 10; data were published by Rowe and others (1991, tables 20, 21)]

Fallon agricultural area

2. Old Reservoir
3. Soda Lake Drain
4. Upper L Drain
5. South Carson River Drain
6. Sheckler Deep Drain
7. Upper West Side Drain
8. Upper Diagonal 2 Drain
9. Upper Diagonal Drain
10. Gummow Drain
11. Carson Lake I Extension Drain
12. Carson Lake Drain
13. Carson Lake 1A Drain
14. A1 Drain
15. Mussi Drain
16. F2 Drain
17. Shaffner Drain
18. ERB Drain
19. Lower Soda Lake Drain
20. Paiute Extension Branch 1 Drain
21. Harmon I Deep Drain
22. S-Line Reservoir
23. New River Extension Drain
24. Upper Harmon Deep Drain
25. Harmon 2 Drain
26. New River Drain
27. Upper New River Drain
28. LD Drain
29. Mid L Drain
30. L3 Drain
31. L2 Drain
32. Upper Diagonal Drain[1]
33. Middle L Drain
34. LB Drain
35. L Branch 1 Deep Drain
36. Lower L Drain
37. Mid Carson Lake Drain
38. A-Line Canal
39. Pierson Drain
40. J1 Deep Drain
41. Yarbrough Drain
42. Carson Lake Branch 3 Drain
43. Carson Lake Branch 1 Drain
44. Downs Drain
54. R2 Drain
55. Upper Paiute Drain
58. Harmon Deep Drain
59. S2G Drain
60. Harmon Reservoir
61. S1B Drain
62. Lower Diagonal 1 Drain
63. Lower Diagonal Drain

Carson Lake area

45. Lower Carson Lake Drain
46. Holmes Branch 2 Drain
47. Holmes Drain
48. NE Carson Lake
67. Carson Lake, Sprig Pond

Stillwater Wildlife Management Area

49. Patrick Drain
50. Kent Lake Extension Drain
51. Lower Stillwater Slough
52. Paiute Branch 3 Drain
53. S1 Deep Drain
56. S2C Drain
57. Upper Stillwater Slough
64. Paiute Branch 1 Drain
65. Stillwater Point Reservoir
66. Upper TJ Drain
76. Big Indian Lake
77. Cattail Lake
78. D-Line Canal
79. Dry Lake East
80. Dry Lake West
81. Hunter Drain
82. Hunter Drain South[1]
83. Lead Lake
84. Likes Lake
85. Papoose Lake
86. Paiute Drain
87. Pintail Bay
88. South Lead Lake
89. South Nutgrass
90. Stillwater Point Reservoir
91. Swan Lake Check
92. TJ Drain
93. Tule Lake
94. West Nutgrass

Fernley Wildlife Management Area

68. Lower A-Drain[1]
69. Upper A-Drain[1]
70. Fernley drain, west
71. Fernley WMA, East Pond
72. Fernley WMA, North Pond
73. Fernley WMA, South Pond

Background and other sites

1. Sheckler Reservoir
74. North Mahala Slough
75. South Mahala Slough
95. Carson River Below Lahontan

[1] Some sites were mislabeled in tables 20 and 21 of Rowe and others (1991): Site 32, Upper Diagonal Drain (sample number 88926), was mislabeled as site 63, Lower Diagonal Drain; site 82, Hunter Drain South (sample 89058), was mislabeled as site 81; site 68, Lower A-Drain (samples 89067 and 89202), was mislabeled as Fernley Drain; and site 69, Upper A-Drain (sample 88171), was mislabeled as Fernley South Drain.

APPROACH AND METHODS

On the basis of the findings of Hoffman and others (1990) that concentrations of selenium and mercury in filtered water samples were low, it was thought that selenium, and possibly mercury, were moving through biological pathways and that transport was through organic rather than inorganic forms. No uniform set of plants or animals was found in all study sites, and living organisms were not always available; therefore, organically rich detritus was sampled to provide continuity throughout the study areas. Because it is lightweight, detritus transports easily down irrigation drainage ditches through routine operational releases and other peak flows. Living plant material, principally filamentous algae, and drift also were collected where available. Definitions of detritus and drift and the sampling procedures used are described by Rowe and others (1991, p. 11-13).

Qualitative analysis of the digestive tracts in birds was made to verify that organisms included in trace-element analysis were indeed diet items of migratory birds. The birds were primarily coots (*Fulica americana*), black-necked stilts (*Himantopus mexicanus*), and avocets (*Recurvirostra americana*), but ducks also were examined. The extent of digestion of the gut contents was a major variable because the elapsed time between collection and preservation varied widely.

A one-time estimate of the standing crop of vegetation (filamentous algae and submergent vascular plants) throughout the TJ Drain system was made August 27, 1990. The drain was divided into 1.0-mi segments, and the width of the drain was measured at 0.5-mi intervals. Transects were made at the midpoint of each segment as determined by measuring with a vehicle odometer. The transects consisted of either three or five evenly spaced sample sites across the width of the drain. Five samples were collected where drain width exceeded 10 ft. A Surber square-foot sampler was used to define the individual sample area. The attached net captured the vegetation after it was severed by the investigator and released into the current. Composite plant samples were frozen in 1-gal jars. Samples were later thawed, hand rinsed, drained, and weighed wet. Dry weights were obtained by drying in an oven at 104°C until weight remained constant.

To determine the source areas of selenium and mercury, sample points were selected throughout the drainage system in such a way that each point represented drainage from about a 1-2-mi^2 area of irrigated land. From these sites, organic detritus and filamentous algae, if present, were collected and analyzed. Although many of the managed wetland units were dry because of the drought, the wetlands sampled contained water. To identify source areas where selenium and mercury are most available biologically, the data sets for both detritus and filamentous algae were displayed through a geographic-information system.

RESULTS

Examination of the upper digestive tracts of 66 migratory birds from Carson Lake and Stillwater WMA confirmed that algae, vegetation, mixed drift, and insects were consumed by migratory birds in the study area. Content of the digestive tracts varied considerably between species and between individuals of the same species. Leeches (*hirunids*), ostracods, and daphnids (*Daphnia magna*) found in drift were not found in gut contents, but these relatively soft-bodied organisms may have been fragmented in the gizzard and therefore difficult to identify. Seeds of emergent vascular plants were found in digestive tracts of ducks. Digestive tracts of 11 black-necked stilts were examined and found to contain primarily water boatmen (*corixids*). No adult brine flies (*Diptera*)were found in the black-necked stilt digestive tracts examined, but stilts have been observed feeding heavily on brine flies at times in Hunter Drain (Steven P. Thompson, Stillwater WMA, U.S. Fish and Wildlife Service, oral commun., 1988).

Because plant material dominated the biomass in the drains and wetlands, detritus was assumed to be primarily of plant origin, with associated microorganisms, and did indeed contain recognizable plant and animal parts. Organically rich sediment, varying in depths at the soil-water interface, was the lightest fraction of the bottom sediment (typically 85-90 percent water). Because of its light-brown color, detritus appeared to be oxygenated and in chemical contact with the water column. Detritus was in direct contact with the most abundant benthic insect larvae in the study areas, midge (*Chironomus sp.*) and brine flies; invertebrates were not typically found in anaerobic sediments below the detrital layers at the sampled locations. All data collected were reported by either Hoffman and others (1990) or Rowe and others (1991).

Detritus sampling was a significant factor in establishing the biological pathways by which selenium and mercury moved into wildlife. A total of 112 composite detritus samples from all sites were analyzed. The concentrations of selenium and mercury, respectively, ranged from <0.09 to 8.04 and from <0.04 to 97.8 µg/g, dry weight.

Exceptionally high concentrations of mercury (26-98 µg/g) were found in three detritus samples from Indian Lakes within Stillwater WMA and from ten irrigation-drain samples. Long and Morgan (1990, p. 41) suggest that concentrations greater than or equal to 1.0 µg/g mercury, dry weight, in sediment would adversely affect exposed invertebrates. This criterion was exceeded in 47 of 112 (42 percent) of the detritus samples analyzed (Rowe and others, 1991).

Lemly and Smith (1987, p. 9) predicted reproductive failure or mortality in fish and waterfowl exposed to sediment containing selenium concentrations greater or equal to 4.0 µg/g, dry weight, because of food-chain bioaccumulation. This criterion was exceeded in only 4 of the 112 detritus samples (4 percent); 3 of those were from Fernley WMA.

Filamentous algae were not present at all detritus sampling sites. The concentrations of mercury and selenium in 87 algae samples ranged from <0.02 to 10.4 and from <0.06 to 5.6 µg/g, dry weight, respectively. The criteria used to evaluate mercury and selenium concentrations in algae are based on dietary bioaccumulation in fish and birds. The effect criterion for mercury in bird diets is 0.39 µg/g, dry weight (Heinz, 1979, p. 395; Hoffman and others, 1990, p. 26), and for selenium in fish diet is 5.0 µg/g, dry weight (Lemly and Smith, 1987, p. 9) The mercury criterion was exceeded in 49 of 87 (56 percent) filamentous algae samples. The selenium criterion was exceeded in only two samples.

Samples of drift were taken for qualitative biological pathway evaluations. The rates of drift movement were not quantified and the data are qualitative. Most of the 55 samples were taken from the Lead Lake, Fernley WMA, and Hunter Drain systems. Sample contents and volumes were highly variable between sites and within sites sampled at various times, but most macroinvertebrates and aquatic plants were in drift. The exception—emergent aquatic plants—were not identified in drift, but may have contributed to the undifferentiated detritus parts. Dominant parts of drift included algae, submergent vascular plants, detritus, daphnids, ostracods, amphipods (*Gammarus sp.*), corixids, chironomids, brine flies, leeches, and odonates.

Selenium was detected in most drift samples, with the highest concentration (10.0 µg/g, dry weight) found in daphnids from South Pond in Fernley WMA. Eight of the 55 (15 percent) samples contained concentrations of selenium greater than the 5.0 µg/g dietary criterion that has been shown to cause reproductive failure or mortality in fish through food-chain bioconcentration (Lemly and Smith, 1987, p. 9). Concentrations in seven of these samples were greater than 7.0 µg/g, dry weight. This concentration in the diet also may cause reproductive failure or mortality in some migratory birds (G.J. Smith, U.S. Fish and Wildlife Service, oral commun., 1989).

Although no attempt was made to establish contaminant loading estimates in aquatic drift, a one-time estimate of the standing crop of all vegetation in TJ drain was made August 27, 1990. The instantaneous standing crop of vegetation in the 17-mi-long TJ Drain was about 15,500 kg (17 tons), dry weight. Using the selenium concentration range of 0.61-2.1 µg/g for vegetation, there was at that time between 9.5 and 31.9 g of selenium fixed in vegetation. This estimate was made several days after a high discharge event when there was visual evidence that large quantities of algae and vascular plants had recently been flushed downstream. Selenium is also found in invertebrates and other animals and in detrital matter, but no attempt was made to estimate the standing crop of these groups because little remained after the discharge.

Brine fly adults were sampled where available. Six of the twelve samples (50 percent) contained selenium in excess of the 7.0 µg/g, dry weight, dietary criterion for birds (G.J. Smith, U.S. Fish and Wildlife Service, oral commun., 1989).

Bioaccumulation and Biomagnification of Selenium and Mercury

Selenium concentrations in filtered water samples from the general study area, including the lower TJ Drain and Lead Lake, were at or below the analytical reporting limit of 1.0 µg/L (Hoffman and others, 1990, p. 77). Higher selenium concentrations, as much as 46 µg/L, were found in filtered sample water from the headwaters of the TJ Drain system (Tokunaga and Benson, 1991, p. 20, 28). Similarly, in California at the Grasslands Water District and Kesterson National Wildlife Refuge, dissolved selenium was quickly absorbed into biota, primarily plants, resulting in low concentrations in water (Presser and Ohlendorf, 1987, p. 811, 815).

Selenium and mercury are not homogeneously distributed in the water or surficial soils within the study area (U.S. Bureau of Reclamation, 1987, p. B14; Ronald R. Tidball, U.S. Geological Survey, oral commun., 1989). Selenium concentrations in water are not a reliable indicator of the magnitude of contamination of selenium in organic matter (Hoffman and others, 1990, p. 77). Evidence of bioaccumulation of mercury in biota was found where dissolved mercury concentrations in water were below the analytical reporting limit (1990, p. 36, 60).

Both selenium and mercury are known to bioaccumulate in detritus. Lemly and Smith (1987, p. 5) discussed the potential importance of detritus pathways leading to fish and migratory birds in situations where selenium may not be detectable in water. Depending on waterway morphology and flow, detritus may accumulate and be available to consumers for several years.

Maximum observed concentrations of selenium and mercury in detritus were 8 and 98 µg/g, dry weight, respectively. Sites with high selenium concentrations in detritus correlate with sites where Hoffman and others (1990, p. 67-70) found high selenium concentrations in juvenile bird livers and muscle. The highest mercury concentration found in detritus was from Indian Lakes, which corresponds to data from Cooper and others (1985, p. 57) that showed elevated mercury concentrations in fish and fish fillets from Indian Lakes. Detritus appears to be a useful medium with which to qualitatively assess the spatial distribution of biologically available selenium and mercury.

Filamentous algae and vascular aquatic plants also may bioaccumulate selenium (Presser and Ohlendorf, 1987, p. 811; Lemly and Smith, 1987, p. 4) or mercury (Eisler, 1987, p. 21), or selenium and mercury (Hoffman and others, 1990, p. 62). In this study, concentrations of selenium and mercury in algae were similar to those in detritus at the same locations. Algae were widespread and nearly as useful as detritus in understanding the dynamics of selenium and mercury movement in irrigation drains and wetlands.

Biomagnification of selenium was documented in juvenile black-necked stilts from Lead Lake (Hoffman and others, 1990, p. 67). Data from the following section on waterfowl production show that selenium concentrations in juvenile bird (cinnamon teal [*Anas cyanoptera*] and coot) tissues were greater than 10,000 times those found in water, and that some migratory birds had accumulated selenium to levels at which mortality or reproductive impairment would be expected.

In the Stillwater study area, bioaccumulation appeared to occur primarily in the irrigation drains. Biomagnification also occurred in drains, but was more evident in the initial wetlands, as was the situation in Grasslands Water District, California, where the largest concentrations of selenium were found in animal tissue collected in the initial wetlands to receive selenium-bearing water (Presser and Ohlendorf, 1987, p. 815).

Selenium and Mercury Pathways

Algae and other aquatic vegetation have been observed moving down the drains. Much of this movement appears to be associated with peak discharges resulting from operational spills or heavy rainfall. Assessments of the vegetation standing crops in TJ Drain followed a large storm that apparently had flushed large quantities of vegetation downstream to Lead Lake. About 15,500 kg, dry weight, of vegetation remained.

The biological components actually being transported were isolated in the drift samples. The irregular mixture of vegetation and invertebrates in these samples makes the data appropriate only for qualitative evaluation. Analysis of drift samples indicates that selenium was being bioaccumulated in plants, biomagnified in invertebrates, and that both components in drift and detritus were transported to wetlands by drain flow.

Figure 11 illustrates the flow path of selenium in particulate matter to downgradient wetlands. The TJ Drain appears to be the largest contributor of selenium to Lead Lake. Paiute Diversion Drain seasonally supports large biomasses of algae and vascular plants, which also may be an important source of selenium to Lead Lake. Biological samples from wetland sites downgradient of Lead Lake, which are maintained by Lead Lake water, were generally lower in selenium than those from Lead Lake.

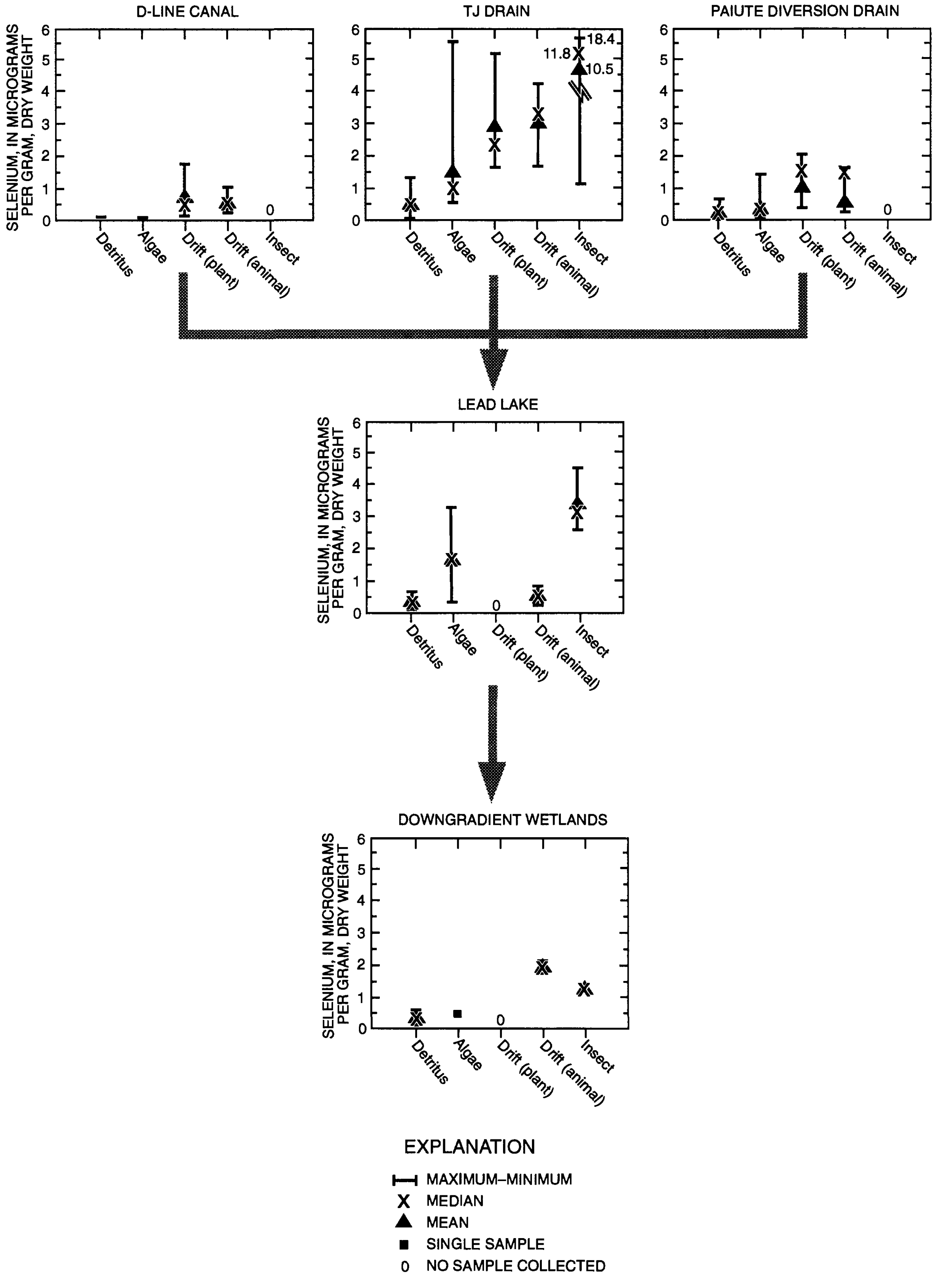


Figure 11. Generalized flow path in Stillwater Wildlife Management Area and selenium concentrations in composite samples from input drains, Lead Lake, and downgradient wetlands. Data from Hoffman and others (1990, table 19) and Rowe and others (1991, tables 20 and 21).

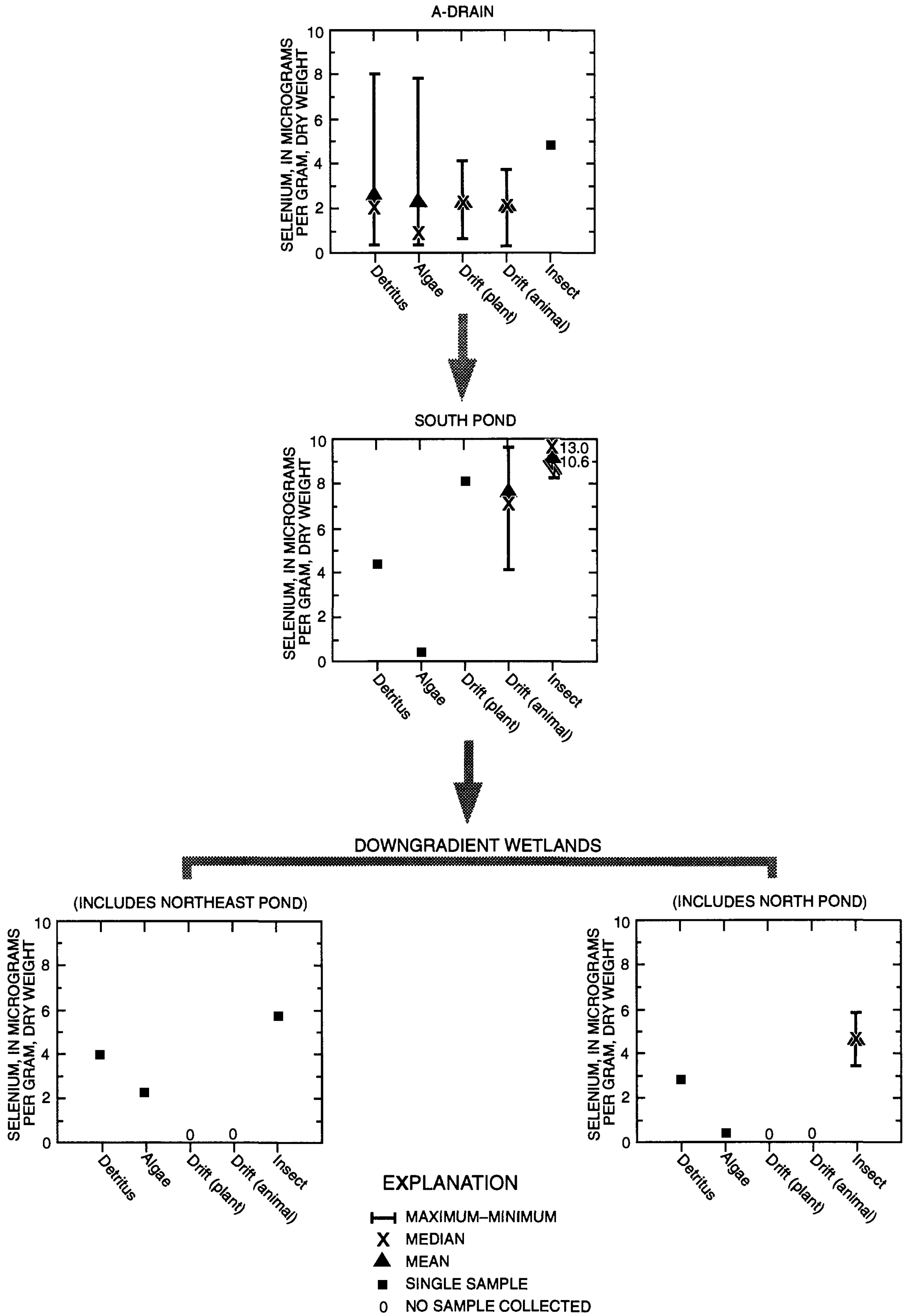


Figure 12. Generalized flow path and selenium concentrations in composite samples from A-Drain, South Pond, and downgradient wetlands, Fernley Wildlife Management Area. Data from Hoffman and others (1990, table 19) and Rowe and others (1991, tables 20 and 21).

Water is lost through evapotranspiration from Lead Lake and from managed ponds or wetlands downgradient to the extent that dissolved solids are concentrated two to six times in each successive wetland (U.S. Fish and Wildlife Service, 1988, p. 51). This process is cumulative, and dissolved-solids concentrations in the last wetland frequently exceed concentrations in seawater. Data on organically bound selenium from this study and on dissolved selenium (Hoffman and others, 1990, p. 37) do not conform to the simple evaporative patterns predicted from these concentration processes. Dissolved selenium is not a conservative variable; it was least present in water in the downgradient wetlands where dissolved solids were the most concentrated. Presser and Ohlendorf (1987, p. 811) also found that selenium distribution in sequentially arranged ponds was inversely related to that which would be predicted through evaporative models. Therefore, evapotranspiration does not appear to be an important mechanism contributing to selenium concentration in surface water.

Inorganic sediment deposition also is not believed to be playing an important role in selenium pathways within Lead Lake. Organic pathways were probably more effective at removing selenium from water than inorganic processes in the Grasslands Water District (Presser and Ohlendorf, 1987, p. 811). Although Lead Lake has received selenium-bearing drift for 7 years from TJ Drain and for more than 40 years from Paiute Diversion Drain, selenium concentrations in the upper 2-3.5 in. of the bottom sediment in Lead Lake averaged only 0.65 μg/g (mg/kg: Hoffman and others, 1990, p. 113), near the median range of concentrations in various organisms and detritus in Lead Lake. These concentrations suggest that, in this system, selenium is not accumulating or immobilizing in inorganic sediment over the long term. Roots of aquatic plants could have an active role in mobilizing selenium out of inorganic sediment (Lemly and Smith, 1987, p. 4), but most of the rooted aquatic plant beds have perished from Lead Lake over the last 30 years.

Organically bound selenium concentrations recorded in biota from Lead Lake are similar to those of the imported organisms and detritus found in drains (fig. 11). This indicates that an equilibrium between import and export exists in Lead Lake, which has been continuously flooded for about 10 years. Even with significant evaporative concentration processes, selenium concentrations in organisms from wetlands downgradient from Lead Lake are lower than those within the lake. Selenium volatilization may be a component of such an equilibrium (Ohlendorf, 1989, p. 139). Frankenberger and Thompson-Eagle (1989, p. 2) have documented volatilization of selenium from saline evaporation-pond water. The bacteria and fungi that were acting on the available selenium to cause this process were particularly effective in protein-rich environments. Organic detritus is a protein source and may facilitate volatilization of selenium in the study areas.

The selenium hazard to fish and wildlife in the Lead Lake system is largely determined by both concentration and length of exposure. Habitat size and configuration are important physical variables that determine the extent of wildlife use and exposure. The TJ Drain system is about 17 mi long, contains about 20 surface acres of flowing water, and attracts small numbers of migratory birds, primarily ducks. Conversely, Lead Lake covers about 1,000 acres and supports thousands of waterfowl. Because of relatively limited use, direct exposure of waterfowl to selenium in the TJ Drain is of lesser concern.

Concentrations of selenium in all types of biological samples from Fernley WMA were generally greater than those from the Lead Lake system. The relative concentrations of selenium in various organisms and spatial relationships are shown in figure 12. The relative concentrations of selenium in A-Drain, South Pond, and downgradient wetlands resemble the pattern described for the Lead Lake system but, like the Grasslands Water District (Presser and Ohlendorf, 1987, p. 815), bioaccumulation was greatest in the initial wetland.

Concentrations of selenium in drift, algae, and adult brine flies in Hunter Drain in Stillwater WMA (fig. 13) are comparable to those in both Fernley WMA and TJ Drain.

Mercury released to the Carson River in the late 1800's by gold and silver milling practices has contaminated the river sediment downstream of the Comstock mining district near Virginia City, Nev. (Smith, 1943; Cooper and others, 1985). Contaminated sediment is present over a large part of the Carson Desert, terminus of the Carson River. Mercury was deposited in the area through flooding prior to the construction of Lahontan Dam in 1915, and remains biologically available within Indian Lakes to this day. However, it appears that mercury export through the D-line Canal, a ditch that feeds the Indian Lakes, was minimal. The median concentration of mercury in composite detritus samples from

Indian Lakes, in Stillwater WMA, was about 53 µg/g, dry weight. In contrast, the median mercury concentration in detritus from the D-line Canal downstream from Indian Lakes was only 0.13 µg/g, which is comparable to background sites and at a level about 400 times lower than that in Indian Lakes.

Mercury concentrations found in 89 detritus and 76 algae samples from drains within the area ranged from <0.04 to 38.6 µg/g, and <0.02 to 10.4, respectively. Most of these high concentrations were from drainage/wetland systems not previously studied in detail for selenium accumulation. The mechanism(s) through which mercury was sorbed onto or into detritus and algae was not determined. Although drift was not examined in these drains, mercury was found in algae and detritus from drift moving down the drains in the area. Many of the samples with high mercury concentrations are from sites in drains upgradient of Carson Lake. Juvenile migratory birds have been shown to bioaccumulate mercury in Carson Lake (Hoffman and others, 1990, p. 61). Adult shoveler ducks (*Anas clypeata*) feeding in Carson Lake have also been shown to bioaccumulate mercury. (See the section in this report, "Mercury and Selenium in Edible Tissue of Waterfowl.")

In the three drainage systems examined in detail (Paiute, D-Line, and TJ Drains), biological pathways—particularly detritus and algae—were associated with the transport of selenium from irrigated lands (source areas) through drains to managed wetlands. Mercury also accumulated in detritus and algae in some drains. Because detritus and algae are transported in drift in similar drains in this project, biological pathways are thought to be involved in the movement of mercury from source areas to managed wetlands.

Duration of Selenium Contamination

Paiute Drain, which discharges to Lead Lake, provides additional information on the possible duration of selenium contamination in agricultural drainages. The Paiute Drain system (fig. 8) was constructed between 1913 and 1950. Median concentrations of selenium in animal drift from Paiute Drain were approximately half those of nearby younger TJ Drain and slightly less than half those from Hunter Drain (figs. 11 and 13).

The release of high concentrations of selenium in the shallow ground water, if present, in the first 1-5 years following construction of new drains, followed by the long-term release of lower concentrations would be expected (Gilliom and others, 1989, p. 123). The water-quality history of Paiute Drain is unknown, but it is apparent that after more than 40 years, selenium continues to be released into the water, incorporated into biota, and transported to Lead Lake (fig. 11). Similarly, after about 40 years of irrigation drainage, soils near Hunter Drain continue to release selenium into the water, and bioaccumulation occurs in this drain (fig. 13).

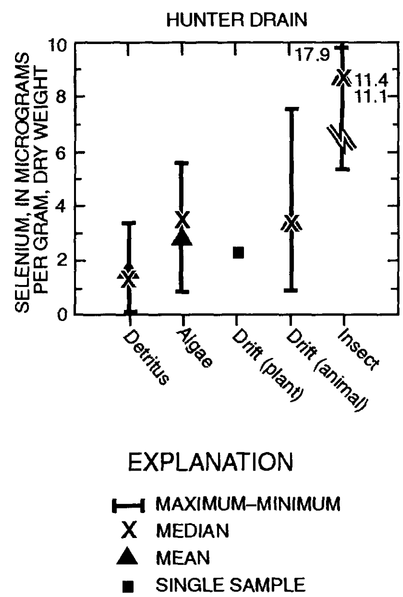


FIGURE 13. Selenium concentrations in composite samples from Hunter Drain in Stillwater Wildlife Management Area.

Contaminant Source Areas

Selenium and mercury are heterogenously distributed in irrigated soils within the Newlands Project area (R.R. Tidball, U.S. Geological Survey, oral commun., 1989). Here, source areas associated with biological pathways are irrigated land upgradient from or at sampling sites in drains that carried concentrations equal or exceeding 1.0 μg/g, dry weight, of selenium or mercury.

Figure 14 shows the spatial distribution of selenium in detritus throughout the study area Selenium concentrations in algae generally correlate with those in detritus. Areas having selenium concentrations ≥1.0 μg/g, dry weight, were defined as source areas or areas of high selenium availability. The 1.0 μg/g concentration was selected principally because of the capacity of selenium to biomagnify up the food chain. This concentration was the average for plants from TJ Drain. Four general source areas of high selenium concentration were identified: near the city of Fallon, north and east of S-Line Reservoir, Fallon Indian Reservation, and the Fernley agricultural area (fig. 9). Areas of high concentrations of arsenic and boron in detritus and algae coincided with the same areas of high selenium concentration (Rowe and others, 1991).

Areas having mercury concentrations ≥ 1.0 μg/g, dry weight, in detritus, were defined as source areas or areas of high mercury availability. Mercury concentrations greater than 1.0 μg/g were found in both detritus (fig. 15) and algae in an area near Carson Lake along the historical Carson River channel, south of Fallon. This fan-shaped area, generally northwest of Carson Lake, is the area which may have received sediment from the Carson River prior to 1900, before the construction of dams that diverted the flow of the river (see fig. 2). Other areas of high concentrations are along the Carson River, Stillwater Slough, and New River Drain.

The occurrence of selenium and mercury in detritus and algae associated with irrigation drainage is indicative of the bioavailability of those elements, and suggests an upgradient source. Furthermore, the magnitude of bioaccumulation at one site can be compared with another to indicate source areas of more or less contamination. Additional information on the annual load of organically bound selenium and mercury from the agricultural drains to receiving wetlands would be more definitive.

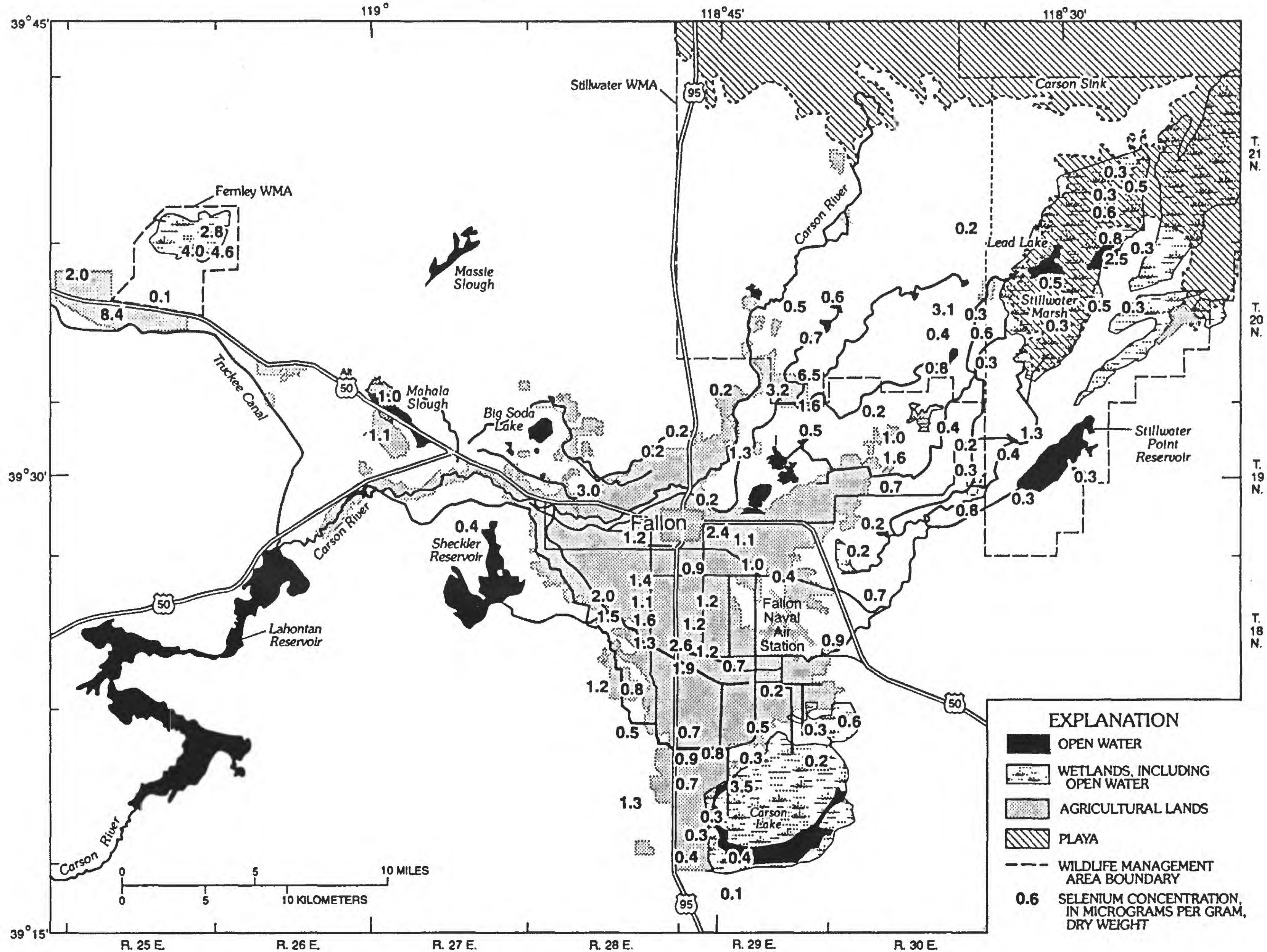


FIGURE 14. Concentrations of selenium in detritus samples from in and near Stillwater and Fernley Wildlife Management Areas. (Map modified from Seiler and Allander, in press.)

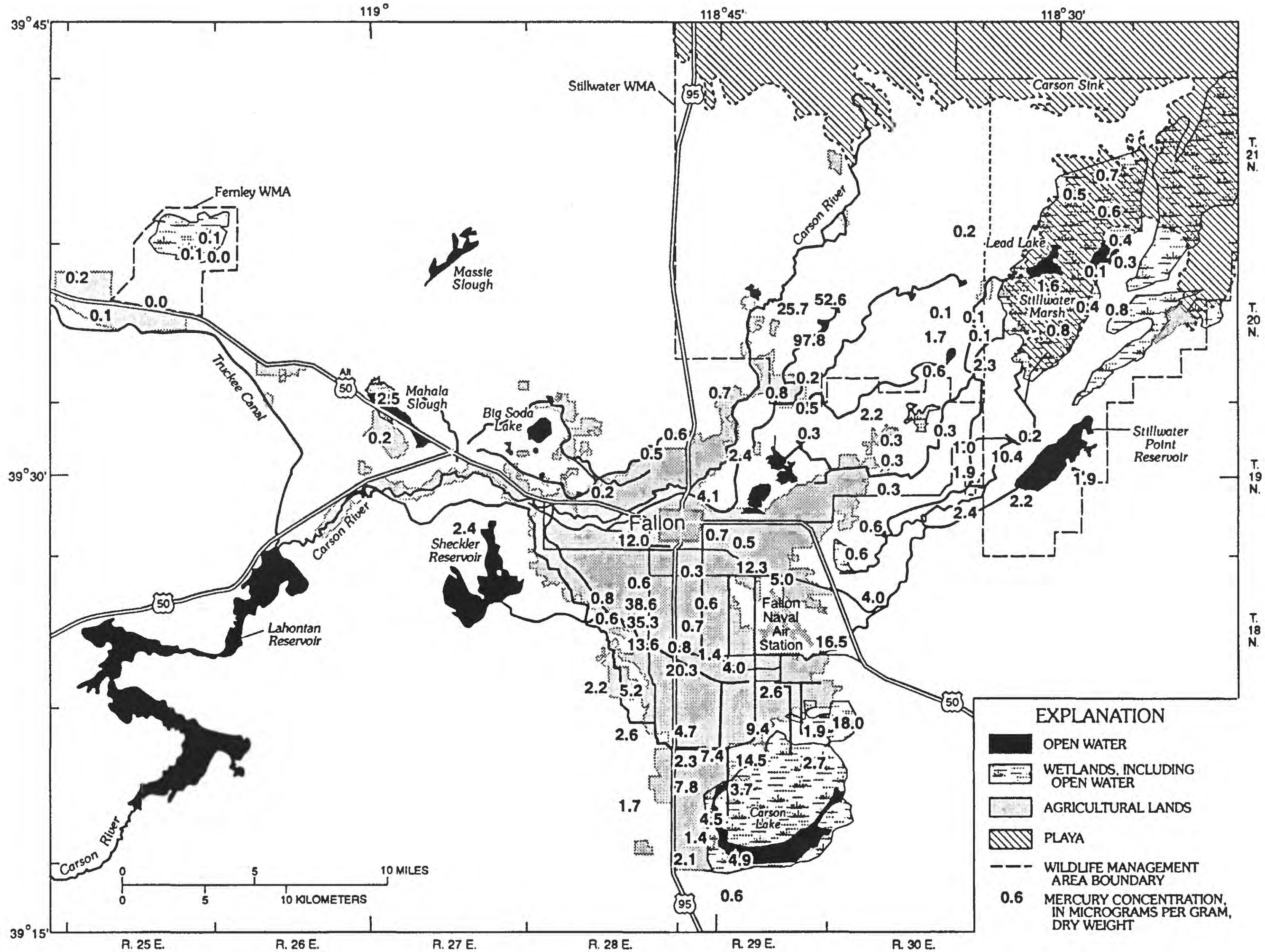


FIGURE 15. Concentrations of mercury in detritus samples from in and near Stillwater and Fernley Wildlife Management Areas and adjacent wetlands. (Map modified from Seiler and Allander, in press.)

(p. 55 follows)

EFFECTS OF BORON, MERCURY, AND SELENIUM ON WATERFOWL PRODUCTION

Waterfowl are typically most sensitive to environmental stress during their reproductive phases (egg formation, incubation, and juvenile growth) and are more vulnerable to the effects of trace elements during that time. Trace-element toxicity is a possible contributing factor to the steady decline of waterfowl production in wetlands that are maintained by drainage from the Newlands Irrigation Project. Livers from juvenile waterfowl and shorebirds collected from these wetlands in 1986-87 contained concentrations of boron, mercury, and selenium that have been associated with various harmful effects (Hoffman and others 1990, p. 53, 60, 67). The available literature concerning the effects of trace-element concentrations in eggs and juvenile waterfowl is limited, but some information is available about the effect levels of boron, mercury, and selenium. Concentrations of 20 trace elements were determined in this study in 1988 and presented by Rowe and others (1991, table 20). Of those elements, this section will consider only boron, mercury, and selenium. Evaluations of these three elements in eggs and juvenile duck livers and of hatch success, teratogenesis, nest success, and duckling production were made to determine the extent of the effects of these trace- elements on waterfowl reproduction.

APPROACH

Six separate wetlands were included in the waterfowl production study: Stillwater, Fernley, and Humboldt Wildlife Management Areas (WMAs); Carson Lake; and Massie and Mahala Sloughs (fig. 1). All of these areas receive irrigation drainage, ground water, or both, from U.S. Bureau of Reclamation Projects. Humboldt WMA is maintained primarily by irrigation return flow from the Humboldt Irrigation Project. The other five study areas receive irrigation return flow from the Newlands Irrigation Project (Hoffman and others, 1990).

Background sites were selected in Carson Valley, outside the study area, and at S-Line Reservoir, near the study area. The wetlands in Carson Valley are about 1,000 ft higher than the valley floors of the study area, thus presenting a history of soil development unaffected by ancient Lake Lahontan. Although S-Line Reservoir is located within the Fallon agricultural area, it is maintained primarily with Carson River water taken from Coleman Diversion Reservoir which contains only small amounts of irrigation return water.

This study was made in 1988 during the second year of a drought. Both irrigation drainwater and operational releases in the study area were greatly reduced because of the tight management of irrigation water; agricultural diversions were reduced to 70 percent of normal in response to the drought. Thousands of wetland acres dried up, reducing nesting and feeding habitat. Waterfowl congregated on the remaining wetlands, where reduced vegetative cover exposed nests to increased predation. Dissolved solids, including trace elements, concentrated in the drainwater and remaining ponds as evaporation reduced the water volume.

The number of samples collected in this study was low, primarily because nests that were not destroyed by predators were extremely difficult to locate in the available habitat. Nest predators were an ongoing problem, commonly destroying previously located nests between study visits.

Nests of six species of ducks—cinnamon teals (*Anas cyanoptera*), gadwalls (*Anas strepera*), mallards (*Anas platyrhynchos*), pintails (*Anas acuta*), redhead ducks, and ruddy ducks (*Aythya americana* and *Oxyura jamaicensis*)—were surveyed, as were nests of American coots (*Fulica americana*).

METHODS

Surveys to locate as many duck and coot nests as possible were conducted, on foot, in the various nesting habitats from early May through June. Peak nesting periods in this area typically range from April 10 to June 20 for ducks and from May 1 to June 15 for coots. All study sites had suitable nesting habitat for the various waterfowl common to the area. Redhead and ruddy ducks and American coots build nests in and over water in bulrush (*Scirpus sp.*) and cattails (*Typha sp.*), and other duck species use a variety of upland and marsh vegetation.

After the nests were located and identified by species, one egg was taken from each nest for trace-element analysis. Two additional eggs were collected from duck nests containing seven or more eggs for artificial incubation under controlled conditions to determine hatch success. Nests with six or fewer eggs were revisited and, if the clutch size had increased to seven, the additional two eggs were collected at that time. Coot eggs were not collected for artificial incubation because the incubator was adjusted for duck eggs and coots were of secondary interest. All eggs were marked for identification and floated in water to determine incubation stage and expected hatch dates (Westerkov, 1950). Eggs collected for trace-element analysis were immediately refrigerated and those for incubation were placed in a Koolatron (a temperature-controlled, padded container) for transportation to the incubator.

Eggs for trace-element analysis were volumetrically measured by water displacement. The eggs were then opened, using a scalpel cleansed with acetone followed by a distilled- or deionized-water rinse. The circumference of the egg was scribed with the scalpel and the contents dropped directly into a nitric-acid-washed, pre-weighed, 2-oz glass jar. The degree of embryonic development was recorded, and the samples were weighed, labeled, and frozen for shipment. All egg samples were analyzed for trace-elements by the U.S. Fish and Wildlife Service, Patuxent Analytical Control Facility, Laurel, Md., or their contract laboratories. The resulting data had appropriate quality-assurance documentation attached. All trace elements were analyzed by inductively coupled plasma emission spectroscopy, except for mercury, which was analyzed by the cold-vapor technique, and selenium, which was analyzed by hydride generation (U.S. Fish and Wildlife Service, 1985).

The incubator (a Petersime Model No. 4) was checked twice daily to monitor temperature and humidity and to remove any newly hatched ducklings. Eggs that remained unhatched approximately 10 days after expected hatch dates, and eggs that pipped but did not hatch, were removed and refrigerated for examination. Embryos and ducklings were examined for gross abnormalities of the eyes, beak, wings, and feet, and were then frozen in individual plastic bags. Mallard eggs of known age from game-farm birds maintained on a contaminant-free diet were used as incubation controls.

Coot nests were rechecked prior to expected hatch date and a second egg was collected from those nests still containing eggs. Embryos from these eggs were examined for deformities and then frozen.

To determine if juvenile birds accumulated trace elements after hatching, livers of juvenile ducks, coots, and black-necked stilts (*Himantopus mexicanus*) from both background and study-area wetlands were analyzed. Sixty preflight ducklings, 45 juvenile coots, and 12 juvenile stilts were collected from the study areas, and 5 ducklings and 6 coots were collected from the background areas. The birds were frozen and later partially thawed for dissection. The liver from each bird was removed using a scalpel rinsed with acetone followed by a distilled- or deionized-water rinse. The liver was placed in a nitric-acid washed, preweighed, 2-oz glass jar, then weighed, labeled, and frozen for shipment. Juvenile stilt livers were so small that two were combined to make a minimum sample weight; therefore, each data point for liver (figs. 16, 17) represents two birds.

Nest success is defined as the probability of production of one or more live juveniles from a nesting attempt. The nest-success rate was calculated by dividing the number of successful nests by the total number of nests. To determine nest success, each duck nest was checked at 7-10 day intervals for evidence of successful hatching. A feathered-egg membrane pulled away from the shell was used as an indicator of successful hatching. Coot nests were rechecked at least once, either at hatching time or just after.

Duckling production was estimated by conducting brood surveys in each study area from the fourth week of June through the third week of July—the peak hatching period Survey routes were predetermined to include areas of high brood use, particularly shorelines and clumps of vegetation, and to ensure adequate visibility by the investigator. Surveys started about 6:00 a.m., during the most active feeding time. Most routes were walked or driven, using binoculars or a spotting scope as necessary. In areas inaccessible by land, an airboat was used. Data on duck species, duckling numbers, and age classes at each site were recorded.

Hatch Success and Teratogenesis

Duck eggs for incubation and subsequent examination were collected from 41 nests; two eggs per nest were collected where possible. A total of 81 eggs were incubated—69 from study-area sites and 12 from background sites. Eggs from the background sites had a hatch-success rate of 92 percent. Of the 69 eggs from the study sites, 62 hatched—a success rate of 90 percent. This rate for artificially incubated duck eggs is in the range expected for eggs from healthy populations (Charles J. Henny, U.S. Fish and Wildlife Service, oral commun., 1990). Hatch success is summarized in table 16 by species and area.

The eight eggs that failed to hatch—seven from the study area and one from the background site—were examined for deformities. Five contained embryos, all well developed with no gross deformities (Charles J. Henny, U.S. Fish and Wildlife Service, oral commun., 1990). The three eggs that failed to develop may have been infertile. The 62 ducklings were also examined and no gross external deformities were observed. Thirty-one coot eggs from separate nests were collected just prior to expected hatch. Of these eggs, 24 had well-developed embryos and all 31 appeared to be normal. Based on these data, levels of trace elements in the eggs probably were below effect levels for teratogenesis and mortality.

Trace Elements in Eggs

Eisler (1990, 1987, and 1985) has summarized the available literature on the effects of boron, mercury, and selenium, respectively, on wildlife, including specific references for duck eggs (table 18). One of the common effects that each of these trace elements has on embryonic development is reduced hatch weight.

Concentrations of trace elements were determined for 62 duck eggs and 68 coot eggs. Five of the duck eggs were from the background site in Carson Valley and were well below the effect level for boron, mercury, and selenium. These data are reported by Rowe and others (1991, table 20) and are summarized in table 17 in this report.

Table 16. Hatch success of artificially incubated waterfowl eggs collected in the study area, 1988

[In each two-line data group, the top number (in parentheses) is total incubated eggs; bottom number is percentage hatched; --, no data; WMA, Wildlife Management Area]

Location	Cinnamon teal	Gadwall	Mallard	Pintail	Redhead	Ruddy	Total, all species
			Study Sites				
Stillwater WMA	(6) 100	(2) 100	(4) 75	(4) 100	(2) 100	(2) 100	(20) 95
Fernley WMA	(6) 100	--	--	--	(6) 67	--	(12) 83
Humboldt WMA	(2) 100	(2) 100	(3) 100	--	(8) 88	--	(15) 93
Mahala Slough	(10) 80	(2) 100	(2) 100	--	--	(4) 75	(18) 89
Massie Slough	(2) 100	--	--	--	(2) 100	--	(4) 100
TOTAL	(26) 92	(6) 100	(9) 89	(4) 100	(18) 83	(6) 83	(69) 90
			Background Site				
Carson Valley	--	--	(12) 92	--	--	--	(12) 92

The primary effects of high levels of boron are on growth, behavior, and brain biochemistry (Eisler, 1990, p. 19). Mallard ducklings from eggs with a boron concentration of 13 µg/g, dry weight, showed reduced hatch weights (Smith and Anders, 1989, p. 945). Eggs with boron concentrations of 49 µg/g, dry weight, showed significantly reduced hatch rates and juveniles from those eggs had higher mortality rates (Smith and Anders, 1989, p. 945). These effect levels came from feeding studies and are the arithmetic means resulting from experimental concentrations in feed, so no data on intermediate concentrations are available.

The boron concentrations found in eggs in this study (maximum, 19.4 µg/g, dry weight) were all well below the level known to adversely affect hatch success (49 µg/g, dry weight). This correlates with the incubator hatch rate of 90 percent observed in this study. Three redhead duck eggs (5 percent), all collected in Mahala Slough, had boron concentrations above 13.0 µg/g, dry weight, the level associated with reduced hatch weight. Overall, coot eggs had higher boron concentrations than duck eggs. Ten percent of the coot eggs, primarily those from Fernley WMA, had boron concentrations above the effect level for reduced hatch weight.

Table 17. Summary of data on concentrations of boron, mercury, and selenium, including minimums, maximums, and means for waterfowl eggs collected in the study area

[Abbreviations: µg/g, micrograms per gram; C. teal, cinnamon teal; WMA Wildlife Management Area.]

Location	Species	Number of samples	Trace-element concentration (µg/g, dry weight)								
			Boron			Mercury			Selenium		
			Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean
			Study Sites								
Stillwater WMA	C. teal	3	3.2	4.8	4.1	1.6	6.2	4.5	2.0	2.3	2.2
	Gadwall	1	2.0	2.0	2.0	0.5	0.5	0.5	1.8	1.8	1.8
	Mallard	2	1.7	2.2	2.0	1.7	3.7	2.7	2.1	2.1	2.1
	Pintail	2	2.3	3.7	3.0	0.4	0.5	0.5	2.5	2.9	2.7
	Redhead	1	5.7	5.7	5.7	1.7	1.7	1.7	1.8	1.8	1.8
	Ruddy	2	1.1	1.2	1.2	0.2	0.5	0.4	2.1	2.9	2.5
Fernley WMA	Coot	25	5.5	15.7	9.3	0.1	0.4	0.1	4.4	12.2	8.7
	C. teal	2	1.7	4.8	3.3	0.2	0.7	0.5	7.6	10.1	8.8
	Mallard	1	3.1	3.1	3.1	0.2	0.2	0.2	8.9	8.9	8.9
	Redhead	3	3.2	5.4	4.2	<0.1	0.1	0.3	8.8	10.9	9.9
Humboldt WMA	Coot	27	4.4	14.3	7.7	0.1	1.36	0.2	1.9	4.48	2.9
	C. teal	2	2.8	5.2	4.0	0.4	0.5	0.5	2.8	3.7	3.2
	Gadwall	1	3.9	3.9	3.9	0.2	0.2	0.2	3.1	3.1	3.1
	Mallard	1	10.2	10.2	10.2	0.5	0.5	0.5	3.0	3.0	3.0
	Redhead	6	3.1	9.6	9.6	0.1	1.74	0.5	3.0	3.7	3.2
Mahala Slough	C. teal	9	1.5	7.6	4.1	0.2	1.1	0.6	2.0	7.4	4.4
	Gadwall	1	7.6	7.6	7.6	0.3	0.3	0.3	4.6	4.6	4.6
	Mallard	3	<0.8	0.9	0.6	0.3	2.1	1.1	1.3	8.4	4.0
	Redhead	7	4.2	19.4	11.7	0.1	1.2	0.6	1.2	8.5	3.7
	Ruddy	7	<0.8	3.1	2.1	0.1	2.7	0.6	1.2	5.7	3.3
Massie Slough	C. teal	1	1.7	1.7	1.7	0.1	0.1	0.1	8.0	8.0	8.0
	Redhead	1	1.8	1.8	1.8	0.4	0.4	0.4	3.6	3.6	3.6
	Ruddy	1	2.1	2.1	2.1	0.1	0.1	0.1	7.4	7.4	7.4
			Background Site								
Carson Valley	Mallard	5	<0.8	1.9	0.7	0.2	0.6	0.3	0.7	2.3	1.4

Table 18. Effect levels and characteristics of boron, mercury, and selenium concentrations in waterfowl eggs and juvenile waterfowl livers

[µg/g, micrograms per gram]

Constituent	Eggs		Liver tissue	
	Effect level (µg/g, dry weight)	Characteristic sign or result	Effect level (µg/g, dry weight)	Characteristic sign or result
Boron[1]	13 49	Reduced hatch weight Reduced hatch rate and juvenile survival	17	Reduced reproduction and duckling growth
Mercury[2]	3.1	Reduced hatch rate and juvenile survival	4.3	Reduced reproduction and survival rates
Selenium[3]	15	Teratogenesis and embryo mortality	9	Reduced reproduction and juvenile survival

[1] Smith and Anders, 1989.
[2] Heinz, 1979.
[3] Lemly and Smith, 1987.

Mercury concentrations of 3.1 µg/g, dry weight, in black duck (*Anas rubripes*) eggs are associated with significantly reduced hatch rate and duckling survival (Heinz, 1979, p. 398). Mercury residues found in duck and coot eggs in this study were low except in those collected in Stillwater WMA. Three (27 percent) of the 11 duck eggs from Stillwater WMA had mercury concentrations above the effect level. The nest siblings of these eggs—cinnamon teal and pintail—hatched in the incubators.

All selenium concentrations found in duck and coot eggs were below 15 µg/g, dry weight. The level at which teratogenesis or embryo mortality (Lemly and Smith, 1987, p. 9), or reproductive problems in the laying hen (Heinz and others, 1989, p. 427) can be expected is 15-18 µg/g, dry weight.

The concentrations of potentially harmful trace elements in duck and coot eggs in all areas were low, with the exception of mercury in those from Stillwater WMA and, with that exception, probably are not a factor in waterfowl production. The adults of these species normally do not overwinter in these wetlands; they arrive shortly before nesting and apparently are not sufficiently exposed to the trace elements to accumulate enough to deposit significant amounts in their eggs.

Trace Elements in Juvenile Duck Livers

Eisler (1990, 1987, and 1985) summarized the available literature on the effects and concentrations of boron, mercury, and selenium in livers of juvenile waterfowl (table 18).

It was assumed that juvenile waterfowl collected preflight had been feeding in the vicinity and that the trace-element concentrations in their tissues reflected existing conditions in the wetlands. Juvenile ducks, coots, and black-necked stilts were taken from study areas where eggs had been collected, and livers were analyzed for concentrations of trace elements. Data were obtained for 65 preflight ducklings, 52 juvenile coots, and 12 juvenile stilts. Complete data are presented by Rowe and others (1991, table 20) and are summarized in this report (table 19).

In boron feeding studies, mallard ducklings with liver concentrations of 17 µg/g, dry weight, showed reduced weight gain (Smith and Anders, 1989, p. 945). In this study, boron concentrations in black-necked stilt livers (6 composite samples) were below 4 µg/g, dry weight. Concentrations of boron in duck livers also were below the effect level, varying widely throughout the study area from 1.1 to 15.1 µg/g. Coot livers showed higher boron concentrations than duck livers, as high as 34.5 µg/g. Concentrations of boron were at or above effect level in 10 percent of the coot livers. The highest boron concentrations were from Fernley WMA, where three of the seven coots collected had concentrations above effect level, and reduced growth rates could be expected. Boron levels in juvenile birds were not high enough to affect waterfowl reproduction

Levels of boron concentration varied; in some instances concentrations were higher in eggs than in juvenile livers from the same area. These data suggest that some adult birds may have acquired boron elsewhere in the flyway prior to their arrival and egg laying in the study areas.

Table 19. Summary of data on concentrations of boron, mercury, and selenium, including minimums, maximums, and means, for juvenile waterfowl livers collected in the study

[Abbreviations: µg/g, micrograms per gram; C. teal, cinnamon teal; WMA, Wildlife Management Area.]

Location	Species	Number of Samples	Trace-element concentration (µg/g, dry weight)								
			Boron			Mercury			Selenium		
			Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean
			Study Sites								
Stillwater WMA	Coot	10	5.8	22.1	11.1	2.0	5.7	4.0	3.4	5.9	4.4
	C. teal	5	<2.0	4.4	3.2	1.4	3.5	2.2	4.0	6.8	5.6
	Mallard	2	5.1	9.8	7.5	2.7	3.0	5.6	7.4	9.0	8.2
	Pintail	1	5.9	5.9	5.9	1.8	1.8	1.8	6.6	6.6	6.6
	Redhead	2	5.0	6.0	5.5	2.4	6.4	4.4	6.7	13.9	10.3
	Ruddy	1	1.8	1.8	1.8	2.1	2.1	2.1	6.4	6.4	6.4
Fernley WMA	Coot	7	7.5	34.5	16.8	0.3	0.5	0.4	26.4	36.0	30.9
	C. teal	4	2.4	12.1	8.1	<0.1	0.1	0.1	26.4	35.3	30.3
	Stilt	3	2.0	3.5	2.5	0.4	1.0	0.7	17.0	35.3	28.4
Humboldt WMA	Coot	12	<2.0	8.0	4.5	0.2	3.2	0.7	7.8	13.0	10.1
	C. teal	6	1.8	5.1	3.2	0.4	1.6	1.0	11.9	17.5	15.1
	Gadwall	1	4.0	4.0	4.0	0.8	0.8	0.8	16.0	16.0	16.0
	Mallard	7	2.7	10.0	5.9	0.3	0.7	0.5	7.2	23.0	12.8
	Redhead	3	3.0	6.1	4.8	0.6	0.8	0.8	9.1	13.3	11.6
Carson Lake	Coot	10	1.6	6.8	4.9	2.0	8.8	5.3	2.6	6.3	4.4
	C. teal	1	7.9	7.9	7.9	5.6	5.6	5.6	8.7	8.7	8.7
	Mallard	4	<2.0	5.3	3.9	1.4	6.0	3.4	4.4	6.6	5.1
	Redhead	4	0.8	15.1	5.9	0.9	5.1	2.3	4.4	7.0	5.6
	Ruddy	4	2.1	3.9	3.3	<0.1	2.4	1.5	6.9	8.2	7.6
	Stilt	1	1.7	1.7	1.7	7.9	7.9	7.9	13.2	13.2	13.2
Mahala Slough	Coot	6	3.7	7.0	5.4	0.2	1.19	0.8	7.6	15.0	9.6
	Redhead	3	3.7	9.3	6.5	0.1	0.7	0.4	8.2	20.4	13.5
	Ruddy	6	<0.8	2.4	1.4	0.1	0.5	0.2	5.4	10.4	7.9
	Stilt	2	3.4	3.8	3.6	2.0	13.9	8.0	52.0	102.0	77.0
Massie Slough	Redhead	7	4.4	13.6	6.8	<0.1	0.5	0.3	17.5	43.5	30.7
			Background Sites								
Carson Valley	Mallard	4	1.2	2.8	2.2	1.3	2.4	1.6	2.3	3.5	3.0
S-Line Reservoir	Coot	7	1.6	7.3	3.9	0.8	4.3	2.9	2.9	5.3	4.3
	C. teal	1	1.5	1.5	1.5	9.4	9.4	9.4	5.6	5.6	5.6

Data are not available for establishing an effect level for mercury concentration in juvenile waterfowl liver, but for adult mallard liver, an effect level of 4.3 μg/g, dry weight, for decreased reproductive success can be derived from existing data (Heinz, 1979, p. 396; Hoffman and others, 1990, p. 26). Stilt, duck, and coot livers from Fernley WMA and Humboldt WMA and Massie and Mahala Sloughs had mercury concentrations generally below 1 μg/g. Moderate concentrations of mercury in ducks, coots, and stilts were found in Carson Valley (mean 1.6 μg/g, dry weight) and in S-Line Reservoir (3.67 μg/g, dry weight). Mercury concentrations were above the effect level in 70 percent of the coots and 31 percent of the ducks from Carson Lake and 40 percent of the coots from Stillwater WMA. The highest mercury concentration found in this study, 13.9 μg/g, dry weight, was in a stilt sample (livers of two birds, combined) from Mahala Slough, an anomalous sample because no other samples from Mahala Slough showed high mercury concentrations, and Mahala Slough had not received direct Carson River water containing mining discharge. One of the birds in this sample probably was an adult that had moved into Mahala Slough from one of the nearby mercury-source areas.

The incidence of elevated mercury concentrations in birds was restricted to areas that had received mercury-contaminated sediment of the Carson River before construction of the Newlands Irrigation Project, including Carson Lake and Stillwater WMA. Mercury concentrations in bird livers from those areas are high enough that a negative effect on waterfowl production could be expected.

Data in the literature on the biological effects of selenium on waterfowl are more available. The effects are primarily on reproduction, but selenium toxicosis has been documented elsewhere since the 1930's. Selenium concentrations of 9 μg/g, dry weight, in livers of adult birds are known to be associated with decreased reproductive success and reduced juvenile survival (table 19; Lemly and Smith, 1987, p. 8). Selenium concentrations in livers establish 95 percent of equilibrium with dietary concentration in 7-8 days and return to background level 9-10 weeks after dietary intake stops (Heinz and others, 1990, p. 376). Selenium concentrations in livers of preflight juveniles, therefore, reflect the selenium in the food chain in the area.

Selenium concentrations in bird livers from Carson Valley and S-Line Reservoir (background sites), and Carson Lake were below the effect level for reduced reproduction (9.0 μ/g, dry weight). Twenty percent of the ducks from Stillwater WMA had selenium concentrations at or above effect level (fig. 16). In birds from Mahala Slough, concentrations of selenium above the effect level were found in 56 percent of the ducks, 33 percent of the coots, and both stilt samples. Concentrations in the two stilt samples (composite) were 52.0 and 102.0 μg/g (mean, 77 μg/g, dry weight), the highest found in this study and in the range where toxicosis and mortality have been documented elsewhere (Heinz and others, 1987, p. 429; 1988, p. 566). Livers from 83 percent of the ducks and 67 percent of the coots collected in Humboldt WMA had selenium concentrations above the effect level, as high as 23 μg/g, dry weight. High concentrations of selenium were also found in livers of cinnamon teal (mean, 30.0 μg/g, dry weight) and coots (mean, 30.9 μg/g, dry weight) from Fernley WMA, and in redhead ducks (mean, 30.7 μg/g, dry weight) from Massie Slough.

The two areas with the highest average concentrations of selenium in bird livers were Fernley WMA and Massie Slough, where every bird taken had concentrations between 17.0 and 43.5 μg/g (mean, 30.4 μg/g, dry weight).

Decreased survival of juvenile birds is anticipated in areas where liver concentrations are consistently above effect levels. Decreased juvenile survival and, thus, decreased waterfowl production, is anticipated in Fernley and Humboldt WMAs and Mahala and Massie Sloughs, and to a lesser extent in Stillwater WMA.

The concentrations of selenium residues found in juvenile livers were consistently higher than those found in eggs from the same areas, indicating juvenile uptake in all areas.

The combined effect of concentrations of two or more trace elements is not well understood. High boron concentrations were found with high selenium concentrations only in Fernley WMA. Selenium and boron have been considered to biochemically operate independently (Patuxent Wildlife Research Center, 1987, p. 28), but there is recent evidence that boron and selenium interact synergistically to produce more severe toxicological effects (David Hoffman, Patuxent Wildlife Research Center, written commun., 1990). With the exception of the anomalous stilt sample from Mahala Slough, high concentrations of mercury and selenium, in eggs or juvenile livers, were not found in the same areas.

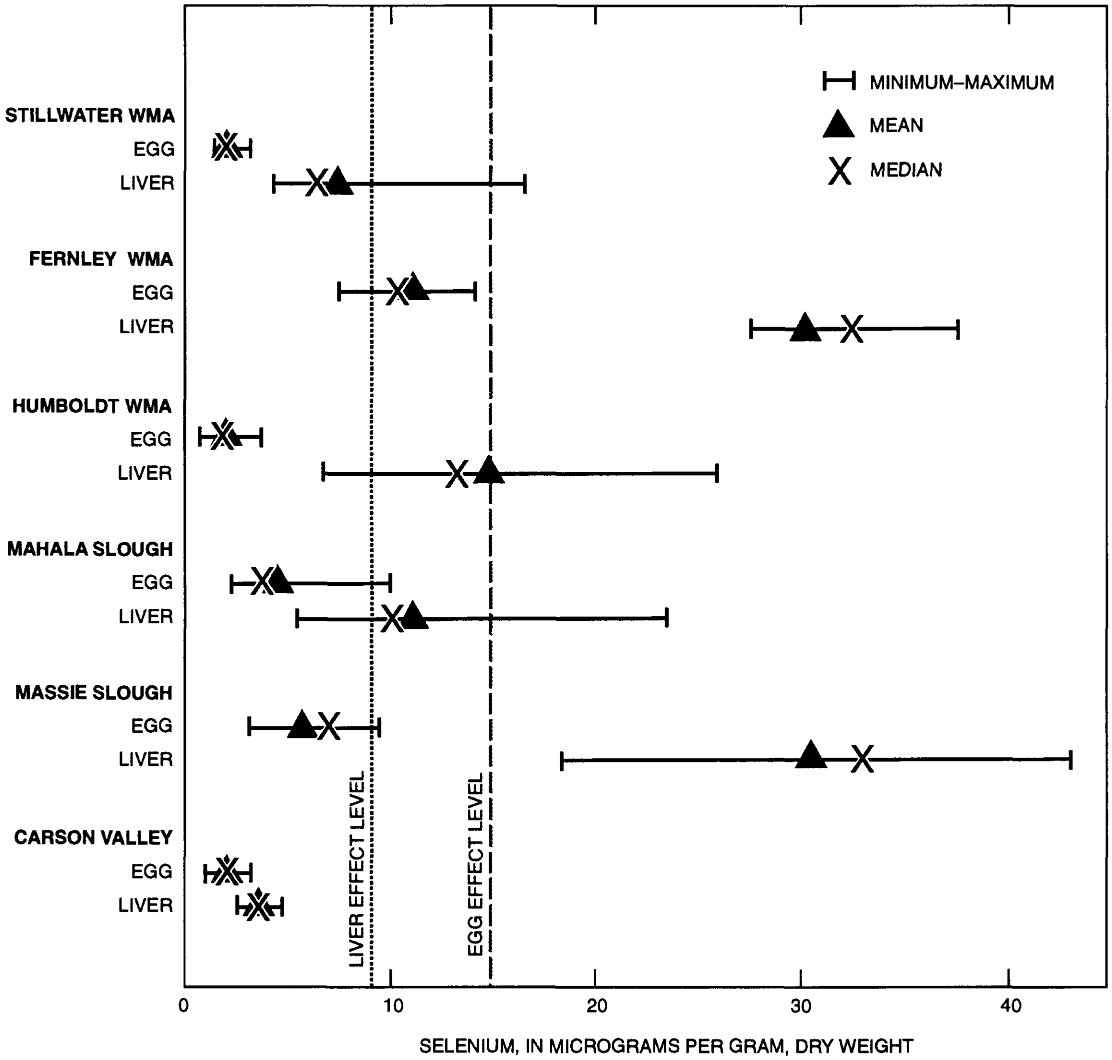


Figure 16. Concentrations of selenium in duck eggs and liver tissue of juvenile ducks collected in Stillwater, Fernley, and Humboldt Wildlife Management Areas, Mahala and Massie Sloughs, and at the background site. Effect levels for eggs and liver, 9 and 15 micrograms per gram, respectively, are described by Lemly and Smith (1987, table 2) and are summarized in table 18 of this report. Abbreviation: WMA, Wildlife Management Area.

Nest Success

Nest-success rate, by species, for each study area is summarized in table 20. The nest-success data from all study sites were combined, because of small sample sizes, and compared with data from nesting surveys conducted in Stillwater WMA in 1968-1970 (Napier, 1974), 1983 (Evans, 1983, p. 18-19), and 1987-1988 (Stillwater WMA, unpublished data); these comparisons are shown in table 21. The nest-success rate found in this study, 26 percent for the combined study sites, is comparable to the success rate of 25 percent determined for Stillwater WMA by the WMA staff. The nest-success rate at Stillwater has shown a general downward trend from 43-52 percent in 1968-70 to 25 percent in 1988.

No relation could be established between the nest-success rates in the various study sites and the trace-element concentrations in eggs or juvenile livers from those sites. The nest failure observed during this study appeared to be caused primarily by predation exacerbated by loss of habitat and the lack of vegetative cover in and near the remaining wetlands.

Table 20. Nest success of ducks in the study area, 1988

[In each two-line data group, the top number (in parentheses) is total nests examined; bottom number is percentage of nests hatching at least one young; --, no data; WMA, Wildlife Management Area.]

Location	Cinnamon teal	Gadwall	Mallard	Pintail	Redhead	Ruddy	Total
			Study Sites				
Stillwater WMA	(3) 100	(1) 0	(2) 50	(2) 100	(1) 0	(2) 0	(11) 55
Fernley WMA	2) 0	--	(1) 0	--	(3) 0	--	(6) 0
Humboldt WMA	(2) 0	(1) 100	(1) 100	--	(6) 50	--	(10) 0
Mahala Slough	(9) 22	(1) 0	(3) 33	--	(1) 0	(5) 20	(19) 21
Massie Slough	(1) 100	--	--	--	(1) 100	(1) 0	(3) 67
TOTAL	(17) 35	(3) 33	(7) 43	(2) 100	(12) 33	(8) 13	(49) 26
			Background Site				
Carson Valley	--	--	(6) 83	--	--	--	(6) 83

Waterfowl Production

A survey of waterfowl breeding pairs, conducted annually in mid-May by the Nevada Department of Wildlife, found 6,810 breeding pairs of various species of ducks in the study area in 1988 (Nevada Department of Wildlife, unpublished data, 1988). The low number of duck nests (49) found in this study, and the difficulty experienced in finding even that number, suggests that most of the breeding pairs present in mid-May did not nest. Nesting habitat in the study area and in other traditional nesting areas is greatly reduced from that available in the recent past (U.S. Department of the Interior, 1988). This lack of habitat may be preventing many pairs from nesting.

Observed duckling production from all study areas except Stillwater WMA was 579 birds. Brood production in Stillwater WMA, estimated by the Stillwater staff, was about 1,800 ducklings (U.S. Fish and Wildlife Service, Fallon, Nev., unpublished data, 1988), for a total estimated production of about 2,400 waterfowl (table 22). The brood counts in Fernley WMA, Humboldt WMA, and Carson Lake were close to estimates made by the Stillwater WMA staff. Duckling production in 1988 was considerably lower than production experienced in previous years (U.S. Fish and Wildlife Service, Fallon, Nev., unpublished data, 1988).

Waterfowl production typically fluctuates, and is dependant on a variety of factors, including the availability of suitable wetland nesting habitat and predator populations. Decreased water flow to the wetlands, management choices about use of the remaining water, and elevated dissolved-solids concentrations—shown to cause vegetative losses (U.S. Fish and Wildlife Service, 1988, p. 74)—are all probable contributing factors to decreases in nesting habitat. The continuing decrease in waterfowl production observed in the study area in recent years is associated with decreased feeding and nesting habitat that results in waterfowl nesting in marginal areas, and to increased predation rates resulting from a concentration of predators foraging in reduced wetland areas.

Table 21. Nest-success rates from various other studies and from the present study

[In each two-line data group, the top number (in parentheses) is total nests examined; bottom number is percentage of nests hatching at least one young.]

Duck species	Other studies (Stillwater Wildlife Management Area)				Present study (all sites)
	1968-1970[1]	1983[2]	1987[3]	1988[3]	1988
Cinnamon Teal	(43-56) 49-56	(100) 28	(7) 29	(25) 32	(17) 35
Gadwall	(19-42) 16-43	(23) 13	(17) 6	(26) 8	(3) 33
Mallard	(1-7) 0-43	(7) 57	(5) 20	(15) 33	(7) 43
Pintail	(2-4) 50-80	(6) 17	(5) 0	(7) 29	(2) 100
Redhead	(4-7) 57-100	(11) 91	(7) 14	(3) 67	(12) 33
Ruddy	(1-3) 67-100	(5) 60	(2) 0	(1) 0	(8) 13
TOTAL	(87-118) 43-54	(152) 32	(43) 33	(77) 25	(49) 26

[1] Napier, 1974.
[2] Evans, 1983.
[3] Unpublished data from U.S. Fish and Wildlife Service, Fallon, Nev.

Table 22. Estimated waterfowl production in the study area, 1988

Site	Cinnamon teal	Gadwall	Mallard	Pintail	Redhead	Ruddy	Other	Total
Stillwater WMA[1]	300	310	66	173	150	690	82	1,771
Fernley WMA	31	0	11	0	0	0	0	42
Humboldt WMA	45	36	17	0	0	0	0	98
Mahala Slough	12	14	12	6	12	30	0	86
Massie Slough	50	26	50	8	50	5	0	189
Carson Lake	45	18	24	1	32	44	0	164
TOTAL	483	404	180	188	244	769	82	2,350

[1] Estimates from U.S. Fish and Wildlife Service, Fallon, Nev. (unpublished data). All other data are from this study.

MERCURY AND SELENIUM IN EDIBLE TISSUE OF WATERFOWL

The reconnaissance study during 1986-87 found indications that juvenile migratory birds, including waterfowl, in and near Stillwater WMA and Carson Lake were accumulating mercury and selenium in liver and muscle tissue and some of the concentrations exceeded established criteria for public-health warnings (Hoffman and others, 1990, p. 60-62, 66-70). Waterfowl taken from these areas are routinely consumed by hunters. Further sampling done in October 1987 and in 1989 for the detailed study found continued high concentrations of mercury and selenium in edible tissues of waterfowl that could affect public health. Accumulation of selenium and mercury between waterfowl species within wetland systems and between different wetlands supporting the same species varied. This study focused primarily on Stillwater WMA and Carson Lake, the largest public waterfowl-hunting areas associated with the Newlands Irrigation Project, but because waterfowl are also hunted at nearby Fernley WMA and at Massie and Mahala Sloughs, those areas were included in the present study (fig. 1).

APPROACH AND METHODS

Ducks of various ages harvested in mid-October 1989 at the beginning of waterfowl-hunting season, were considered to be representative of waterfowl consumed by humans. Most ducks evaluated in this study were contributed by hunters passing through USFWS check stations near Stillwater WMA and Carson Lake during the opening weekends of the waterfowl seasons; species included were shovelers (*Anas clypeata*), mallards (*Anas platyrhynchos*), green-winged teals (*Anas crecca*), canvasbacks (*Aythya valisineria*), and redhead ducks (*Aythya americana*). Shovelers, late summer-early fall migrants that rarely nest in the study area, were collected from Carson Lake during August 1989 soon after they arrived and again in mid-October 1989. The mercury and selenium concentrations of the August birds served as a baseline against which to establish the increase in concentration of these trace elements between August and the mid-October hunting season. Green-winged teals also were collected in October 1989 from Carson Lake.

Whole ducks were identified, tagged, stored on ice in the field, and frozen in the laboratory on the date of collection. The ducks were thawed and later dissected. To prevent cross contamination, rubber gloves were worn and stainless-steel instruments were rinsed in acetone, then distilled or deionized water. Tissue samples were weighed and refrozen in labeled, nitric-acid-rinsed 2-oz jars. Concentrations of mercury and selenium in muscle, liver, and skin were evaluated.

Tissue samples were analyzed by the U.S. Fish and Wildlife Service, Patuxent Analytical Control Facility, Laurel, Md., or its contract laboratories. The resulting data had appropriate quality-assurance documentation attached. Mercury was analyzed by cold-vapor techniques and selenium was analyzed by hydride generation. Associated procedures used are described by the U.S. Fish and Wildlife Service (1985). Data were reported by the laboratory in micrograms per gram, dry weight. Because the purpose of this section of the report is to compare concentrations found with established criteria or action levels for human consumption, all data have been converted to wet weight, by dividing by 3.6 (Lemly and Smith, 1987, p. 7). An action level is a concentration of a contaminant in animal tissue used for human food at which responsible agencies take action. It is derived from a hazard-risk assessment and includes a safety margin for the population deemed most likely to consume the food involved. Typically, with wildlife not involved in commerce, the action taken is posting of public health warnings. Analysis of variance was used to examine temporal relations of contaminant levels. A statistical significance of 0.1 was selected.

Action levels established by the U.S. Food and Drug Administration (FDA) and the State of California were used to evaluate the potential threat to human health. The U.S. FDA (1984, p. 1) action level for mercury in edible animal tissue is 1.0 µg/g, wet weight, and the State of California action level for selenium is 2.0 µg/g, wet weight (Fan and others, 1988, p. 544).

During the October 1987 sampling period, mallards, redheads, and shovelers were collected from both Carson Lake and Stillwater WMA. Canvasbacks were collected only from Stillwater WMA. The data were published by Rowe and others (1991). The action level for mercury concentrations was exceeded in almost half of the ducks sampled. Selenium concentrations exceeded the action level in less than 15 percent of the samples. Mercury and selenium concentrations were highest in liver, lower in muscle, and least in skin samples. Concentrations in skin never exceeded the action levels. Muscle is of greatest concern because it is the preferred tissue of most humans who consume ducks; liver and skin are less frequently eaten.

Mercury Accumulation in Waterfowl

Hoffman and others (1990, p. 60-62) found that mercury concentrations in liver and muscle tissue of juvenile mallards and liver tissue of coots (*Fulica americana*) and black-necked stilts (*Himantopus mexicanus*) collected in 1986 and 1987 from Carson Lake exceeded the action level. Juvenile birds, too young to migrate, were assumed to have acquired most of this mercury locally through diet. Henny and Herron (1989, p. 1043) documented accumulation of mercury in white-faced ibis (*Plegadis chihi*) which feed in fields near Carson Lake. Mercury concentrations in liver tissue of juvenile black ducks (*Anas rubripes*) were found to nearly double in 4 weeks when the ducks were fed a diet containing 3 µg/g mercury, and the rate of accumulation in liver was greater than in muscle (Finley and Stendell, 1978, p. 60).

Mercury concentrations in shoveler ducks collected in October 1987 from Carson Lake exceeded the action level (or public-health criterion of 1.0 µg/g, wet weight; 3.6 µg/g, dry weight) in every liver sample and in 90 percent of the muscle samples (fig. 17). Mercury concentrations in shoveler muscle and liver from the October 1987 data set were the basis for the Nevada State Health Officer's decision to post a public-health warning specific to shoveler ducks at Carson Lake in 1989. The mean concentrations of mercury in shoveler muscle and liver from Carson Lake were, respectively, about 6 and 18 times the action level of 1.0 µg/g, wet weight. At the request of the State Health Officer, shovelers were again analyzed by the Department of the Interior in 1989. The concentrations of mercury in muscle were lower, but still averaged three times the action level, and the public-health warning stayed in effect. Concentrations exceeding the action level for mercury were also found in some shovelers from Stillwater WMA (fig. 17), in some green-winged teal from Carson Lake, and in mallards from both locations (fig. 18).

The shoveler ducks collected from Carson Lake in mid-August 1989 for baseline data had a mean concentration of mercury in muscle tissue of 1.1 µg/g, wet weight (fig. 17), significantly lower ($p=<0.05$) than the 5.9 µg/g mercury level found in shoveler ducks collected in October 1987. A lesser, but still significant difference ($p=0.08$) was found between the shovelers collected in August 1989 and those collected in October 1989. The shovelers collected in October 1989 contained a mean of 2.8 µg/g, wet weight, mercury in muscle tissue.

The significant difference ($p=<0.05$) found between the 1987 and the 1989 mercury concentrations in shovelers from Carson Lake may be related to wetland availability. In 1987, both the Island Unit and Sprig Pond in Carson Lake contained water, but in 1989 only Sprig Pond was flooded. Composite sediment data gathered by Hoffman and others (1990, p. 113) indicated that mercury was twice as abundant in the Island Unit (18 mg/kg) as in Sprig Pond (9 mg/kg). The time of shoveler arrival and exposure (up to 8 weeks) was similar in both years.

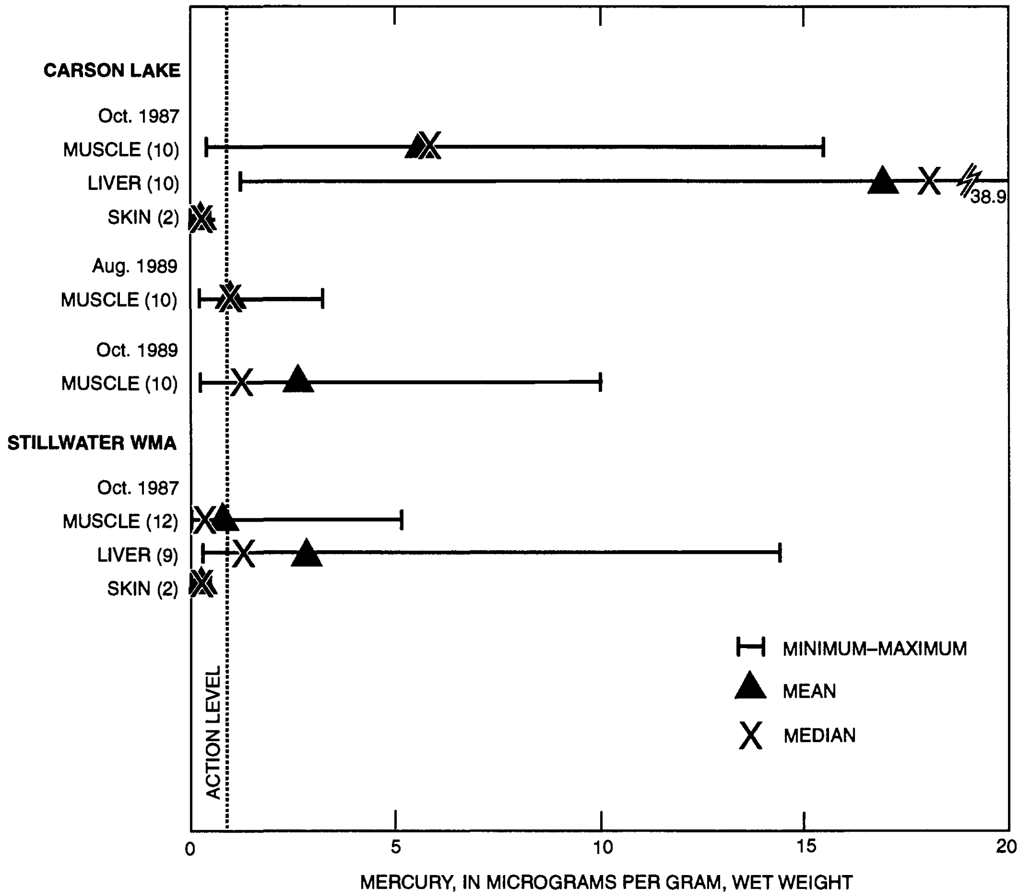


FIGURE 17. Concentrations of mercury in edible portions of shoveler ducks collected at Stillwater WMA and Carson Lake. Number of samples in parentheses. The action level is 1.0 microgram per gram, wet weight (U.S. Food and Drug Administration, 1984, p. 1).

Differences in mercury concentrations between duck species was apparent in October 1987, when the widest variety of species was collected (figs. 17 and 18). Shovelers from Carson Lake had the highest concentrations of mercury, with all liver samples and 90 percent of the muscle samples exceeding the action level. Mercury concentrations in mallards from Carson Lake exceeded the action level in 70 percent of the liver and 20 percent of the muscle samples. Mean and median mercury concentrations were below the action level in muscle tissue of green-winged teal, redheads, and canvasbacks (fig. 18).

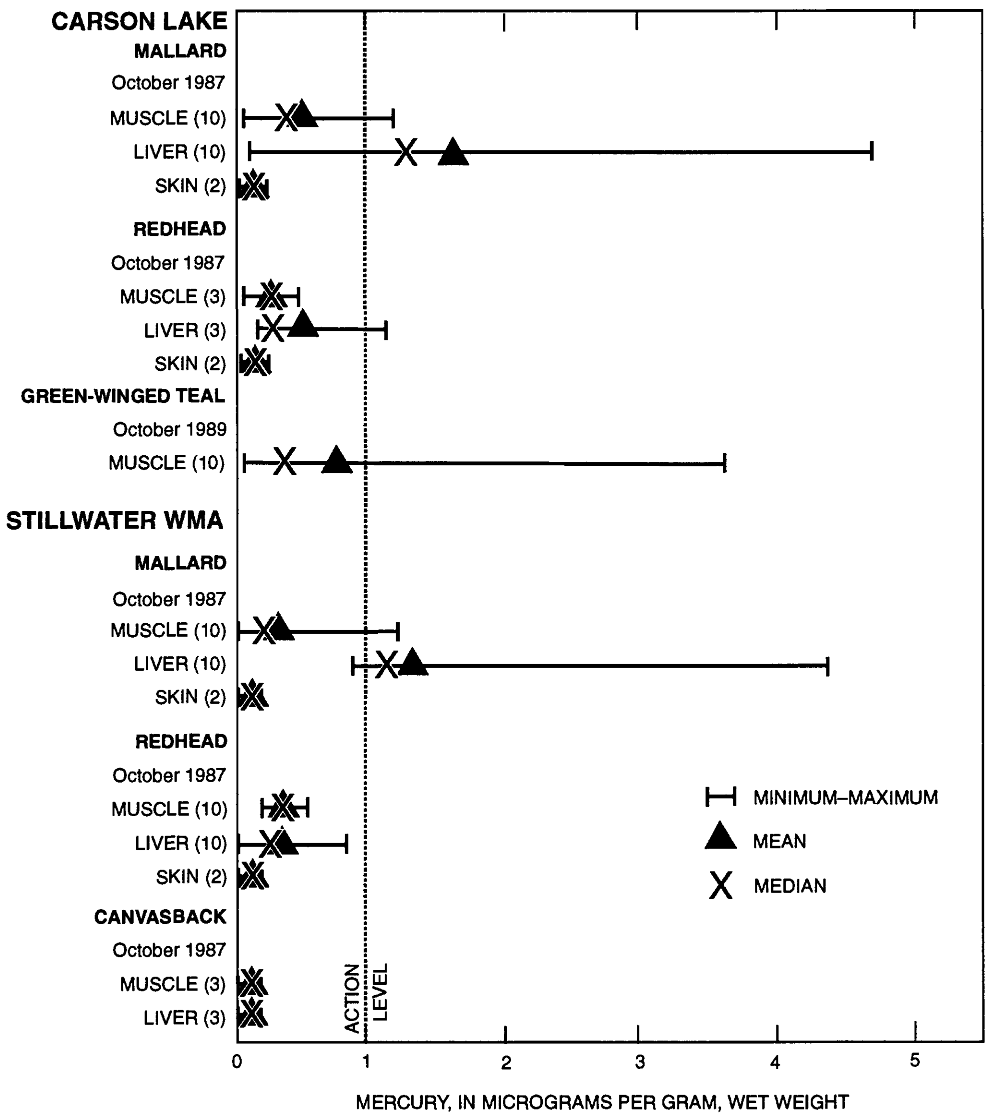


Figure 18. Concentrations of mercury in edible portions of various duck species collected at Stillwater WMA and Carson Lake during the opening week of waterfowl season, October 1987. Number of samples in parentheses. The action level is 1.0 microgram per gram, wet weight (U.S. Food and Drug Administration, 1984, p. 15).

Selenium Accumulation in Waterfowl

Although Hoffman and others (1990, p. 66-70) reported selenium concentrations much higher than the action level of 2.0 µg/g, wet weight, in liver tissue of various juvenile migratory birds from the study area, less than 15 percent (8 of 58) of livers from various waterfowl collected in October 1987 from Carson Lake and Stillwater WMA exceeded 2.0 µg/g. However, within this data set, 10 shoveler livers taken on October 17, 1987, contained a mean selenium concentration of 2.2 µg/g and ranged from 0.8 to 3.8 µg/g. Shovelers are fall migrants believed to have been in these wetlands up to 6 weeks prior to collection. None of the muscle samples from these birds contained selenium concentrations above 2.0 µg/g (Rowe and others, 1991, p. 118-151).

Fernley WMA could not be evaluated during the 1989 hunting season because it was dry by mid-October and, therefore, contained no ducks. However, during July 1988, juvenile cinnamon teal (*Anas cyanoptera*) and coots with mean selenium concentrations in livers of 8.4 and 8.6 µg/g, wet weight, respectively, were collected from Fernley WMA. Selenium in livers of juvenile redhead ducks from Massie and Mahala Sloughs averaged 8.5 µg/g and 3.7 µg/g, wet weight, respectively, as reported in the preceding section on waterfowl production. In a study of mallards, Heinz and others (1990, p. 374) found that after 81 days on a selenium-rich diet, concentrations of selenium were slightly higher in muscle than in liver tissues. This suggests that by October, at the start of hunting season, if the juvenile ducks had remained in Fernley WMA and Massie Slough they might have had selenium concentrations in muscle that were higher than the concentrations found in July (>8.0 µg/g), which was already more than four times the established human-health criteria.

Differing lengths of waterfowl residence in the wetlands may account for some of the variability in the data from this study. Other important factors determining intake of potentially toxic constituents are dietary preferences and the specific wetlands selected by birds.

(p. 71 follows)

OVERALL SUMMARY OF EFFECT OF IRRIGATION DRAINAGE ON BIOTA

Important findings of the five biologically related study elements addressing possible adverse effects of irrigation drainage on biota in and near Stillwater WMA are summarized here. The goal of the investigation was to (1) determine the effects of irrigation drainage on migratory waterfowl that frequent these wetlands and (2) provide information for resource-management decisions and subsequent remediation strategies. Major findings of the studies, in order of presentation, are:

- Historical wetlands in Carson Lake, Stillwater Marsh, and Carson Sink averaged about 150,000 acres. Under the Operating Criteria and Procedures mandates, fully implemented in 1992, only about 10 percent, or 15,000 wetland acres, are projected to remain in nearly permanent impoundments.
- Average dissolved-solids concentration in drainwater entering these wetlands has increased about seven-fold from the estimated historical 170 mg/L to a current average of 1,170 mg/L. This increase in concentration has occurred largely through evapotranspiration associated with irrigated agriculture. Although the annual dissolved-solids load has decreased in these now-isolated wetlands, the increased concentrations have adversely affected the various plants, invertebrates, fish, and wildlife that were once abundant in the historical wetlands.
- During acute-toxicity tests, organisms in control water survived wide fluctuations in specific conductance that ranged from 410 to 27,500 μS/cm (252 to 17,900 mg/L dissolved solids). Organisms in undiluted drainwater samples subjected to similar fluctuations in dissolved-solids concentrations did not survive. Surface water from TJ and Hunter Drains and ground water from a shallow well near TJ Drain were acutely toxic to all test organisms.
- Analysis of undiluted drainwater showed that four potentially toxic elements—arsenic, boron, lithium, and molybdenum—were representative of the overall levels of toxicity in the water tested. Strong, positive relations were found between the aggregate of arsenic, boron, lithium, and molybdenum and both daily and average dissolved-solids concentration. Thus, specific conductance may be a useful measure of surface-water quality for management of fish and invertebrate populations in Stillwater WMA.
- Within drains, both mercury and selenium are being bioaccumulated in plants and plant detritus and biomagnified in invertebrates by factors of up to 10,000 times the concentrations measured in associated drainwater.
- Mercury and selenium are being transported in living organisms and their detritus. Transport is from irrigated land, by way of irrigation drains, to large wetlands where most migratory birds, fish, and other wildlife are exposed to these elements.
- Source areas of mercury and selenium in the Newlands Irrigation Project area are areas generally upgradient from sampling sites in drains where concentrations exceeded concern levels of 1.0 μg/g, dry weight, in detritus.
- Although selenium continues to be released from some irrigated soils more than 40 years after initiation of irrigation, and is being carried by plants and invertebrates through the drains, no evidence was found to indicate a long-term build up of selenium in the sediment and biota of downgradient wetlands.

- Potential contaminant concentrations in waterfowl eggs from the study sites were generally below published effect-level criteria and would not be expected to affect bird production. Consistent with these data, no teratogenesis was observed in embryos or hatchlings. The hatch rate of waterfowl eggs was normal, 90 percent or more, at both study and background sites.

- Concentrations of boron, mercury, and selenium at adverse effect levels were found in juvenile migratory birds from several study sites. Concentrations of selenium as high as 30 µg/g were found in duck and coot livers from Fernley WMA and Massie Slough, and 77 µg/g in black-necked stilt livers from Mahala Slough. Decreased survival and, thus, production may be expected among birds containing selenium concentrations in this range.

- Nest success was poor throughout the study areas, averaging only 26 percent. Loss of feeding habitat and nesting cover caused by drought, increased dissolved-solids concentrations, and predation are the primary reasons for reduced nest success. Thus, waterfowl production is now much reduced compared to historical conditions.

- Shoveler ducks harvested by hunters from Carson Lake had accumulated mercury in muscles and livers, 5.9 and 17.8 times greater, respectively, on average, than the 1.0 µg/g, wet weight, action level for human consumption. In response to the elevated mercury concentrations, the State of Nevada posted human-health warnings at Carson Lake specific to shoveler ducks.

- Concentrations of selenium in juvenile-waterfowl livers from Fernley WMA and Massie Slough in July 1988 averaged more than 8.0 µg/g, wet weight, or more than four times the 2.0 µg/g, wet weight, criterion for human consumption. Because of drought, no birds were present in these areas in October 1989 for collection and evaluation; thus, the potential for human-health concerns in these areas could not be fully evaluated.

REFERENCES CITED

American Society for Testing Materials, 1988, Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians: Philadelphia, ASTM Designation E 729-88, 20 p.

Bailey, Vernon, 1898, Nevada: Carson Lake Valley (Wadsworth, Ragtown and Stillwater) Physiography: Unpublished manuscript, Smithsonian Institution Archives, Record Unit 7176, U.S. Fish and Wildlife Service, 1860-1961, Field Reports, Box 69, Folder 14, 3 p.

Brown, W.M., Nowlin, J.O., Smith, L.H., and Flint, M.R., 1986, River quality assessment of the Truckee and Carson River system, California and Nevada—Hydrologic characteristics: U.S. Geological Survey Open-File Report 84-576, 201 p.

Burton, R.F, 1963, The City of the Saints and across the Rocky Mountains to California: New York, Alfred A. Knopf, Inc., facsimilie of the 1861 edition, 654 p.

California Department of Water Resources, 1991, Carson River atlas: Sacramento, Calif., State of California, 126 p.

Claassen, H.C., 1982, Guidelines and techniques for obtaining water samples that accurately represent the water chemistry of an aquifer: U.S. Geological Survey Open-File Report 82-1024, 49 p.

Cooper, J.J., Thomas, R.O., and Reed, S.M., 1985, Total mercury in sediment, water, and fishes in the Carson River drainage, west-central Nevada: Nevada Division of Environmental Protection, Carson City, Nev., 96 p.

DeQuille, Dan, 1963, Washoe rambles: Los Angeles, Westernlore Press, reproduction of a series of articles published in 1861, 169 p.

Drews, M.P., 1988, Freshwater molluscs, *in* Raven, Christopher, and Elston, R.G., eds., Preliminary investigations in Stillwater Marsh—Human prehistory and geoarchaeology: Portland, Oreg., U.S. Fish and Wildlife Service, p. 328-338.

Dwyer, F.J., Burch, S.A., and Ingersoll, C.G., 1990, Investigation on the combined toxicity of trace elements and salinity to aquatic organisms at Stillwater Wildlife Management Area: Columbia, Mo., U.S. Fish and Wildlife Service, Final Report, 47 p.

Dwyer, F.J., Burch, S.A., Ingersoll, C.G., and Hunn, J.B., 1992, Toxicity of trace elements and salinity mixtures to striped bass (*Morone saxatilis*) and Daphnia magna: Environmental Toxicology and Chemistry, v. 11, p. 513-520.

Eisler, Ronald, 1985, Selenium hazards to fish, wildlife, and invertebrates—A synoptic review: U.S. Fish and Wildlife Service Biological Report 85 (1.5), 57 p.

——1987, Mercury hazards to fish, wildlife, and invertebrates—A synoptic review: U.S. Fish and Wildlife Service Biological Report 85 (1.10), 90 p.

——1990, Boron hazards to fish, wildlife and invertebrates—A synoptic review: U.S. Fish and Wildlife Service Biological Report 85 (1.20), 32 p.

Evans, C., 1983, Waterfowl nesting at Stillwater marsh in relation to predation and habitat factors affecting nest site selection: U.S. Fish and Wildlife Service, Unpublished Report, 45 p.

Fan, A.M., Book, S.A., Neutra, R.R., and Epstein, D.M., 1988, Selenium and human health implications in California's San Joaquin Valley: Journal of Toxicology and Environmental Health, v. 23, p. 539-569.

Finley, M.T., and Stendell, R.C., 1978, Survival and reproductive success of black ducks fed methyl mercury: Environmental Pollution, v. 16, p. 51-64.

Frankenberger, W.T., and Thompson-Eagle, E.T., 1989, *In situ* volatilization of selenium–II evaporation ponds, Executive Summary, San Joaquin Valley Drainage Program: Sacramento, Calif., U.S. Bureau of Reclamation, 6 p.

Frisbie, H.R., LaCamera, R.J., Riek, M.M., and Wood D.B., 1984, Water-resources data for Nevada, Water Year 1983: U.S. Geological Survey Water-Data Report NV-83-1, 328 p.

Gilliom, R.J., and others, 1989, Preliminary assessment of sources, distribution, and mobility of selenium in the San Joaquin Valley, California: U.S. Geological Survey Water-Resources Investigations Report 88-4186, 129 p.

Glancy, P.A., and Katzer, T.L., 1976, Water-resources appraisal of the Carson River basin, western Nevada: Nevada Division of Water Resources, Reconnaissance Report 59, 126 p.

Greenspan, R.L., 1988, Fish remains, *in* Raven, Christopher, and Elston, R.G., eds., Preliminary investigations in Stillwater Marsh—Human prehistory and geoarchaeology: Portland, Oreg., U.S. Fish and Wildlife Service, p. 312-326.

Hart, R.A., and Bixby, F.L., 1922, Report of review of proposed drainage plans for the drainage of a portion of the Newlands Irrigation District at Fallon, Nevada: U.S. Bureau of Reclamation, Smithsonian Institution, Unpublished Manuscript, 27 p.

Heinz, G.H., 1979, Methylmercury—Reproductive and behavioral effects on three generations of mallard ducks: Journal of Wildlife Management, v. 43, no. 2, p. 394-401.

Heinz, G.H., Hoffman, D.J., and Gold, L.G., 1988, Toxicity of organic and inorganic selenium to mallard ducklings: Archives of Environmental Contamination and Toxicology, v. 17, p. 561-568.

——1989, Impaired reproduction of mallards fed an organic form of selenium: Journal of Wildlife Management, v. 53, no. 2, p. 418-428.

Heinz, G.H., Hoffman, D.J., Krynitsky, A.J., and Weller, D.M.G, 1987, Reproduction in mallards fed selenium: Environmental Toxicology and Chemistry, v. 6, p. 423-433.

Heinz, G.H., Pendleton, G.W., Krynitsky, A.J., and Gold, L.G., 1990, Selenium accumulation and elimination in mallards: Archives of Environmental Contamination and Toxicology, v. 19, p. 374-379.

Henny, C.J., and Herron, G.B., 1989, DDE, selenium, mercury, and white-faced ibis reproduction at Carson Lake, Nevada: Journal of Wildlife Management, v. 53, no. 4, p. 1032-1045.

Hoffman, R.J., in press, Detailed study of irrigation drainage in and near Wildlife Management Areas, west-central Nevada, 1987-90. Part C—Summary of irrigation-drainage effects on water quality, bottom sediment, and biota: U.S. Geological Survey Water-Resources Investigations Report 92-4024C.

Hoffman, R.J., Hallock, R.J., Rowe, T.G., Lico, M.S., Burge, H.L., and Thompson, S.P., 1990, Reconnaissance investigation of water quality, bottom sediment, and biota associated with irrigation drainage in and near Stillwater Wildlife Management Area, Churchill County, Nevada 1986-87: U.S. Geological Survey Water-Resources Investigations Report 89-4105, 150 p.

Ingersoll, C.G., Dwyer, F.J., Burch, S.A., Nelson, M.K., Buckler, D.R., and Hunn, J.B., 1992, The use of freshwater and saltwater animals to distinguish between the toxic effects of salinity and contaminants in irrigation drain water: Environmental Toxicology and Chemistry, v. 250, p. 503-511.

Kelly, R.L., 1988, Archaeological context, *in* Raven, Christopher, and Elston, R.G., 1988, Preliminary investigations in Stillwater Marsh—Human prehistory and geoarchaeology: U.S. Fish and Wildlife Service, v. 1, p. 5-20.

Knapton, J.R., Jones, W.E., and Sutphin, J.W., 1988, Reconnaissance investigation of water quality, bottom sediment, and biota associated with irrigation drainage in the Sun River area, west-central Montana, 1986-87: U.S. Geological Survey Water-Resources Investigations Report 87-4244, 78 p.

Lambing, J.H., Jones, W.E., and Sutphin, J.W., 1988, Reconnaissance investigation of water quality, bottom sediment, and biota associated with irrigation drainage in Bowdoin National Wildlife Refuge and adjacent areas of the Milk River basin, northeastern Montana, 1986-87: U.S. Geological Survey Water-Resources Investigations Report 87-4243, 71 p.

Lemly, A.D., and Smith, G.J., 1987, Aquatic cycling of selenium—Implications for fish and wildlife: U.S. Fish and Wildlife Service leaflet 12, 10 p.

Lico, M.S., 1992, Detailed study of irrigation drainage in and near Wildlife Management Areas, west-central Nevada, 1987-1990. Part A—Water quality, sediment composition, and hydrogeochemical processes in Stillwater and Fernley Wildlife Management Areas: U.S. Geological Survey Water-Resources Investigations Report 92-4024A, 65 p.

Long, E.R., and Morgan, L.G., 1990, The potential for biological effects of sediment-sorbed contaminants tested in the National Status and Trends Program: National Oceanic and Atmospheric Administration Technical Memorandum NOSOMA 52, 175 p.

Morrison, R.B., 1964, Lake Lahontan—Geology of southern Carson Desert, Nevada: U.S. Geological Survey Professional Paper 401, 156 p.

Napier, L.D., 1974, Report of wildlife management study—Duck nesting success on the Stillwater Marsh: Fallon, Nev., U.S. Fish and Wildlife Service, Unpublished final report, 34 p.

Ohlendorf, H.M., 1989, Bioaccumulation and effects of selenium in wildlife: Soil Science Society of America and American Society of Agronomy, Special Publication no. 23, p. 133-177.

Patuxent Wildlife Research Center, 1987, Effects of irrigation drainage contaminants on wildlife: Laural Md., U.S. Fish and Wildlife Service, Annual Report FY 1987, 31 p.

Peden, M.E., 1986, Methods for collection and analysis of precipitation: Champaign, Ill., Illinois State Water Survey, 260 p.

Peterson, D.A., Jones, W.E., and Morton, A.G., 1988, Reconnaissance investigation of water quality, bottom sediment, and biota associated with irrigation drainage in the Kendrick Reclamation Project area, Wyoming, 1986-87: U.S. Geological Survey Water-Resources Investigations Report 87-4255, 57 p.

Presser, T.S., and Ohlendorf, H.M., 1987, Biogeochemical cycling of selenium in the San Joaquin Valley, California, USA: Environmental Management, v. 11, no. 6, p. 805-821.

Pupacko, A., LaCamera, R.J., Riek, M.M., and Swartwood, J.R., 1989, water resources data, Nevada, water year 1988: U.S. Geological Survey Water-Data report NV-88-1, 265 p.

Radtke, D.B., Kepner, W.G., and Effertz, R.J., 1988, Reconnaissance investigation of water quality, bottom sediment, and biota associated with irrigation drainage in the Lower Colorado River Valley, Arizona, California, and Nevada, 1986-87: U.S. Geological Survey Water-Resources Investigations Report 88-4002, 77 p.

Raven, Christopher, and Elston, R.G., 1988, Preliminary Investigations in Stillwater Marsh—Human prehistory and geoarchaeology: U.S. Fish and Wildlife Service, v. 1 and 2.

——1989, Preliminary Investigations in Stillwater Marsh—Human prehistory and geoarchaeology: U.S. Fish and Wildlife Service, v. 3.

——1990, Preliminary Investigations in Stillwater Marsh—Human prehistory and geoarchaeology: U.S. Fish and Wildlife Service, v. 4.

Rowe, T.G., and Hoffman, R.J., 1990, Wildlife kills in the Carson Sink, western Nevada, winter of 1986-87, *in* Carr, J.E., Chase, E.B., and Paulson, R.W. and Moody, D.W., comps., National water summary 1987—Hydrologic events and water supply and use: U.S. Geological Survey Water-Supply Paper 2350, p. 37-40.

Rowe, T.G., Lico, M.S., Hallock, R.J., Maest, A.S., and Hoffman, R.J., 1991, Physical, chemical, and biological data associated with irrigation drainage in and near Stillwater, Fernley, and Humboldt Wildlife Management Areas and Carson Lake, west-central Nevada, 1987-1989: U.S.Geological Survey Open-File Report 91-185, 199 p.

Rush, F.E., 1968, Index of hydrographic areas in Nevada: Nevada Division of Water Resources Information Report 6, 38 p.

Russell, I.C., 1885, Geological history of Lake Lahontan—A Quaternary lake of northwestern Nevada: U.S. Geological Survey Monograph 11, 288 p.

——1895, Present and extinct lakes of Nevada: American Book Company, Reprinted by Nevada Historical Society, 1976, as Camp Nevada Monograph No. 3, 132 p.

Schmitt, D.N., 1988, Mammalian fauna, *in* Raven, Christopher, and Elston, R.G., eds., Preliminary investigations in Stillwater Marsh—Human prehistory and geoarchaeology: Portland, Oreg., U.S. Fish and Wildlife Service, v. 1, p. 262-290.

Schroeder, R.A., Palawski, D.U., and Skorupa, J.P., 1988, Reconnaissance investigation of water quality, bottom sediment, and biota associated with irrigation drainage in the Tulare Lake Bed area, southern San Joaquin Valley, California, 1986-87: U.S. Geological Survey Water-Resources Investigations Report 88-4001, 86 p.

Seiler, R.L., and Allander, K.K., in press, Water-level changes and directions of ground-water flow in the shallow aquifer, Fallon area, Churchill County, Nevada: U.S. Geological Survey Water-Resources Investigation Report 93-4118.

Setmire, J.G., Wolfe, J.C., and Stroud, R.K., 1990, Reconnaissance investigation of water quality, bottom sediment, and biota associated with irrigation drainage in the Salton Sea area, California, 1986-87: U.S. Geological Survey Water-Resources Investigations Report 89-4102, 68 p.

Simpson, J.H., 1876, Report of explorations across the Great Basin of the Territory of Utah for a direct wagon-route from Camp Floyd to Genoa, in Carson Valley, in 1859: U.S. Army Engineer Department; reprinted 1983 by University of Nevada Press, Reno, Nev., Vintage Nevada Series, 518 p.

Smith, G., 1985, The paleontology of Hidden Cave—Fish, *in* Thomas, D.H., ed., The archaeology of Hidden Cave, Nevada: Anthropological Papers of the American Museum of Natural History, v. 61, no. 1, p. 171-178.

Smith, G.H., 1943, The history of the Comstock Lode, 1850-1920; University of Nevada Bulletin, Geology and Mining Series no. 37, 305 p.

Smith, G.J., and Anders, V.P., 1989, Toxic effects of boron on mallard reproduction: Environmental Toxicology and Chemistry, v. 8, p. 943-950.

Spence, M.L., and Jackson, Donald, 1973, The expeditions of John Charles Fremont: Chicago, University of Illinois Press, v. 2., 519 p.

Sperry, C.C., 1929, Report on Carson Sink (Churchill Co.) Nevada—Its duck food resources and value as a Federal Migratory Bird Refuge site: Smithsonian Institution Archives, Unpublished manuscript, 3 p.

Stephens, D.W., Waddell, Bruce, and Miller, J.B., 1988, Reconnaissance investigation of water quality, bottom sediment, and biota associated with irrigation drainage in the Middle Green River basin, Utah, 1986-87: U.S. Geological Survey Water-Resources Investigations Report 88-4011, 70 p.

Steward, A.N., Denis, L.J., and Gilkey, H.M., 1963, Aquatic plants of the Pacific Northwest with vegetative keys (2nd ed.): Corvallis, Oregon State University Press, 261 p.

Stewart, R.E., and Kantrud, H.A., 1972, Vegetation of prairie potholes, North Dakota, in relation to quality of water and other environmental factors: U.S. Geological Survey Professional Paper 585-D, 36 p.

Taylor, R.E., 1972, The effects of increasing salinity on the Pyramid Lake fishery: Reno, University of Nevada, Agricultural Experiment Station Bulletin 15, 8 p.

Tokunaga, Tetsu, and Benson, S.M., 1991, Evaluation of management options for disposal of salt and trace element laden agricultural drainage water from the Fallon Indian Reservation, Fallon, Nevada: Lawrence Berkeley Laboratory, under contract with U.S. Bureau of Reclamation, final report 165 p.

U.S. Bureau of Reclamation, 1987, Fallon Indian Reservation water quality report: Sacramento, U.S. Bureau of Reclamation, 62 p., 3 appendices.

U.S. Department of the Interior, 1988, Final operating agreement and procedures, record of decision, Newlands Project, Nevada-California: Washington, D.C., 22 p., 5 appendices.

U.S. Environmental Protection Agency, 1985, Methods for measuring the acute toxicity of effluents to freshwater and marine organisms (3d ed.): Cincinnati, Ohio, Environmental Monitoring and Support Laboratory, EPA/600/4-85/013, 216 p., 5 appendices.

——1986, Quality criteria for water: Washington, D.C., U.S. Office of Water Regulations and Standards, Report EPA-440/5-86-001, [453 p.]

U.S. Fish and Wildlife Service, 1952, Narrative report, Stillwater Wildlife Management Area January-April 1952: Fallon, Nev., U.S. Fish and Wildlife Service, unpublished report, 33 p.

——1969, Refuge narrative report: Fallon, Nev., U.S. Fish and Wildlife Service, unpublished report, 42 p.

——1985, Procedures for resource contaminant assessment contract analytical work: Washington D.C., Habitat Resources Instructional Memorandum, 203 p.

——1988, Record of decision, draft wetlands analysis—Newlands Project operating criteria and procedures: Fallon, Nev., U.S. Fish and Wildlife Service, 141 p.

U.S. Food and Drug Administration, 1984, Compliance policy guide for methyl mercury in fish: Report 7108.07, Federal Register, v. 49, no. 45663, 1 p.

U.S. Geological Survey, 1978, Water resources data, Nevada, Water Year 1977: U.S. Geological Survey Water Data Report NV-77-1, 334 p.

Van Denburgh, A.S., and Arteaga, F.E., 1985, Revised water budget for the Fernley area, west-central Nevada: U.S. Geological Survey Open-File Report 84-712, 17 p.

Warburton, J.A., and others, 1990, Studies of climatic variations and their impact in the Great Basin: Final report on Contract NA 87AA-D-CP114 by Desert Research Institute, Reno, Nev., for National Oceanic and Atmospheric Administration, 94 p.

Wells, F.C., Jackson, G.A., and Rogers, W.J., 1988, Reconnaissance investigation of water quality, bottom sediment, and biota associated with irrigation drainage in the Lower Rio Grande Valley and Laguna Atascosa National Wildlife Refuge, Texas, 1986-87: U.S. Geological Survey Water-Resources Investigations Report 87-4277, 89 p.

Wershaw, R.L., Fishman, M.J., Grabbe, R.R., and Lowe, L.E., eds., 1987, Methods for determination of organic substances in water and fluvial sediments: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A3, 80 p.

Westerkov, K., 1950, Methods for determining the age of gamebird eggs: Journal of Wildlife Management, v. 14, p. 56-61.

(p.77 follows)

SUPPLEMENTAL WATER-QUALITY DATA FROM TOXICITY STUDY

Table 23. Water-quality measurements for daily composite water samples from Paiute Diversion Drain, August 1988

[Concentrations in milligrams per liter except as indicated. All mercury concentrations were below analytical reporting limit. Abbreviations: µg/L, micrograms per liter; µS/cm, microsiemens per centimeter at 25°C; NTU, Nephelometric turbidity units; ppt, parts per thousand.]

Date	Specific conductance (µS/cm)	Field pH	Onsite mobile lab pH	Turbidity (NTU)	Dissolved oxygen	Calcium	Magnesium
August 10	420	7.5	8.2	31	6.8	33	11
11	370	8.4	8.2	35	8.9	31	10
12	480	8.3	8.4	33	7.7	35	12
13	390	8.0	8.3	32	7.3	33	11
14	400	8.3	8.1	16	9.7	35	12
15	400	8.5	8.4	15	8.9	36	12
16	400	8.5	8.5	25	8.8	35	11
17	383	8.6	8.6	20	9.0	35	13
18	430	8.6	8.6	18	8.7	36	11

Date	Hardness	Sodium	Potassium	Alkalinity	Sulfate	Chloride	Silica
August 10	120	72	3.8	142	85	63	5.8
11	110	62	2.8	111	68	58	6.3
12	130	84	3.9	146	64	50	7.0
13	120	68	4.9	133	60	55	6.7
14	130	75	5.4	138	72	60	6.6
15	140	79	5.3	145	72	60	6.9
16	130	72	4.4	158	52	65	6.8
17	130	82	4.7	135	52	70	7.2
18	130	73	3.9	157	60	65	6.6

Date	Salinity (ppt)	Nitrate as N	Ammonia as N	Phosphorus	Arsenic	Barium	Boron
August 10	0.2			0.1	0.01	0.08	0.58
11	.2			<.1	.02	.08	.52
12	.3	<0.01	0.16	.1	.02	.08	.76
13	.2			.1	.02	.08	.58
14	.1	<.01	.06	.1	.02	.09	.60
15	.1			.1	.02	.08	.63
16	.1			.1	.02	.07	.60
17	.1	<.01	.04	.2	.02	.07	.55
18	.2			<.1	.02	.08	.61

Date	Lithium	Molybdenum	Selenium (µg/L)	Strontium	Vanadium	Zinc
August 10	0.06	0.02	<0.3	0.37	0.01	0.07
11	.05	<.02	.3	.35	.01	.03
12	.06	.02	.3	.40	.01	.03
13	.06	<.02	.3	.37	.01	.05
14	.06	.02	.3	.40	.01	.04
15	.06	.02	.3	.41	.01	.05
16	.06	<.02	.3	.38	.01	.03
17	.06	<.02	.3	.41	.01	.02
18	.06	.02	.3	.40	.01	.03

Table 24. Water-quality measurements for daily composite water samples from TJ Drain, August 1988

[Concentrations in milligrams per liter except as indicated. All mercury concentrations were below analytical reporting limit. Abbreviations: µg/L, micrograms per liter; µS/cm, microsiemens per centimeter at 25°C; NTU, Nephelometric turbidity units; ppt, parts per thousand.]

Date	Specific conductance (µS/cm)	Field pH	Onsite mobile lab pH	Turbidity (NTU)	Dissolved oxygen	Calcium	Magnesium
August 10	8,100	8.5	8.5	2	7.4	180	200
11	12,000	8.5	9.0	3	8.4	260	320
12	11,800	8.6	8.5	2	8.7	240	300
13	14,900	8.5	8.3	3	8.7	310	410
14	11,000	8.6	8.4	2	9.2	230	290
15	6,100	8.4	8.1	4	8.3	140	160
16	7,200	8.4	8.3	4	8.9	160	180
17	13,700	8.5	8.4	3	8.7	290	360
18	14,300	8.6	8.4	8	10.9	210	250

Date	Hardness	Sodium	Potassium	Alkalinity	Sulfate	Chloride	Silica
August 10	1,300	2,000	29	232	1,400	2,700	2.1
11	1,900	3,500	42	289	2,900	4,800	.9
12	2,000	3,300	40	263	2,900	4,900	2.0
13	2,500	4,700	57	319	3,600	6,200	.6
14	1,800	3,400	43	276	1,600	4,700	.1
15	1,100	1,900	29	224	880	3,800	3.7
16	1,300	1,900	29	217	1,500	5,800	2.9
17	1,900	4,000	50	292	1,600	4,900	.8
18	1,700	2,900	39	244	1,600	4,800	3.8

Date	Salinity (ppt)	Nitrate as N	Ammonia as N	Phosphorus	Arsenic	Barium	Boron
August 10	6.5			0.7	0.11	0.08	7.8
11	10.0			1.0	.13	.11	13
12	10.0	<0.01	0.11	.8	.12	.11	12
13	13.0			1.0	.17	.10	17
14	9.2	<.01	.02	.8	.15	.07	12
15	5.0			.4	.13	.05	6.8
16	4.1			.4	.11	.05	6.8
17	8.9	<.01	.16	1.0	.14	.11	14
18	9.2			.8	.12	.08	10

Date	Lithium	Molybdenum	Selenium (µg/L)	Strontium	Vanadium	Zinc
August 10	0.40	0.32	1.2	4.0	0.02	0.05
11	.62	.54	1.3	6.2	.02	.07
12	.57	.45	1.2	5.8	.02	.09
13	.76	.67	1.6	7.9	.02	.01
14	.55	.45	1.1	5.7	.02	.03
15	.34	.27	1.1	3.2	.02	.02
16	.38	.38	.9	3.5	.01	.02
17	.67	.67	1.3	7.0	.02	.02
18	.46	.46	1.5	4.9	.02	.02

Table 25. Water-quality measurements for daily composite water samples from D-Line Canal, August 1988

[Concentrations in milligrams per liter except as indicated. All mercury concentrations were below analytical reporting limit. Abbreviations: µg/L, micrograms per liter; µS/cm, microsiemens per centimeter at 25°C; NTU, Nephelometric turbidity units; ppt, parts per thousand.]

Date	Specific conductance (µS/cm)	Field pH	Onsite mobile lab pH	Turbidity (NTU)	Dissolved oxygen	Calcium	Magnesium
August 10	600	8.6	8.7	4	9.2	30	11
11	490	9.3	9.0	5	10.0	26	11
12	610	9.2	9.0	4	9.5	28	11
13	400	9.2	9.0	3	9.2	26	10
14	425	9.2	8.9	4	10.3	29	11
15	380	9.0	8.1	3	9.8	28	10
16	350	9.0	9.1	6	10.0	27	10
17	445	8.7	8.7	9	9.0	32	10
18	470	9.4	9.3	9	9.9	30	9

Date	Hardness	Sodium	Potassium	Alkalinity	Sulfate	Chloride	Silica
August 10	94	110	9.4	149	110	50	6.9
11	100	110	8.6	156	110	12	7.0
12	110	100	8.3	165	60	50	6.6
13	100	97	8.2	155	56	43	5.9
14	120	110	8.4	157	130	47	5.9
15	110	84	6.3	135	68	40	6.0
16	98	87	6.2	125	47	36	7.2
17	130	96	5.4	174	100	45	7.3
18	110	83	6.5	113	48	45	7.6

Date	Salinity (ppt)	Nitrate as N	Ammonia as N	Phosphorus	Arsenic	Barium	Boron
August 10	0.5			<0.1	0.05	0.06	0.76
11	.4			<.1	.05	.05	.78
12	.5	<0.01	0.10	<.1	.05	.05	.74
13	.0			<.1	.05	.04	.79
14	.2	.01	.04	<.1	.04	.04	.81
15	.1			<.1	.04	.05	.70
16	.0			<.1	.04	.05	.77
17	.0	.01	.05	<.1	.05	.05	.75
18	.1			<.1	.04	.05	.62

Date	Lithium	Molybdenum	Selenium (µg/L)	Strontium	Vanadium	Zinc
August 10	0.06	0.02	0.3	0.37	0.02	0.03
11	.06	.02	.3	.34	.02	.01
12	.06	.02	.3	.36	.02	.01
13	.06	.02	.3	.34	.02	.01
14	.06	.02	.3	.37	.02	.01
15	.05	.02	.3	.34	.01	.01
16	.05	.02	.3	.34	.01	.01
17	.05	.02	.3	.36	.01	.04
18	.05	<.02	.3	.34	.02	.02

Table 26. Water-quality measurements for daily instantaneous water samples from Lead Lake, August 1988

[Concentrations in milligrams per liter except as indicated. All mercury concentrations were below analytical reporting limit. Abbreviations: μg/L, micrograms per liter; μS/cm, microsiemens per centimeter at 25°C; NTU, Nephelometric turbidity units; ppt, parts per thousand.]

Date	Specific conductance (μS/cm)	Field pH	Onsite mobile lab pH	Turbidity (NTU)	Dissolved oxygen	Calcium	Magnesium
August 10	6,100	8.9	8.8	62	9.2	67	96
11	6,000	8.8	9.2	96	7.2	69	97
12	5,900	8.9	8.9	59	6.8	71	100
13	5,300	8.8	8.8	63	5.6	65	92
14	5,600	8.6	8.8	53	7.1	68	98
15	5,100	8.9	8.8	37	5.7	64	90
16	5,300	8.9	8.9	53	6.9	67	96
17	5,900	8.9	9.0	50	8.5	68	103
18	5,300	8.9	8.9	43	8.6	74	103

Date	Hardness	Sodium	Potassium	Alkalinity	Sulfate	Chloride	Silica
August 10	580	1,000	32	227	430	1,300	6.9
11	570	1,000	32	272	600	1,400	7.5
12	630	1,100	33	257	680	1,200	6.9
13	580	1,000	31	266	400	1,700	6.8
14	600	1,100	33	256	520	1,400	6.8
15	600	980	30	249	470	1,300	6.3
16	610	1,000	31	258	360	1,800	6.4
17	640	1,100	33	258	410	1,800	7.3
18	680	1,100	29	227	560	1,800	5.1

Date	Salinity (ppt)	Nitrate as N	Ammonia as N	Phosphorus	Arsenic	Barium	Boron
August 10	3.5			0.2	0.12	0.14	6.0
11	3.5			.2	.12	.13	5.4
12	3.8	<0.01	0.14	.2	.13	.13	5.6
13	3.2			.2	.11	.12	5.2
14	3.5	.01	.14	.2	.11	.13	5.5
15	4.2			.2	.10	.12	5.0
16	3.1			.2	.11	.13	5.3
17	3.5	.05	.24	.4	.11	.13	5.7
18	3.3			.2	.10	.11	5.4

Date	Lithium	Molybdenum	Selenium (μg/L)	Strontium	Vanadium	Zinc
August 10	0.36	0.11	0.3	1.9	0.02	0.02
11	.36	.11	.3	1.9	.02	.04
12	.37	.12	.3	1.9	.02	.01
13	.34	.11	.3	1.8	.02	.02
14	.37	.12	.3	1.9	.02	.02
15	.34	.11	.3	1.8	.02	.02
16	.36	.11	.3	1.8	.02	.02
17	.39	.12	.3	2.0	.02	.04
18	.34	.13	.3	2.0	.02	.02

Table 27. Water-quality measurements for daily composite water samples from Hunter Drain, August 1988

[Concentrations in milligrams per liter except as indicated. All mercury concentrations were below analytical reporting limit. Abbreviations: µg/L, micrograms per liter; µS/cm, microsiemens per centimeter at 25°C; NTU, Nephelometric turbidity units; ppt, parts per thousand.]

Date	Specific conductance (µS/cm)	Field pH	Onsite mobile lab pH	Turbidity (NTU)	Dissolved oxygen	Calcium	Magnesium
August 10	12,000	8.3	8.2	14	7.0	190	100
11	13,900	8.4	8.9	7	6.2	220	130
12	27,500	8.5	8.4	37	7.4	350	230
13	22,000	8.8	8.7	13	6.3	380	240
14	25,000	8.7	8.5	8	5.6	390	250
15	8,000	8.7	8.6	14	7.5	160	100
16	410	8.0	8.0	19	8.2	32	10
17	600	8.3	8.2	14	8.4	35	13
18	1,200	8.2	8.3	10	8.8	38	15

Date	Hardness	Sodium	Potassium	Alkalinity	Sulfate	Chloride	Silica
August 10	860	3,600	89	223	1,200	4,200	5.0
11	1,100	4,500	110	250	1,300	7,000	13
12	2,300	7,600	180	242	2,000	11,000	14
13	1,800	8,200	200	255	2,900	10,000	13
14	200	8,600	210	276	2,900	13,000	14
15	660	3,200	76	179	1,200	3,700	9.0
16	110	98	4.8	255	52	84	7.6
17	140	180	4.9	124	130	190	6.8
18	170	200	4	102	200	310	6.8

Date	Salinity (ppt)	Nitrate as N	Ammonia as N	Phosphorus	Arsenic	Barium	Boron
August 10	9.5			0.6	0.06	0.10	20
11	12			.6	.07	.09	25
12	28	<0.01	0.30	.6	.11	.10	43
13	18			.8	.17	.09	46
14	23	.04	.10	.9	.19	.09	49
15	7.0			.6	.06	.07	18
16	.0			<.1	.02	.08	.81
17	1.0	.05	.15	<.1	.02	.05	1.3
18	1.0			<.1	.03	.05	1.2

Date	Lithium	Molybdenum	Selenium (µg/L)	Strontium	Vanadium	Zinc
August 10	1.0	0.46	2.2	5.0	0.02	0.09
11	1.2	.59	2.3	6.0	.02	.06
12	2.0	1.1	2.1	9.6	.02	.01
13	2.2	1.2	3.5	10.2	.03	.06
14	2.3	1.3	3.6	10.4	.02	.01
15	.86	.47	2.1	4.1	.02	.08
16	.06	.02	<.3	.39	.01	.02
17	.08	.04	<.4	.51	.01	.02
18	.08	.06	.4	.89	.02	.02

Table 28. Water-quality measurements for daily composite water samples from Stillwater Point Diversion Drain, August 1988

[Concentrations in milligrams per liter except as indicated. All mercury concentrations were below analytical reporting limit. Abbreviations: µg/L, micrograms per liter; µS/cm, microsiemens per centimeter at 25°C; NTU, Nephelometric turbidity units; ppt, parts per thousand.]

Date	Specific conductance (µS/cm)	Field pH	Onsite mobile lab pH	Turbidity (NTU)	Dissolved oxygen	Calcium	Magnesium
August 10	700	8.4	8.4	39	9.0	48	14
11	550	8.5	8.3	36	8.5	47	14
12	620	8.3	8.4	35	8.4	45	13
13	710	8.4	8.4	32	8.3	51	18
14	470	8.2	8.4	54	8.9	43	12
15	520	8.3	8.3	25	8.7	42	13
16	510	8.4	8.4	37	8.9	43	12
17	580	8.6	8.4	23	9.6	44	13
18	720	8.5	8.5	21	8.4	47	14

Date	Hardness	Sodium	Potassium	Alkalinity	Sulfate	Chloride	Silica
August 10	140	140	8.8	234	130	60	13
11	200	150	8.8	227	140	63	14
12	150	130	8.6	114	120	63	12
13	150	270	13	312	110	58	12
14	150	120	8.6	211	110	50	12
15	150	140	7.9	210	120	55	11
16	140	120	7.7	210	140	55	12
17	160	120	7.3	221	150	43	12
18	160	160	8.6	245	140	48	13

Date	Salinity (ppt)	Nitrate as N	Ammonia as N	Phosphorus	Arsenic	Barium	Boron
August 10	0.5			0.2	0.05	0.07	0.99
11	.5			.2	.05	.07	1.2
12	.5	<0.01	0.21	.3	.04	.07	1.1
13	.8			.3	.04	.06	1.8
14	.5	.05	.50	.2	.04	.07	.86
15	.2			.2	.04	.07	.99
16	.2			.2	.04	.07	.88
17	.2	.04	.44	.2	.04	.07	.91
18	.4			.2	.05	.07	1.1

Date	Lithium	Molybdenum	Selenium (µg/L)	Strontium	Vanadium	Zinc
August 10	0.06	0.03	0.4	0.52	0.02	0.04
11	.07	.03	.5	.51	.02	.06
12	.06	.03	<.4	.48	.02	.05
13	.10	.05	.6	.67	.02	.10
14	.06	.03	.5	.46	.02	.04
15	.06	.03	<.4	.47	.02	.06
16	.06	.03	.5	.45	.02	.07
17	.06	.03	<.4	.46	.02	.04
18	.07	.04	.4	.52	.02	.04

Table 29. Water-quality measurements for daily instantaneous water samples from Stillwater Point Reservoir, August 1988

[Concentrations in milligrams per liter except as indicated. All mercury concentrations were below analytical reporting limit. Abbreviations: µg/L, micrograms per liter; µS/cm, microsiemens per centimeter at 25°C; NTU, Nephelometric turbidity units; ppt, parts per thousand.]

Date	Specific conductance (µS/cm)	Field pH	Onsite mobile lab pH	Turbidity (NTU)	Dissolved oxygen	Calcium	Magnesium
August 10	2,510	9.1	8.9	410	8.8	35	18
11	1,950	9.0	9.0	580	8.9	24	20
12	1,710	9.1	9.1	390	8.2	35	16
13	2,270	9.1	9.1	280	8.3	26	18
14	2,000	9.1	9.0	200	8.3	30	18
15	2,210	9.2	9.1	260	8.2	22	18
16	1,590	9.1	9.1	190	8.8	36	16
17	1,720	9.0	9.1	240	8.4	31	17
18	2,020	9.2	9.2	170	10.2	28	21

Date	Hardness	Sodium	Potassium	Alkalinity	Sulfate	Chloride	Silica
August 10	160	360	17	261	120	300	11
11	160	480	21	263	240	310	11
12	160	310	15	289	150	310	12
13	150	440	19	298	180	550	10
14	170	410	18	300	140	380	10
15	140	460	20	292	190	480	10
16	170	310	15	291	100	480	10
17	160	340	15	292	190	410	11
18	130	420	18	235	190	450	11

Date	Salinity (ppt)	Nitrate as N	Ammonia as N	Phosphorus	Arsenic	Barium	Boron
August 10	1.7			<0.1	0.09	0.10	2.2
11	1.2			<.1	.11	.11	3.0
12	1.3	<0.01	0.15	<.1	.08	.11	2.3
13	1.5			<.1	.11	.10	2.9
14	1.2	.01	.18	<.1	.10	.10	2.6
15	1.9			<.1	.11	.09	2.9
16	.9			<.1	.08	.09	2.0
17	1.1	<.01	.18	<.1	.09	.09	2.2
18	1.2			<.1	.10	.12	2.7

Date	Lithium	Molybdenum	Selenium (µg/L)	Strontium	Vanadium	Zinc
August 10	0.11	0.05	0.5	0.57	0.03	0.01
11	.12	.07	.6	.55	.03	.01
12	.10	.05	.6	.54	.03	.05
13	.13	.06	.4	.54	.03	.02
14	.12	.06	.4	.55	.03	.01
15	.13	.07	.5	.52	.03	.01
16	.10	.05	.6	.54	.02	.01
17	.11	.05	.5	.53	.02	.02
18	.13	.06	.4	.57	.03	.02

Total Dissolved Solids (TDS)

An aesthetic objective of *≤500 mg/L* has been established for total dissolved solids (TDS) in drinking water. At higher levels, excessive hardness, unpalatability, mineral deposition and corrosion may occur. At low levels, however, TDS contributes to the palatability of water.

Definition

Total dissolved solids (TDS) comprise inorganic salts and small amounts of organic matter that are dissolved in water. The principal constituents are usually the cations calcium, magnesium, sodium and potassium and the anions carbonate, bicarbonate, chloride, sulphate and, particularly in groundwater, nitrate (from agricultural use).

Occurrence

Total dissolved solids in water supplies originate from natural sources, sewage, urban and agricultural runoff and industrial wastewater. In Canada, salts used for road deicing can contribute significantly to the TDS loading of water supplies. Concentrations of TDS in water vary owing to different mineral solubilities in different geological regions. The concentration of TDS in water in contact with granite, siliceous sand, well-leached soil or other relatively insoluble materials is usually below 30 mg/L.[1] In areas of Precambrian rock, TDS concentrations in water are generally less than 65 mg/L.[2] Levels are higher in regions of Palaeozoic and Mesozoic sedimentary rock, ranging from 195 to 1100 mg/L[2] because of the presence of carbonates, chlorides, calcium, magnesium and sulphates.[1,3] Concentrations of TDS in some streams and small lakes in the arid western regions of Canada and the United States are often as high as 15 000 mg/L.[3,4]

Concentrations of TDS, expressed as the sum of its constituents, were below 500 mg/L in 36 of 41 rivers monitored in Canada.[5] In a survey of the Great Lakes, TDS levels ranged from 61 to 227 mg/L.[6] The levels of TDS in all of the Great Lakes except Lake Superior increased between 1900 and 1970. A threefold increase in chlorides and a twofold increase in sulphates, sodium and potassium in Lakes Erie and Ontario[7] increased the TDS concentration in those lakes by 50 to 60 mg/L.[6,8–10]

Concentrations of TDS in drinking water in Canada are generally below 500 mg/L but are considerably higher in some locations, particularly the arid western regions. Levels of TDS in Newfoundland and Labrador were below 500 mg/L in 96% of 103 communities sampled from 1969 to 1989 (range 10 to 2263 mg/L; average 146 mg/L).[11] In Quebec, samples of distributed water taken at 19 plants between 1987 and 1989 contained TDS at mean concentrations ranging from 58 to 213 mg/L.[12] Concentrations of TDS in distributed water from 31 plants in Ontario during 1987 and 1988 ranged from 91 to 470 mg/L.[13] In Manitoba, TDS concentrations measured during 1988 in the treated water of 168 communities ranged from 56 to 2510 mg/L; concentrations were less than 500 mg/L in 19% of these communities.[14] Levels of TDS in 1978 samples of community drinking water taken between 1970 and 1989 in Saskatchewan ranged from 6.5 to 5376 mg/L.[15] Concentrations of TDS in 54% of 1042 communities surveyed in Alberta in October 1989 were below 500 mg/L (range <100 to 1000 mg/L).[16] In British Columbia, concentrations of TDS in individual well water supplies ranged from 120 to 4662 mg/L; those in community (generally surface water) supplies were commonly less than 500 mg/L.[17]

Analytical Methods and Treatment Technology

The method most commonly used for the analysis of TDS in water supplies is the measurement of specific conductivity with a conductivity probe that detects the presence of ions in water. Conductivity measurements are converted to TDS values by a factor that varies with the type of water.[18,19] The practical quantitation limit for TDS in water by this method is 10 mg/L.[20] High TDS concentrations can also be measured gravimetrically, although this method excludes volatile organics.[21] The constituents of TDS can also be measured individually.

Total dissolved solids are not appreciably removed using conventional water treatment processes. In fact, the addition of chemicals during conventional water treatment generally increases the TDS concentration.[22] Certain treatment processes, such as lime–soda ash softening and sodium exchange zeolite softening, may slightly decrease or increase the TDS concentration, respectively.[23] Demineralization processes are required for significant TDS removal. Although the technology is available to reduce TDS levels significantly, the economic cost may be a major constraint.[23] Reverse osmosis and electrodialysis would probably be the most economical processes for removing TDS from public water supplies.[24]

Health Considerations

Recent data on health effects associated with the ingestion of TDS in drinking water have not been identified; however, associations between various health effects and hardness, rather than TDS content, have been investigated in many studies. These data are discussed in the section on hardness. As well, some of the individual components of TDS can have effects on human health. Effects that can be attributed to specific constituents are discussed in separate reviews for those constituents.

In early studies, inverse relationships were reported between TDS concentrations in drinking water and the incidence of cancer,[25] coronary heart disease,[26] arteriosclerotic heart disease[27] and cardiovascular disease.[28,29] Total mortality rates were reported to be inversely correlated with TDS levels in drinking water.[29,30]

Conversely, a summary of an Australian study reported that mortality due to all categories of ischaemic heart disease and acute myocardial infarction was increased in a community with higher levels of soluble solids, calcium, magnesium, sulphate, chloride and fluoride, alkalinity, total hardness and pH, when compared with a community in which levels were lower.[31] No attempts were made to relate mortality due to cardiovascular disease to other potential confounding factors. The results of a limited epidemiological study in the former Soviet Union indicated that the average number of “cases” of inflammation of the gall bladder and gallstones over a five-year period increased with the mean level of dry residue in the groundwater.[32] It should be noted, however, that the number of “cases” varied greatly from year to year in one district, as did the concentration of dry residue in each district, and no attempt was made to take into account possible confounding factors.

Other Considerations

The presence of dissolved solids in water may affect its taste.[33–42] The palatability of drinking water has been rated, by panels of tasters, according to TDS level as follows: excellent, less than 300 mg/L; good, between 300 and 600 mg/L; fair, between 600 and 900 mg/L; poor, between 900 and 1200 mg/L; and unacceptable, greater than 1200 mg/L.[37] Water with extremely low TDS concentrations may also be unacceptable because of its flat, insipid taste.

In addition to palatability, certain components of TDS such as chlorides, sulphates, magnesium, calcium and carbonates also affect corrosion or encrustation in water distribution systems.[21] High TDS levels (above 500 mg/L) result in excessive scaling in water pipes, water heaters, boilers and household appliances such as tea kettles and steam irons.[43] Such scaling can shorten the service life of these appliances.[44]

Rationale

1. The most important aspect of TDS with respect to drinking water quality is its effect on taste. The palatability of drinking water with a TDS level less than 600 mg/L is generally considered to be good. Drinking water supplies with TDS levels greater than 1200 mg/L are unpalatable to most consumers.

2. Concentrations of TDS above 500 mg/L result in excessive scaling in water pipes, water heaters, boilers and household appliances.

3. An aesthetic objective of ≤500 mg/L should ensure palatability and prevent excessive scaling. However, it should be noted that at low levels TDS contributes to the palatability of drinking water.

References

1. Rainwater, F.H. and Thatcher, L.L. Methods for collection and analysis of water samples. Geological Survey Water-Supply Paper, U.S. Department of the Interior. U.S. Government Printing Office, Washington, DC (1960).

2. Garrison Investigative Board. Water quality report (Appendix A). Garrison Diversion Study, report to the International Joint Commission (1977).

3. Durfor, C.J. and Becker, E. Constituents and properties of water. In: Water quality in a stressed environment: readings in environmental hydrology. W.A. Pettyjohn (ed.). Burgess Publishing Company, Minneapolis, MN (1972).

4. Rawson, D.S. and Moore, J.E. The saline lakes of Saskatchewan. Can. J. Res., 22: 141 (1944).

5. Fisheries and Environment Canada. Surface water quality in Canada. An overview. Water Quality Branch, Inland Waters Directorate (1977).

6. Upper Lakes Reference Group. The waters of Lake Huron and Lake Superior. Vols. I to III, Parts A and B. Report to the International Joint Commission (1977).

7. Great Lakes Water Quality Board. Great Lakes water quality 1974. Appendix A. Water Quality Objectives Subcommittee report to the International Joint Commission (1975).

8. Beeton, A.M. Indices of Great Lakes eutrophication. Publ. No. 15, Great Lakes Research Division, University of Michigan, Ann Arbor, MI (1966).

9. Kormondy, E.J. Concepts of ecology. Prentice-Hall, Englewood Cliffs, NJ. p. 182 (1969).

10. Vaughn, J.C. and Reed, P.A. Quality status of southern Lake Michigan. J. Am. Water Works Assoc., 62: 103 (1972).

11. Dominie, K. Personal communication. Newfoundland and Labrador Department of Environment and Lands (1989).

12. Durocher, H. Personal communication. Ministère de l'Environnement, Gouvernement du Québec (1990).

13. Vajdic, A. Personal communication. Ontario Ministry of the Environment (1989).

14. Rocan, D. Personal communication. Manitoba Department of Environment (1989).

15. Nargang, D. Personal communication. Saskatchewan Department of the Environment and Resource Management (1989).

16. Spink, D. Personal communication. Alberta Ministry of Environmental Protection (1989).

17. Willoughby, B.A. Personal communication. B.C. Ministry of Health (1989).

18. Ontario Ministry of the Environment. Outlines of analytical methods. A guide to the occurrence, significance, sampling and analysis of chemical and microbial parameters in water. February (1975).

19. Singh, T. and Kalra, Y.P. Specific conductance method for in situ estimation of total dissolved solids. J. Am. Water Works Assoc., 67(2): 99 (1975).

20. Forbes, M. Personal communication. Canada Centre for Inland Waters, Burlington, Ontario (1988).

21. Sawyer, C.N. and McCarty, P.L. Chemistry for sanitary engineers. 2nd edition. McGraw-Hill Series in Sanitary Science and Water Resources Engineering, McGraw-Hill, Toronto (1967).

22. Department of National Health and Welfare. Water treatment principles and applications: a manual for the production of drinking water. Canadian Water and Wastewater Association (1993).

23. Canadian Council of Resource and Environment Ministers. Total dissolved solids. In: Canadian water quality guidelines. Prepared by the Task Force on Water Quality Guidelines. Environment Canada, Ottawa, March (1987).

24. Clark, J.W., Viessman, W., Jr. and Hammer, M.J. Water supply and pollution control. 3rd edition. Harper & Row Publishers, New York, NY (1977).

25. Burton, A.C. and Cornhill, J.F. Correlation of cancer death rates with altitude and with the quality of water supply of the 100 largest cities in the United States. J. Toxicol. Environ. Health, 3(3): 465 (1977).

26. Schroeder, H.A. Relation between mortality from cardiovascular disease and treated water supplies. Variation in states and 163 largest municipalities. J. Am. Med. Assoc., 172: 1902 (1960).

27. Schroeder, H.A. Municipal drinking water and cardiovascular death rates. J. Am. Med. Assoc., 195: 125 (1966).

28. Sauer, H.I. Relationship between trace element content of drinking water and chronic disease. Univ. Ill. Bull., 71(108): 39 (1974).

29. Craun, G.F. and McCabe, L.J. Problems associated with metals in drinking water. J. Am. Water Works Assoc., 67: 593 (1975).

30. Crawford, M., Gardner, M.J. and Morris, J.N. Mortality and hardness of local water supplies. Lancet, i: 827 (1968).

31. Meyers, D. Mortality and water hardness. Lancet, i: 398 (1975).

32. Popov, V.V. Cholelithiasis and calculi, hardness of drinking water. Gig. Sanit., 33(6): 416 (1970).

33. Bruvold, W.H. and Pangborn, R.M. Rated acceptability of mineral taste in water. J. Appl. Psychol., 50(1): 22 (1966).

34. Bruvold, W.H., Ongerth, H.J. and Dillehay, R.C. Consumer attitudes toward mineral taste in domestic water. J. Am. Water Works Assoc., 59: 547 (1967).

35. Bruvold, W.H. Scales for rating the taste of water. J. Appl. Psychol., 52: 245 (1968).

36. Bruvold, W.H. Mineral taste and the potability of domestic water. Water Res., 4: 331 (1970).

37. Bruvold, W.H. and Ongerth, H.J. Taste quality of mineralized water. J. Am. Water Works Assoc., 61: 170 (1969).

38. Cox, G.J., Nathans, J.W. and Vonau, N. Subthreshold-to-taste thresholds of sodium, potassium, magnesium and calcium ions in water. J. Appl. Physiol., 8: 283 (1955).

39. Bryan, P.E., Kuzmunski, L.N., Sawyer, F.M. and Feng, T.H. Taste thresholds of halogens in water. J. Am. Water Works Assoc., 65: 363 (1973).

40. Pangborn, R.M. and Bertolero, L.L. Influence of temperature on taste intensity and degree of liking of drinking water. J. Am. Water Works Assoc., 64: 511 (1972).

41. Bruvold, W.H. and Pangborn, R.M. Rated acceptability of mineral taste in water. J. Appl. Psychol., 50: 22 (1966).

42. Pangborn, R.M., Trabue, I.M. and Little, A.C. Analysis of coffee, tea, artificially flavoured drinks prepared from mineralized waters. J. Food Sci., 36: 355 (1971).

43. Tihansky, D.P. Economic damages from residential use of mineralized water supply. Water Resour. Res., 10(2): 145 (1974).

44. McQuillan, R.G. and Spenst, P.G. The addition of chemicals to apartment water supplies. J. Am. Water Works Assoc., 68: 415 (1976).

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 16, No. 10, pp. 2009–2019, 1997
Printed in the USA
0730-7268/97 $6.00 + .00

Produced Water Series

STATISTICAL MODELS TO PREDICT THE TOXICITY OF MAJOR IONS TO *CERIODAPHNIA DUBIA, DAPHNIA MAGNA* AND *PIMEPHALES PROMELAS* (FATHEAD MINNOWS)

DAVID R. MOUNT,*‡ DAVID D. GULLEY,†§ J. RUSSELL HOCKETT,‖ TYLER D. GARRISON‖ and JAMES M. EVANS#
‡U.S. Environmental Protection Agency, 6201 Congdon Boulevard, Duluth, Minnesota 55804
§University of Wyoming, Laramie, Wyoming 82070, USA
‖ENSR Consulting and Engineering, Fort Collins, Colorado 80521, USA
#Gas Research Institute, Chicago, Illinois 60631, USA

(*Received* 29 *April* 1996; *Accepted* 20 *February* 1997)

Abstract—Toxicity of fresh waters with high total dissolved solids has been shown to be dependent on the specific ionic composition of the water. To provide a predictive tool to assess toxicity attributable to major ions, we tested the toxicity of over 2,900 ion solutions using the daphnids, *Ceriodaphnia dubia* and *Daphnia magna,* and fathead minnows (*Pimephales promelas*). Multiple logistic regression was used to relate ion composition to survival for each of the three test species. In general, relative ion toxicity was $K^+ > HCO_3^- \approx Mg^{2+} > Cl^- > SO_4^{2-}$; Na^+ and Ca^{2+} were not significant variables in the regressions, suggesting that the toxicity of Na^+ and Ca^{2+} salts was primarily attributable to the corresponding anion. For *C. dubia* and *D. magna,* toxicity of Cl^-, SO_4^{2-}, and K^+ was reduced in solutions enriched with more than one cation. Final regression models showed a good quality of fit to the data ($R^2 = 0.767$–0.861). Preliminary applications of these models to field-collected samples indicated a high degree of accuracy for the *C. dubia* model, while the *D. magna* and fathead minnow models tended to overpredict ion toxicity.

Keywords—Ions Total dissolved solids Salinity Toxicity *Ceriodaphnia dubia*

INTRODUCTION

Natural fresh waters contain several ionic constituents at greater than trace levels. Indeed, ions such as Na^+, Ca^{2+}, Cl^-, and others are required at a minimum level to support aquatic life, and these major ions are components of most formulas for ''reconstituted'' water used in aquatic toxicity testing [1,2]. However, many natural and anthropogenic sources can increase ion concentrations to levels toxic to aquatic life. Studies of oil and gas produced waters [3–5], irrigation drain waters [6,7], shale oil leachates [8], sediment pore waters [9,10], and industrial process waters [11,12] have shown toxicity caused by elevated concentrations of common ions.

Typically, integrative parameters such as conductivity, total dissolved solids (TDS), or salinity are used as a measure of the concentrations of common ions in fresh waters. While for a given ionic composition there is undoubtedly a correlation between increasing conductivity or TDS and increasing toxicity, these parameters are not robust predictors of toxicity for a range of water qualities. For example, Burnham and Peterka [13] noted that fathead minnows could tolerate TDS concentrations up to 15,000 mg/L in Saskatchewan lakes dominated by Na^+ and SO_4^{2-}, but populations did not persist above 2,000 mg/L in $Na^+/K^+/HCO_3^-$-dominated lakes of Nebraska. In studies of irrigation drain waters, Dickerson et al. [7] found *Ceriodaphnia dubia* 50% lethal concentration (LC50) values corresponding to approximate conductivities of 3,500 to 4,000 μS/cm (calculated), while Jop and Askew [11] showed major ion toxicity to *C. dubia* in an industrial process water with a conductivity of only 1,800 μS/cm (K.M. Jop, personal communication). Studies by Dwyer et al. [14] demonstrated that the toxicity of high TDS waters to *Daphnia magna* and striped bass *Morone saxatilis* was dependent on the specific ionic composition of those waters.

Given the substantial differences in toxicity among major ion salts [15], these differing responses in waters with different ionic compositions are to be expected. Still, they emphasize the inadequacy of generic measures for assessing the potential toxicity of major ions and the need for a broader understanding of major ion toxicity. This paper presents research to develop more comprehensive tools for assessing major ion toxicity. Acute toxicity tests using three freshwater organisms were conducted on solutions enriched with varying combinations of major ions. Results of these tests were incorporated into multivariate logistic regression models that predict survival of the three test species based on major ion concentrations.

MATERIALS AND METHODS

Test organisms

All organisms used in testing were obtained from in-house cultures (ENSR, Fort Collins, CO, USA); daphnids were less than 24 h old at test initiation, while fathead minnows were 1 to 7 d old. *Ceriodaphnia dubia* were cultured in either moderately hard reconstituted water (MHRW) or 20% mineral water [1] at 25°C, while *D. magna* were cultured in hard reconstituted water [1] at 20°C. Fathead minnow brood stock were cultured at 20 to 25°C in tap water that was pretreated with activated carbon. Eggs and larva were held in MHRW; larva were fed brine shrimp nauplii (*Artemia* sp.) twice daily until they were used in testing.

* To whom correspondence may be addressed.
† Deceased.
Presented in part at the 12th Annual Meeting, Society of Environmental Toxicology and Chemistry, Seattle, WA, USA, November 3–7, 1991.

Test procedures

Toxicity tests followed the general guidance of the U.S. Environmental Protection Agency (USEPA) [1,16] for conducting acute whole effluent toxicity tests. All tests were conducted in 30-ml plastic beakers containing 10 ml of test solution and five organisms per chamber. Tests were conducted under a 16-h:8-h light : dark photoperiod; *C. dubia* and fathead minnows were tested at 25°C, while *D. magna* were tested at 20°C. Dilution/control water for all tests was MHRW. Exposure periods were 48 h for *C. dubia* and *D. magna* and 96 h for fathead minnows, with daily observations of mortality. The criteria for death were no visible movement and no response to prodding.

Standard guidance for acute effluent toxicity testing [1] is to withhold food during testing of daphnids, presumably because of concerns that the addition of food might alter the toxicity of the sample. However, in water devoid of food (e.g., reconstituted laboratory water), withholding food likely places some stress on the test organisms. Moreover, effluents and ambient waters, to which the results of these experiments apply, can be expected to contain bacteria, algae, and other sources of food. Hence, addition of daphnid food (yeast/cerophyl/trout chow [YCT] and algae [2]) to clean laboratory water might better simulate the characteristics of field-collected samples. To assess the potential effect of feeding on major ion toxicity, initial tests using *C. dubia* were conducted both with and without feeding. Analysis of these initial experiments (see Results) showed that the addition of food represented only a small influence on *C. dubia* survival. Because the effect of feeding was small and its inclusion was believed to provide a more representative test matrix, remaining *C. dubia* tests included feeding, as did all *D. magna* and fathead minnow tests. For daphnid tests, 100 μl of a 1:1 mix of YCT and algal suspension was added to each test chamber at test initiation. For fathead minnow tests, 100 μl of concentrated brine shrimp nauplii was added after 48 h of exposure, though solutions were not subsequently renewed as recommended by the USEPA [1].

Because toxicity testing of salt solutions was to be completed over several months, we recognized the possibility that systematic drift in test organism sensitivity could bias the results of toxicity tests conducted at different times. In an attempt to account for this potential variability, each set of toxicity tests included a reference toxicant test using NaCl. LC50 values were computed for each of these tests and were included in the statistical modeling as another independent variable. Thus, if drifts in organism sensitivity did occur and were reflected in the response to NaCl, they could be accounted for in the regression modeling.

Chemical measurements

Concentrations of major ions were determined analytically in all stock solutions used in testing. Ca^{2+}, Na^{+}, Mg^{2+}, and K^{+} were determined using inductively coupled plasma emission spectroscopy (ICP) according to USEPA method 200.7 [17]; Cl^{-} and SO_4^{2-} concentrations were determined by anion chromatography [18]; and HCO_3^{-} concentrations were determined indirectly by the measurement of phenolphthalein alkalinity [19]. As HCO_3^{-} is the predominate carbonate species present in the pH range of interest (pH 6.5–9.0), alkalinity equivalents were converted directly to HCO_3^{-} concentration.

Dissolved oxygen (DO) and pH were measured in selected test solutions during actual toxicity testing, primarily on solutions near the threshold for acute toxicity. DO was measured with a Yellow Springs Instrument model 54 DO meter (Yellow Springs, OH, USA) while pH was measured with a Orion pH meter model SA250 (Boston, MA, USA). Measured DO concentrations were always within an acceptable range (>40% saturation) [1]. Measured pH varied according to the components of the solution but was generally between pH 7.5 and 9.0.

Preparation of test solutions

Test solutions were prepared by dissolving individual ion salts in MHRW. Salts used in testing were NaCl, Na_2SO_4, $NaHCO_3$, KCl, K_2SO_4, $KHCO_3$, $CaCl_2$, $CaSO_4$, $MgCl_2$, $MgSO_4$, $CaCO_3$, and $MgCO_3$; all were of reagent grade or better (Sigma Chemical Company, St. Louis, MO, USA). Stock solutions were prepared from these salts by dissolving 10,000 mg/L of a salt in MHRW. $CaSO_4$ was not fully soluble at 10,000 mg/L; for this reason, $CaSO_4$ solutions were filtered through a 1-μm glass fiber filter prior to testing and ion concentrations were measured in filtered solutions. Test solutions using $CaCO_3$ and $MgCO_3$ had pH in excess of 10 and were acidified with HCl or H_2SO_4 until pH stabilized at approximately 8.5.

For tests evaluating only one salt (one cation and one anion), test solutions were prepared by serially diluting the 10,000-mg/L stock solutions with MHRW to develop a series of test concentrations spaced on a 0.5 × dilution factor (i.e., 10,000, 5,000, 2,500, 1,250 mg/L). For tests involving two salts, solutions were prepared by combining equal volumes of the two stock solutions, then diluting as necessary. As testing proceeded and effect thresholds were determined, test concentrations were often spaced much more closely (e.g., 2,500, 2,000, 1,500, 1,000, 500 mg/L) to better define responses near the effect threshold.

All ion concentrations measured in the stock solutions were compared to nominal values. If the measured concentrations differed from the nominal value by more than 20%, the actual measured concentrations were substituted for the nominal concentrations. Aside from $CaSO_4$, which did not completely dissolve, substantial discrepancies between nominal and measured concentrations occurred in two instances, once for a $MgCl_2$ stock solution and once for a $CaCl_2$ stock solution. In some analyses, the measured concentrations of cations and anions (expressed as milliequivalents or meq) in a salt solution were not similar. Because charge balance is a physical/chemical requirement, such solutions were further evaluated to determine which concentration (cation or anion) was closer to the nominal value. In all cases, the cation concentration was closer to the nominal value; based on this, the anion concentration in the stock solution was changed to the concentration (in meq) of the corresponding cation.

To calculate ion concentrations in actual test solutions, the concentrations in the applicable stock solutions were multiplied by the relative proportion of each solution in the test solution. Because the dilution water (MHRW) also contained small concentrations of each ion, these background concentrations were then added to the calculated contributions from the stock solutions.

In cases where an SO_4^{2-} salt (e.g., Na_2SO_4) was combined with a Ca^{2+} salt (e.g., $CaCl_2$), the potential existed for supersaturation of test solutions with respect to $CaSO_4$. This potential was confirmed by the appearance of white precipitates in some test solutions. Because precipitation would affect the dissolved ion concentrations in the test solutions, all ion combinations tested were checked for $CaSO_4$ supersaturation by comparing the nominal test concentrations of Ca^{2+} and SO_4^{2-} with the solubility product for $CaSO_4$ (226.5) calculated from measured concentrations of Ca^{2+} and SO_4^{2-} in a saturated $CaSO_4$ solution. If a particular solution was supersaturated with respect to $CaSO_4$, Ca^{2+} and SO_4^{2-} concentrations were reduced on an equimolar basis until the concentrations reached the calculated saturation point. These corrected concentrations were then used for data analyses.

Replication

To incorporate intertest variability into the data set, emphasis was placed on replication between batches of tests conducted through time rather than on having replicate chambers tested simultaneously. Accordingly, most ion combinations evaluated were tested on at least two and as many as five different occasions (see results). The exception was for two cation/one anion solutions tested with *D. magna* and fathead minnows, and two cation/two anion solutions tested with *C. dubia*; for these tests, duplicate chambers (10 animals total) were tested simultaneously. When calculating LC50 values, replicate tests conducted on different days were analyzed separately, but duplicate chambers tested simultaneously were combined into one analysis.

Data collection, management, and analysis

Data generated by all toxicity tests were entered into a database using Paradox™ 3.1 software (Borland International, Scotts Valley, CA, USA). Regression modeling was based on individual ion concentrations rather than salt concentrations. By converting salts to ion concentrations, we were able to separate out the effects of individual cations and anions instead of the effects of cation–anion pairs. Statistical modeling of the toxicity data consisted of stepwise logistic multiple regression using the LR program within BMDP statistical software [20].

Logistic regression relates binary observations (e.g., alive or dead) to one or more independent variables (in this case, ion concentrations). The completed regression predicts a probability of survival based on concentrations of ions showing relationships to survival. The linear logistic regression model used is of the form

$$\begin{aligned}\mathrm{logit}(P) &= \ln[P/(1 - \mathrm{P})] \\ &= \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_n X_n \end{aligned} \qquad (1)$$

where P = proportion surviving, β = regression coefficient, X = ion concentration, and n = total number of significant terms in the model.

During development of the final models, various data transformations (e.g., log) and independent variable interactions (e.g., $Cl \times SO_4$ interaction) were considered. Each potential model was evaluated using the following criteria: (1) each independent variable in the model must significantly improve the fit of the model to the data ($\alpha = 0.05$); (2) the model should maximize R^2 (maximize the amount of variance in the data that is explained by the model) and minimize the number of independent variables; and (3) the model should provide reasonable predictions even when extrapolating outside the limits of the data used to generate the model.

Data collection and model development were iterative processes in which a series of statistical models (regressions) were developed followed by supplemental data collection. To begin, data were generated for single ion pairs or salts (e.g., Na_2SO_4, $CaCl_2$). Based on these data, an initial regression equation was developed (F_1). Next, additional toxicity data were generated using combinations of two cations and one anion (e.g., Na^+, Ca^{2+}, and SO_4^{2-}) and one cation and two anions (e.g., Na^+, Cl^-, and SO_4^{2-}). The F_1 equation was then used to predict survival for these additional data. In addition, a second regression equation (F_2) was then developed using all data generated to date. The predictive abilities of both models were then compared by examining the relationship between predicted and observed survival for all of the ion combinations tested. If F_2 had notably better predictive ability than F_1, we concluded that important relationships in the data were not accounted for in the F_1 equation. The process was repeated by testing more complex ion solutions and developing additional regression equations, until the incorporation of additional data did not substantially alter the basic equation. This iterative process of data generation, model development, and additional data generation continued throughout model development.

As part of this iterative process, characteristics of specific points that had poor correlation between predicted and observed survival were considered. In some cases, it was found that such data points had poor agreement between replicate tests of the same ion combination, hence it was impossible for the regression equation to fit both responses. In these instances, additional toxicity tests were conducted using that particular combination of ions to better characterize the response. Of 2,904 total data points, 59 were discarded as spurious; of these, 46 were for *C. dubia,* 5 for *D. magna,* and 8 for fathead minnows. Thirty-eight of the 59 discarded points were cases where mortality (typically one or two dead out of five organisms) was observed two or more concentrations below the primary concentration response, suggesting that ion toxicity may not have been the cause of mortality. Though these points may represent innate variability in the survival of test organisms, our intent was to represent mortality due to ion stress; random mortalities at low ion concentrations tended to decrease the slope of the regression model and obscure the response threshold. Of the remaining discarded points, 10 were discarded because the $CaSO_4$ solution was not filtered prior to testing (*C. dubia*); 10 were from a K_2SO_4 dilution series in which there was erratic and substantial mortality without evidence of a concentration response (*C. dubia*); and one was from a test chamber that was spilled after the 24-h observation (*P. promelas*).

In other cases, it was found that outlier points tended to share certain characteristics. For example, it was noted that for *C. dubia,* early regressions showed poor predictive ability for ion combinations containing Cl^- opposed by two cations (e.g., Na^+ and Ca^{2+} with Cl^-); these solutions showed lower toxicity than those with just one Cl^- salt (e.g., NaCl). Further testing with these ion combinations showed that this response was reproducible. To account for this phenomenon, a new variable called NumCat was created. The value of NumCat is equal to the number of cations representing at least 10% of the total molar concentration of cations and present at greater than 100 mg/L. The development and implications of the NumCat variable are discussed in detail in the Results.

In addition to the more rigorous statistical modeling described above, LC50 concentrations were also calculated using a computer program following the trimmed Spearman–Karber method [21]. Independent LC50 values were calculated for each unique (i.e., nonsimultaneous) test of ion toxicity. For

Table 1. Number of ion solutions tested for toxicity[a]

Species	Number of cations/anions[b] 1/1	1/2	2/1	2/2	3/1	4/1	Subtotal	Reference toxicant and controls	Total
Ceriodaphnia dubia	464	449	438	401	108	20	1,887	232	2,119
Daphnia magna	354	147	65	0	0	0	566	122	688
Fathead minnows	242	142	59	0	0	0	451	56	499

[a] Replicate analyses counted separately.
[b] Number of ions enriched above background concentrations.

ion combinations that were tested repeatedly, average LC50s were calculated as the arithmetic mean of the values. In some cases, tests did not capture the effect threshold and an LC50 could only be expressed as a range (e.g., LC50 $<$ 625 mg/L). Where this range did not conflict with the other calculated values, the indefinite value was dropped and the mean was calculated from the remaining values (e.g., 500, 700, and $<$625 would average to 600 with $n = 2$). If the indefinite value represented an extreme value, the mean was calculated as an inequality relative to the mean of the numerical values (e.g., 775, 700, and $<$625 would average to $<$700 with $n = 3$).

RESULTS AND DISCUSSION

In total, survival data were collected for 2,904 ion solutions, excluding reference toxicant tests and controls (Table 1). Data collection and modeling were conducted first for *C. dubia*, and the resulting data set encompasses both greater replication and a greater variety of ion combinations. The full data sets are too extensive to provide here but are provided in print in Mount and Gulley [22].

To present the data in a more condensed form, LC50 values were calculated for all ion solutions tested (Tables 2 and 3). Coefficients of variation for LC50 values for individual ion combinations were typical for acute toxicity tests [1], with means of 17% for *C. dubia* (SD = 14; range 0.0–61), 17% for *D. magna* (SD = 7.5; range 4.8–31), and 24% for fathead minnows (SD = 15; range 1.4–62).

The effect of feeding on the response of *C. dubia* was assessed during the first three sets of tests conducted. In each of these, toxicity of each single salt solution was tested both with and without the addition of food. Average LC50 values for tests with and without feeding were similar (Fig. 1), although there was a tendency for tests without feeding to have slightly lower LC50 values. Logistic regression modeling of these data confirmed this trend; feeding was judged a significant variable by the regression algorithm, with a positive coefficient indicating that feeding did increase overall survival. However, the influence of feeding in the model was quite small, explaining less than 1% of the overall variance. Because we believed that the addition of food might provide a more natural test matrix, all remaining tests were conducted with feeding.

To determine whether the results of reference toxicant tests related to the responses observed in the concurrent exposures to ion combinations, LC50 values were calculated for the reference toxicant tests from the first 11 test groups with *C. dubia* (total of 1,045 ion solutions tested). During this period, 48-h LC50 values for NaCl averaged 1,042 mg/L as Cl^- with a coefficient of variation equal to 24%. The LC50 value from the concurrent reference toxicant test was included as an independent variable for each ion solution and thus considered by the stepwise logistic regression. In this analysis, the reference toxicant variable was not selected as being statistically significant, explaining only 0.12% of the overall variance. From this, we surmised that there was no consistent relationship between the sensitivity of the test organisms (as measured by the reference toxicant test) and the responses of organisms in the concurrent ion exposures. For this reason, the reference toxicant test results were not considered further in subsequent regressions.

As described previously, the development of the final predictive models was an iterative process in which a series of regression models was developed. Initial regressions were developed based on more limited data sets (e.g., results from toxicity tests using single salts only); as data collection proceeded to more complicated solutions (enrichment with three and four ions), these equations were refined. Throughout the project, 74 distinct models were developed and considered. The majority of these models were discarded, either because they were superseded by later models that incorporated larger data sets, or were found to have undesirable characteristics (e.g., poor predictive ability). Several of these analyses involved experimentation with alternative variables or data transformations. To illustrate the model development process, we selected three intermediate models that demonstrate major advances in the model development, including the creation of a new variable, referred to as NumCat. The three example models are referred to as the single salt, double salt, and double salt with NumCat models and are based on 48-h survival data for *C. dubia*.

The single salt model was developed relatively early in the data collection process using 362 data points involving single salt solutions only (i.e., enriched with one cation and one anion; Fig. 2). This regression equation fit the observed survival values very well, with an R^2 value of 0.950. Significant variables in this equation were the concentrations of K^+, Mg^{2+}, HCO_3^-, Cl^-, and SO_4^{2-}; Na^+ and Ca^{2+} were not significant variables indicating that the toxicity of Na^+ and Ca^{2+} salts could be accounted for primarily by the toxicity of the co-occurring anion. No first-order interaction terms (e.g., K $\times$ Cl) were selected as significant.

Data collection was then expanded to include solutions with one cation and two anions and two cations and one anion. When the single salt model was used to predict survival for this expanded data set (1,045 data points) it showed considerably less predictive ability than it had for the smaller initial data set. Accordingly, a new model was developed using data from all test solutions. This double salt model had the same significant variables as did the single salt model but did a better job of predicting survival for the entire data set than did the single salt model. Although it did have better predictive ability for the combined data set, the R^2 value of 0.837 indi-

Table 2. Mean 24-h (upper right) and 48-h (lower left) LC50 values for salt combinations tested with *Ceriodaphnia*[a]

	NaCl	Na_2SO_4	$NaHCO_3$	KCl	K_2SO_4	$KHCO_3$	$CaCl_2$	$CaSO_4$	$MgCl_2$	$MgSO_4$	24-h
	3,380 [3] (3,080–3,540)	3,320 [4] (3,110–3,540)	2,200 [4] (1,770–2,680)	1,650 [2] (1,540–1,770)	>1,800 [1]	1,360 [1]	3,340 [3] (2,960–3,540)	>2,430 [1]	3,230 [4] (3,080–3,460)	3,400 [1]	NaCl
		3,590 [4] (3,540–3,740)	2,800 [5] (2,220–3,540)	1,730 [1]	1,390 [2] (1,020–1,770)	1,300 [1]	4,120 [2] (3,800–4,150)	>4,940 [2] (4,170–>5,700)	3,100 [2] (2,750–3,460)	3,480 [3] (3,080–3,820)	Na_2SO_4
			1,420 [4] (1,240–1,770)	1,200 [1]	1,110 [1]	920 [3] (880–1,000)	2,680 [2] (2,320–3,080)	>1,040 [1]	1,800 [1]	2,210 [1]	$NaHCO_3$
NaCl	1,960 [3] (1,770–2,330)			630 [3] (580–630)	620 [3] (250–880)	550 [3] (290–770)	1,740 [3] (1,690–1,770)	1,580 [1]	1,400 [2] (1,030–1,770)	1,070 [2] (880–1,260)	KCl
Na_2SO_4	3,070 [4] (2,530–3,540)	3,080 [4] (1,770–3,540)			770 [3] (770–780)	390 [3] (290–440)	2,250 [1]	1,140 [3] 480–1,870	>1,550 [1]	1,510 [4] (1,340–1,770)	K_2SO4
$NaHCO_3$	1,890 [3] (1,770–2,030)	2,630 [4] (1,880–3,540)	1,020 [4] (880–1,170)			630 [2] (580–670)	1,910 [1]	1,560 [1]	860 [1]	940 [1]	$KHCO_3$
KCl	1,560 [3] (1,540–1,600)	1,730 [1]	1,140 [1]	630 [3] (580–670)			2,260 [3] (1,770–2,680)	3,880 [2] (3,660–4,100)	3,500 [3] (3,420–3,540)	>3,690 [2] (3,670–>3,700)	$CaCl_2$
K_2SO_4	1,660 [1]	1,590 [3] (1,020–2,000)	<1,000 [1]	480 [3] (250–670)	<680 [3] (<620–710)			>1,940 [4] (>1,940–>1,990)	>2,760 [1]	>5,610 [3] (>2,610–>5,610)	$CaSO_4$
$KHCO_3$	1,360 [1]	1,300 [1]	800 [3] (580–950)	480 [3] (290–580)	390 [3] (290–440)	630 [2] (580–670)			1,270 [3] (880–1,770)	1,560 [3] (1,360–1,770)	$MgCl_2$
$CaCl_2$	3,030 [4] (2,240–3,540)	>3,940 [2] (3,800–>4,080)	<2,640 [2] (<2,250–3,030)	1,730 [3] (1,640–1,770)	1,820 [1]	1,810 [1]	1,830 [4] (1,770–2,030)			1,770 [3] (1,770–1,770)	$MgSO_4$
$CaSO_4$	>2,430 [1]	>4,940 [2] (4,170–>5,700)	>1,040 [1]	1,580 [1]	1,130 [3] (480–1,830)	1,560 [1]	3,050 [2] (2,450–3,660)	>1,910 [4] (1,910–>1,970)			
$MgCl_2$	2,380 [4] (1,770–2,730)	<2,520 [2] (<2,320–2,720)	1,510 [1]	1,270 [3] (1,000–1,770)	1,040 [1]	860 [1]	2,600 [3] (2,430–2,680)	<2,370 [1]	880 [3] (880–880)		
$MgSO_4$	3,250 [1]	3,190 [3] (2,680–3,540)	1,670 [1]	1,060 [2] (880–1,220)	1,480 [4] (1,340–1,770)	940 [1]	>3,690 [2] (3,670–>3,700)	>5,610 [2] (>5,610–>5,610)	1,490 [3] (1,360–1,560)	1,770 [3] (1,770–1,770)	
48-h	NaCl	Na_2SO_4	$NaHCO_3$	KCl	K_2SO_4	$KHCO_3$	$CaCl_2$	$CaSO_4$	$MgCl_2$	$MgSO_4$	

[a] Values are arithmetic means [*n*] (range) expressed as total ion concentrations added in mg/L. Tests with two salts involved 1:1 combinations of stock solutions containing 10,000 mg/L, except $CaSO_4$ (1,970 mg/L).

Table 3. Mean LC50 values for salt combinations tested with *Daphnia magna* and fathead minnows[a]

	Daphnia magna		Fathead minnow		
Salt	24-h	48-h	24-h	48-h	96-h
NaCl	6,380 [2] (6,160–6,600)	4,770 [2] (3,790–5,740)	8,280 [3] (7,240–10,000)	6,510 [3] (6,090–7,070)	6,390 [3] (6,020–7,070)
Na_2SO_4	6,290 [4] (5,790–7,070)	4,580 [4] (4,060–5,360)	>8,080 [3] (7,070–>10,000)	>7,960 [3] (6,800–>10,000)	7,960 [3] (6,800–10,000)
$NaHCO_3$	2,380 [4] (1,900–2,870)	1,640 [4] (1,170–2,030)	4,850 [2] (3,540–6,160)	2,500 [2] (950–4,060)	<850 [3] (<310–1,220)
KCl	740 [5] (580–880)	660 [5] (440–880)	950 [3] (750–1,090)	910 [3] (750–1,090)	880 [3] (750–1,020)
K_2SO_4	850 [4] (670–1,170)	720 [4] (580–880)	990 [4] (770–1,170)	860 [4] (580–1,170)	680 [4] (510–880)
$KHCO_3$	670 [4] (440–880)	650 [4] (380–820)	940 [4] (750–1,340)	820 [4] (750–880)	<510 [4] (<310–750)
$CaCl_2$	3,250 [4] (2,680–4,010)	2,770 [4] (2,330–3,230)	>6,660 [3] (4,700–>10,000)	>6,560 [3] (4,390–>10,000)	4,630 [3] (3,930–5,360)
$CaSO_4$	>1,970 [3] (>1,970–>1,970)	>1,970 [3] (>1,970–>1,970)	>1,970 [2] (>1,970–>1,970)	>1,970 [2] (>1,970–>1,970)	>1,970 [2] (>1,970–>1,970)
$MgCl_2$	1,560 [4] (1,250–1,810)	1,330 [4] (1,170–1,580)	3,520 [3] (2,520–4,490)	2,840 [3] (1,970–3,880)	2,120 [3] (1,580–2,740)
$MgSO_4$	2,360 [4] (2,180–2,500)	1,820 [4] (1,540–2,330)	4,630 [3] (3,180–7,070)	3,510 [3] (3,000–4,350)	2,820 [3] (2,610–3,080)
NaCl/Na_2SO_4	6,140 [2] (5,360–6,930)	5,700 [2] (5,360–6,030)	>9,040 [2] (8,080–>10,000)	>8,460 [2] (6,930–>10,000)	6,090 [2] (6,030–6,160)
NaCl/$NaHCO_3$	4,440 [2] (3,520–5,360)	2,950 [2] (2,830–3,080)	4,580 [2] (3,540–5,630)	3,790 [2] (2,330–5,250)	2,540 [2] (2,330–2,750)
Na_2SO_4/$NaHCO_3$	4,480 [2] (4,060–4,900)	3,180 [2] (2,830–3,540)	5,350 [2] (4,660–6,030)	5,050 [2] (4,060–6,030)	4,060 [2] (3,080–5,040)
KCl/K_2SO_4	740 [2] (600-880)	740 [2] (600–880)	900 [2] (790–1,020)	760 [2] (630–880)	760 [2] (630–880)
KCl/$KHCO5_3$	740 [2] (640–830)	740 [2] (640–830)	800 [2] (770–830)	770 [2] (700–830)	770 [2] (700–830)
K_2SO_4/$KHCO_3$	630 [2] (540–720)	630 [2] (540–720)	1,060 [2] (1,030–1,090)	720 [2] (610–830)	720 [2] (610–830)
$CaCl_2$/$CaSO_4$	3,250 [2] (3,140–3,360)	2,950 [2] (2,760–3,150)	>5,510 [1]	>5,510 [1]	>5,510 [1]
$MgCl_2$/$MgSO_4$	2,110 [2] (1,940–2,280)	1,510 [2] (1,340–1,680)	3,830 [2] (3,790–3,870)	3,330 [2] (3,300–3,370)	2,800 [2] (2,240–3,370)
NaCl/KCl	3,930 [1]	3,930 [1]	1,410 [1]	1,410 [1]	1,410 [1]
NaCl/$CaCl_2$	5,250 [1]	5,250 [1]	8,410 [1]	8,080 [1]	6,460 [1]
NaCl/$MgCl_2$	3,820 [1]	3,070 [1]	5,250 [1]	3,520 [1]	3,160 [1]
KCl/$CaCl_2$	2,620 [1]	2,450 [1]	2,810 [1]	2,810 [1]	2,810 [1]
KCl/$MgCl_2$	2,280 [1]	2,020 [1]	1,580 [1]	1,410 [1]	1,410 [1]
$CaCl_2$/$MgCl_2$	4,850 [1]	4,390 [1]	5,630 [1]	5,250 [1]	5,250 [1]
Na_2SO_4/K_2SO_4	4,800 [1]	4,610 [1]	1,580 [1]	1,580 [1]	1,580 [1]
Na_2SO_4/$MgSO_4$	8,400 [1]	7,980 [1]	8,840 [1]	5,740 [1]	4,800 [1]
K_2SO_4/$CaSO_4$	1,160 [1]	1,200 [1]	1,980 [1]	1,720 [1]	1,720 [1]
K_2SO_4/$MgSO_4$	2,760 [1]	2,210 [1]	1,380 [1]	1,290 [1]	1,290 [1]
$CaSO_4$/$MgSO_4$	>6,470 [1]	>6,470 [1]	NT[b]	NT	NT
$NaHCO_3$/$KHCO_3$	1,220 [1]	1,040 [1]	1,140 [1]	820 [1]	740 [1]

[a] Values are arithmetic means [*n*] (range) expressed as total ion concentrations added in mg/L. Tests with two salts involved 1:1 combinations of stock solutions containing 10,000 mg/L, except for $CaSO_4$ (1,970 mg/L), $MgCl_2$ (5,480 mg/L), and $CaCl_2$ (7,480 mg/L).
[b] Not tested.

cated a lower quality of fit than was observed for the single salt model fit to the initial, less complex data set.

There were two basic explanations for the decreased quality of fit observed with the double salt model: (1) the larger data set contained greater inherent variability (measurement error) and hence it was not possible to achieve as high an R^2 value; or (2) there were important toxic interactions represented in the three ion solutions that were not represented in the solutions containing only a single salt (although the regression algorithm had not selected any interaction terms as being significant). When the ion combinations for which the model made poor predictions were analyzed, some patterns were apparent. In particular, it appeared that the model was overpredicting toxicity for solutions containing two Cl^- salts.

This phenomenon is perhaps best illustrated by data collected for solutions of NaCl and $CaCl_2$ tested both alone and in combination. As explained above, the single salt model indicated that the toxicity of Na^+ and Ca^{2+} salts could be

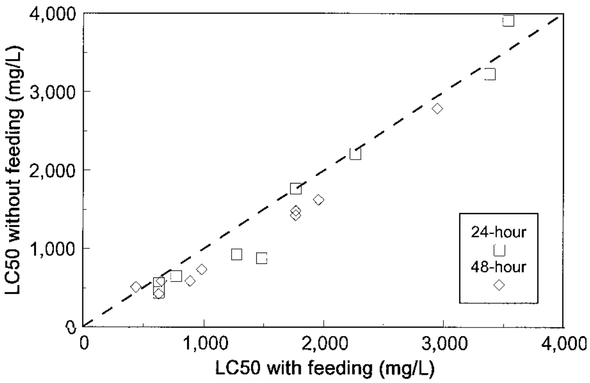


Fig. 1. Average LC50 values for *Ceriodaphnia dubia* exposed to single salts with and without feeding.

adequately explained on the basis of the anion concentration alone; in other words, NaCl and $CaCl_2$ had approximately the same toxicity when expressed on the basis of Cl^-. A plot of these data (Fig. 2) supports this conclusion and also shows a good fit of the single salt model to these data. However, when NaCl and $CaCl_2$ were tested in combination, the resulting solution was less toxic (on the basis of Cl^- concentration) than either of the solutions tested singly. The single salt model was unable to account for this decreased toxicity and, consequently, made poor predictions for the combined NaCl/$CaCl_2$ solutions (Fig. 2). The same trend toward lower toxicity of Cl^- in the presence of two cations was also evident for solutions containing K^+ or Mg^{2+}.

The double salt model compensated for the lower toxicity of two cation solutions but only partially. The double salt model simply fit a shallow response curve between the single cation and two cation data, predicting a ''mean'' probability of survival somewhere between the observed single salt and two salt survival values. While this compromise provided a better overall fit to the data than did the single salt model, it was clearly not a good representation of the response. Given that the regression algorithm did not find any interaction terms to be significant, it appeared that a new variable was required to provide a better fit to the data.

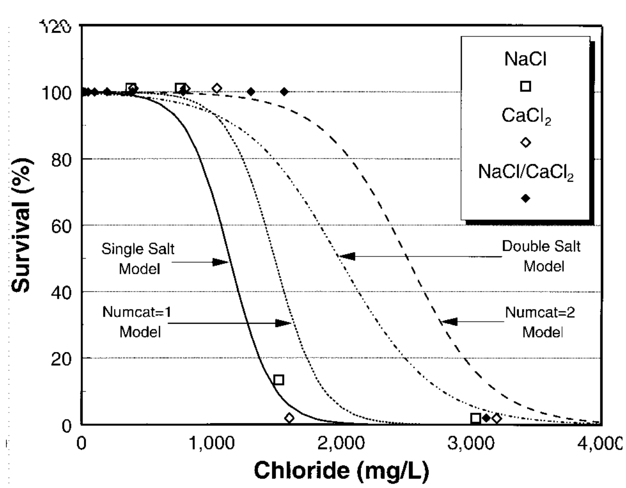


Fig. 2. The 48-h survival of *Ceriodaphnia dubia* exposed to solutions enriched with NaCl, $CaCl_2$, or a 1:1 combination of NaCl and $CaCl_2$, normalized to Cl^- concentration. Curves represent regression model predictions for the single salt, double salt, and double salt with NumCat models. Values at 0% and 100% offset slightly for clarity.

We attempted without success to derive a continuous variable that would respond appropriately to the relative concentration of cations in solution and thus identify the two cation solutions as different than solutions with a single cation. After our lack of success with continuous variables, we created a categorical variable called NumCat. The NumCat variable was intended to simply represent the number of major cations in the solution. For the initial modeling trials, the NumCat variable was arbitrarily defined as the number of cations in the solution that represented at least 10% of the total molar cation concentration and that were also present at a concentration greater than 100 mg/L. Our expectation was that the NumCat variable would show a significant interaction with Cl^- and any other ion whose toxicity was influenced by the number of cations present. The resulting model, called the ''double salt with NumCat'' model, showed a markedly improved fit (R^2 = 0.899); significant terms were the original five ions in the single and double salt models, plus NumCat and the NumCat × Cl, NumCat × SO_4, and NumCat × K interaction terms. The NumCat × Cl term allowed the model to better represent the toxicity of NaCl, $CaCl_2$, and NaCl + $CaCl_2$ solutions shown in Figure 2. NumCat also showed significant (positive) interactions with SO_4^{2-} and K^+, suggesting that the presence of two cations (or one additional cation in the case of K^+) ameliorated the toxicity of these ions as well.

After subsequent data collection and analysis, two additional steps were taken to optimize the NumCat variable. First, we conducted supplemental testing of *C. dubia* exposed to mixtures of three and four Cl^- salts (data not shown). Modeling of these data (NumCat = 3 or 4) yielded a substantial underprediction of toxicity. Direct inspection of these data confirmed that the protective effect observed with two cations did not seem to increase with the addition of three or four cations. Accordingly, we chose to limit the NumCat variable to values of 0, 1, or 2; for solutions where the >10% and >100-mg/L criterion yielded values of 3 or 4, these values were reset to 2.

The second step involved rigorously evaluating the definition criteria for the NumCat variable. Although the NumCat variable was clearly effective at increasing the predictive capability of the model, its original definition had been arbitrary. To provide a stronger technical basis for defining NumCat, we conducted a sensitivity analysis by varying the two components of the NumCat definition, the relative molar concentration (originally >10%), and the absolute concentration (originally 100 mg/L). A complete matrix of relative concentration (0, 5, 10, 15, 20, and 25%) and absolute concentration (0, 100, 200, and 300 mg/L) was modeled using 48-h *C. dubia* data. The resultant models were evaluated based on their R^2 values (Fig. 3). The NumCat criteria that produced the model with the highest R^2 (best fit of the model to the observed data) were the 15% with >100 mg/L (R^2 = 0.8559) and the 10% with >100-mg/L (R^2 = 0.8553) criteria. Given that the difference in R^2 was only 0.0006 (0.06% of the variance) and that we had already worked extensively with the 10% and >100-mg/L criteria, we elected to continue using these criteria in finalizing the model equations.

After completion of data collection, final regression equations were developed to predict *C. dubia* survival after 24 and 48 h of exposure. Through the course of these analyses, several additional variables and data transformations were evaluated and discarded. Aside from the feeding and reference toxicant variables discussed previously, we evaluated the sum of all ions, the sum of all cations, the sum of all anions, and NumAn

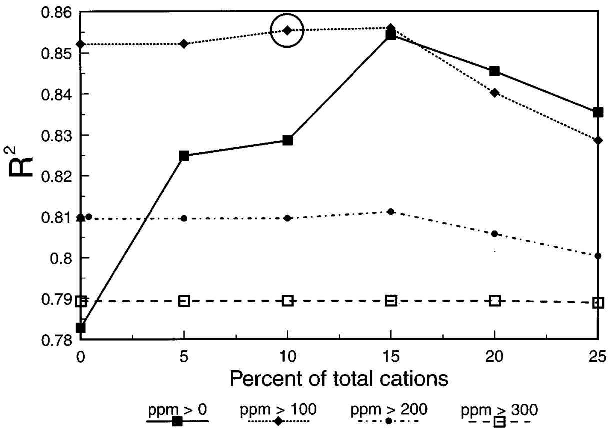


Fig. 3. Effect of varying criteria for the definition of the NumCat variable. Circled point represents the criteria selected initially and maintained for final derivation of the regression equations.

(the anion equivalent of NumCat). First-order interactions of these variables and ion concentrations were also evaluated. None of these variables was selected as significant by the regression algorithm. Models based on log-transformed ion concentrations consistently showed lower R^2 values than those based on untransformed data.

The final 24- and 48-h equations for *C. dubia* had K^+, HCO_3^-, Mg^{2+}, Cl^-, and SO_4^{2-} as significant variables (Table 4). Additionally, NumCat and the interaction terms NumCat × Cl, NumCat × SO_4, and NumCat × K were found to be significant. As had been the case since early in the modeling process, Na^+ and Ca^{2+} concentrations were not significant variables except as they affected the calculation of NumCat. R^2 for the final regressions were 0.861 and 0.842 for the 24-h and 48-h equations.

Model development for *D. magna* proceeded along the same lines as those described for *C. dubia*. The initial model developed using only single salt data fit those data very well (R^2 = 0.97) but was not as good at predicting survival for more complex ion mixtures. As was observed for *C. dubia*, solutions with multiple cations tended to be less toxic than comparable solutions with only one cation. As a result, when all *D. magna* data were analyzed, NumCat was again selected as a significant variable, both by itself and through its interactions with Cl^-, SO_4^{2-}, and K^+ (Table 4). In fact, all significant terms in the *C. dubia* double salt model with NumCat were also significant for *D. magna*. Quality of fit for the *D. magna* models was slightly lower than for the *C. dubia* models, though still quite good (0.812 and 0.799).

As for the daphnids, modeling of the fathead minnow data indicated that toxicity was a function of K^+, Mg^{2+}, HCO_3^-, Cl^-, and SO_4^{2-} concentrations, as neither Na^+ nor Ca^{2+} were selected as significant variables (Table 4). The primary difference in the fathead minnow equations was that NumCat was not a significant variable either by itself or in interaction with other terms. R^2 values for the three regression equations were generally comparable to those for the other models, ranging from 0.767 to 0.832.

Because of the large number of independent variables, the actual response surface of the regression models cannot be easily visualized. Nonetheless, marginal plots of the regression equations can be used to illustrate the relative sensitivity of each species to the various ions (Fig. 4). These plots show that *C. dubia* are, in general, the most sensitive of the three species to major ion toxicity, while fathead minnows are the least sensitive. K^+ was the most toxic ion to all species and SO_4^{2-} the least. The only inconsistency between species was that Mg^{2+} was more toxic than HCO_3^- for *D. magna* and fathead minnows, but the reverse was true for *C. dubia*.

As a means to visually evaluate the fit of the data sets to the regression equations, each regression equation was used to predict the ion concentrations producing 50% survival for each of the ion combinations tested during data collection. These values were then plotted against the average observed LC50 values from Tables 2 and 3 (Fig. 5). These plots indicate good overall agreement between the calculated and predicted LC50 values for all three species. Note, however, that this analysis is not a direct evaluation of quality of fit for the models because it actually compares a point estimate derived from individual logistic regression equations with the arithmetic mean of multiple point estimates for specific ion combinations derived by a different method (trimmed Spearman–Karber LC50 estimation [21]); it is not a plot of raw data versus model predictions. There are other biases in this comparison as well, such as different weighting of observations. Nevertheless, the concordance between the two methods does provide some assurance that the single multiple regression models provide a reasonable representation of the responses to a broad range of ion combinations.

The absence of interaction terms in the final regression equations, aside from those involving NumCat, suggests that

Table 4. Regression coefficients for final regression equations[a]

	Ceriodaphnia dubia		*Daphnia magna*		Fathead minnow		
	24-h	48-h	24-h	48-h	24-h	48-h	96-h
Constant	9.11	8.83	5.91	5.83	5.69	5.51	4.70
K^+	−0.0320	−0.0299	−0.0200	−0.0185	−0.0108	−0.0113	−0.00987
Mg^{2+}	−0.00594	−0.00668	−0.00450	−0.00510	−0.00225	−0.00316	−0.00327
Cl^-	−0.00706	−0.00813	−0.00330	−0.00395	−0.00117	−0.00125	−0.00120
SO_4^{2-}	−0.00424	−0.00439	−0.00204	−0.00255	−0.000728	−0.000750	−0.000750
HCO_3^-	−0.00745	−0.00775	−0.00276	−0.00397	−0.00200	−0.00274	−0.00443
NumCat	0.0332	−0.446	−0.410	−0.511	NS[b]	NS	NS
NumCat*K^+	0.00888	0.00870	0.00778	0.00677	NS	NS	NS
NumCat*Cl^-	0.00196	0.00248	0.00110	0.00146	NS	NS	NS
NumCat*SO_4^{2-}	0.00121	0.00140	0.000998	0.00132	NS	NS	NS
Model R^2	0.861	0.842	0.812	0.799	0.832	0.828	0.767

[a] Units for ion variables are mg/L.

[b] NS indicates that this particular variable was not significant and was excluded from the model.

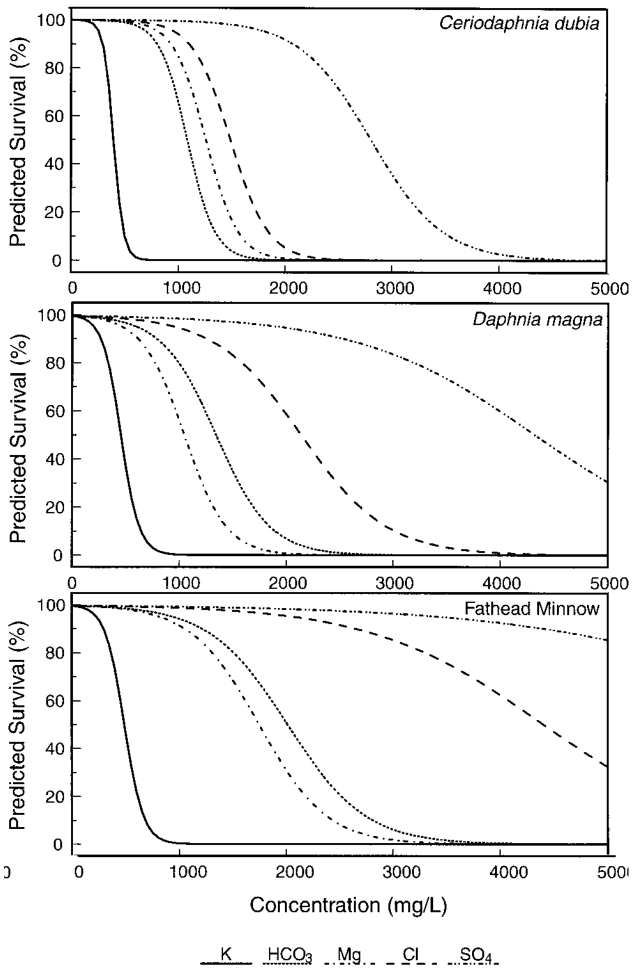


Fig. 4. Marginal plots of regression equations for each of the ions selected as significant. For *Ceriodaphnia dubia* and *Daphnia magna* models, NumCat = 1.

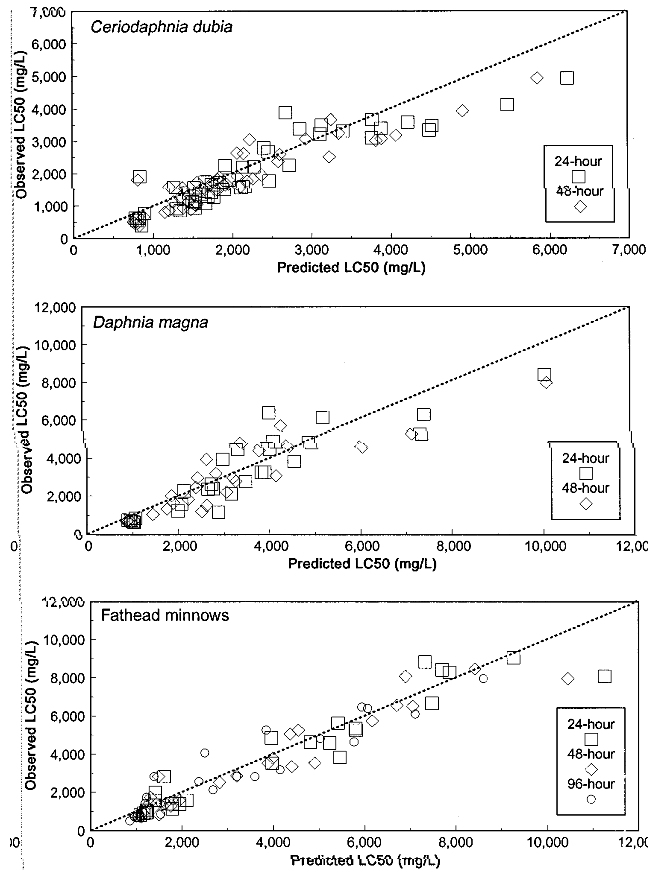


Fig. 5. Relationship between the ion concentrations predicted to cause 50% mortality and the average of LC50 values for individual salts and salt combinations (Tables 2 and 3). Line of unity (slope = 1) added for reference.

assuming additivity among individual ion toxicities is sufficient to describe the toxicities of the ion mixtures, at least from an empirical standpoint. The apparent amelioration of Cl^-, SO_4^{2-}, and K^+ toxicity by multiple cations could be construed as less than additivity. Alternatively, given that Na^+ and Ca^{2+} were not clearly identified as toxic by themselves, it might be more appropriate to consider those ions as water quality variables influencing toxicity, rather than as components of a toxic mixture.

We had little precognition of the important role that the NumCat variable would play in representing the combined toxicity of major ions. In a study of high-TDS irrigation return waters, Dwyer et al. [14] demonstrated that increasing the hardness (Mg^{2+} and Ca^{2+}) of an NaCl-dominated water decreased toxicity to *D. magna* and striped bass. For *D. magna*, this decreased toxicity would be predicted based on the current research, as the addition of hardness to these waters would have increased the value of the NumCat variable, thereby increasing predicted survival. However, results of our study also show that the effect of multiple cations is not an effect of hardness per se. For example, the *C. dubia* 48-h LC50 values for NaCl and $CaCl_2$ were almost identical when expressed on the basis of Cl^- concentration (1,187 and 1,172 mg/L, respectively; Table 2), even though the solutions had greatly different hardness. Moreover, the addition of NaCl to KCl increased the K^+ concentration at the *C. dubia* 48-h LC50 from 329 mg K/L for KCl to 458 mg K/L for an NaCl + KCl mix (Table 2), even though hardness was the same in both solutions.

Despite its importance in modeling the response of *C. dubia* and *D. magna*, the NumCat variable was not selected as significant for fathead minnows. Given that the addition of Mg^{2+}/Ca^{2+} improved survival of striped bass in high TDS solutions tested by Dwyer et al. [14], it seems that the protective effect in multiple cations is not restricted to cladocerans. It is worth noting that combinations of two cations and one anion were only tested once (in duplicate) for fathead minnows. If by chance those test results had a systematic bias, it might mask the presence of a cation-related effect for fathead minnows; coefficients of variation for fathead minnow LC50s were higher than for the other two species. More testing would be required to confirm or deny this possibility.

Though the effect of multiple cations is quite consistent both within the *C. dubia* and *D. magna* data sets and with other research [14], it must be emphasized that its identification and quantification through our modeling is empirical. Notwithstanding the effectiveness of the categorical NumCat variable in modeling our data set, it seems reasonable from a physiological standpoint to assume that the effect is in reality some type of continuous function, rather than the step function represented by our >10% and >100-mg/L criteria. In our modeling efforts, we were unable to devise a continuous variable that corresponded to the observed influence of multiple cations. Nonetheless, with continued research it seems likely that such a relationship could be uncovered and, if so, might provide a more rigorous representation of the actual relationship than that provided by NumCat as currently defined. A better understanding of the mechanisms of major ion toxicity would likely enhance this effort.

As a related matter, even though we conducted a sensitivity analysis to determine the optimum criteria for the NumCat variable, this analysis was subject to bias from the structure of our data set. Specifically, we tested binary combinations of salt solutions in 1:1 ratios only. As such, only certain areas within the total sampling space (all possible ion combinations) were represented in the data set. Thus, there is no assurance that the ion combinations tested were near critical points in the response surface that might alter the apparent thresholds for response. While we believe the NumCat variable is a significant advance in understanding the response of cladocerans to high TDS solutions, it is probably a somewhat crude representation of the actual physiological response.

Because most chemical reactions are related to molar concentrations, an argument could be made for modeling survival on the basis of molar concentrations rather than mass-based concentration. In retrospect, it seems this would have made little difference in the outcome of the modeling. As the equations are based on first-order concentrations of single ions only, transformation between mass-based and molar concentrations is a simple algebraic manipulation and does not affect the nature of the response surface. In fact, the equations in Table 4 can be converted to a molar basis by simply dividing each coefficient by the molecular weight of each ion. Conversion to chemical activity, however, would be much more involved.

Ultimately, the test of the toxicity models we have generated lies in their ability to make accurate predictions for samples outside those used to generate the original data set. Thus far, the equations have performed well in predicting major ion toxicity in field-collected samples, particularly so for the *C. dubia* equations. For example, Mount et al. [4] showed a strong correlation ($R^2 = 0.95$) between predicted and observed survival of *C. dubia* exposed to ambient samples from a watershed receiving oil field-produced waters. The *C. dubia* regression model was a better predictor of survival than any individual ion concentration, illustrating the ability of the model to predict the combined toxic effects of multiple ions. In a separate analysis, Mount et al. [15] showed a strong relationship between predicted and observed survival of *C. dubia* exposed to six produced waters collected from coalbed methane operations in Alabama. Obviously, these comparisons assume that major ions were the primary cause of toxicity in the field-collected samples.

Another application of the ion toxicity models that may prove equally or even more valuable lies in using model predictions to determine whether the presence of toxicants other than major ions is indicated. Research by Tietge et al. [5] both demonstrates this application and provides a rigorous evaluation of the predictive capability of the regression models. Six produced waters from various fossil fuel production sites were tested for toxicity and analyzed for major ion concentrations. The ion toxicity models presented here were used to predict survival of *C. dubia*, *D. magna*, and fathead minnows based on major ion concentrations. Differences between observed and predicted toxicity were used to make inferences as to whether the observed toxicity could be wholly explained by the major ion concentrations alone, or if the presence of other toxicants was indicated. The accuracy of these inferences was then evaluated by conducting Phase I TIE manipulations [16] and by testing the toxicity of laboratory waters reconstituted to the same major ion concentrations. This study indicated that the *C. dubia* model provided highly accurate predictions, while the fathead minnow and *D. magna* models tended to overpredict ion toxicity. The tendency of the *D. magna* and fathead minnow models to overpredict toxicity in field-collected samples was also noted in comparisons made by Mount et al. [4].

Dickerson et al. [7] used the *C. dubia* and fathead minnow models to evaluate toxicity in surface waters influenced by irrigation drain water. Although independent tests were not performed to confirm model predictions, it appeared that predictions by the *C. dubia* model correlated well with observed toxicity. As in the study by Tietge et al. [5], however, the fathead minnow model seemed to overpredict toxicity; several sites had higher observed survival than was predicted by the fathead minnow model.

In summary, applications of the *C. dubia* models and, to a lesser extent the *D. magna* and fathead minnow models, have proven them to be highly effective and comprehensive tools for evaluating major ion toxicity. To date, they have been successfully applied to studies of ambient waters [15], produced waters [4,5], irrigation drain waters [7], water purification byproducts [23], municipal effluents, and effluents from pulp and paper, refining, and manufacturing industries (J.R. Hockett, unpublished data). Because the models represent the combined toxicity of all seven ions, they have much broader application than ion toxicity studies based on generic measures like conductivity or TDS, or studies focusing on certain waters or ion combinations. Application of these models can reduce the need for extensive characterization and fractionation manipulations during TIE studies of high TDS waters [11]. They can also be used to project changes in toxicity resulting from modifications in industrial processes, effluent treatment, or other remedial measures.

Acknowledgement—Primary support for this research was provided by the Gas Research Institute through contract 5091-253-2160 with ENSR Consulting and Engineering. Analytical Technologies, Inc., conducted major ion analyses. J.P. Fillo contributed to the conceptual development of the research and K.A. Barten assisted in toxicity data collection. G.T. Ankley, S.R. Broderius, and A.M. Farag provided helpful comments on earlier versions of the manuscript. Helpful comments by two anonymous reviewers are greatly appreciated. This manuscript was reviewed in accordance with U.S. EPA policy. Mention of trade names does not indicate endorsement by the Gas Research Institute, U.S. EPA, or the Federal Government.

REFERENCES

1. **U.S. Environmental Protection Agency.** 1991. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms, 4th ed. EPA/600/4-90/027. Cincinnati, OH.
2. **U.S. Environmental Protection Agency.** 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, 3rd ed. EPA/600/4-91/002. Washington, DC.
3. **Boelter, A.M., F.N. Lamming, A.M. Farag** and **H.L. Bergman.** 1992. Environmental effects of saline oil-field discharges on surface waters. *Environ. Toxicol. Chem.* **11**:1187–1195.
4. **Mount, D.R., D.D. Gulley** and **J.M. Evans.** 1993. Salinity/toxicity relationships to predict the acute toxicity of produced waters to freshwater organisms. *Proceedings*, 1st Society of Petroleum Engineers/U.S. Environmental Protection Agency Environmental Conference. San Antonio, TX, USA pp. 605–614
5. **Tietge, J.E., J.R. Hockett** and **J.M. Evans.** 1997. Major ion toxicity of six produced waters to three freshwater species: Application of ion toxicity models and TIE procedures. *Environ. Toxicol. Chem.* **16**:2002–2008.

6. **Ingersoll, C.G., F.J. Dwyer, S.A. Burch, M.K. Nelson, D.R. Buckler** and **J.B. Hunn.** 1992. The use of freshwater and saltwater animals to distinguish between the toxic effects of salinity and contaminants in irrigation drain water. *Environ. Toxicol. Chem.* **11**:503–511.
7. **Dickerson, K.K., W.A. Hubert** and **H.L. Bergman.** 1996. Toxicity assessment of water from lakes and wetlands receiving irrigation drainwater. *Environ. Toxicol. Chem.* **15**:1097–1101.
8. **Meyer, J.S., D.A. Sanchez, J.A. Brookman, D.B. McWhorter** and **H.L. Bergman.** 1985. The chemistry and aquatic toxicity of raw oil shale leachates from Piceance Basin, Colorado. *Environ. Toxicol. Chem.* **4**:559–572.
9. **Hoke, R.A., J.P. Giesy, M. Zabik** and **M. Unger.** 1993. Toxicity of sediments and sediment pore waters from the Grand Calumet River—Indiana Harbor, Indiana area of concern. *Ecotoxicol. Environ. Saf.* **26**:86–112.
10. **Hoke, R.A., W.R. Gala, J.B. Drake, J.P. Giesy** and **S. Flegler.** 1992. Bicarbonate as a potential confounding factor in cladoceran toxicity assessments of pore water from contaminated sediments. *Can. J. Fish. Aquat. Sci.* **49**:1633–1640.
11. **Jop, K.M.** and **A.M. Askew.** 1994. Toxicity identification evaluation using a short-term chronic test with *Ceriodaphnia dubia. Bull. Environ. Contam. Toxicol.* **53**:91–97.
12. **van Compernolle, R., P.B. Dorn, S.G. Newman, J.A. King** and **J. Gumulka.** 1993. Demonstration of the absence of underlying acute toxicity in the presence of acutely toxic levels of salts of group 1A and IIA cations. *Abstract*, 14th Annual Meeting, Society of Environmental Toxicology and Chemistry, Houston, TX, USA, November 14–18, p. 91.
13. **Burnham, B.L.** and **J.J. Peterka.** 1975. Effects of saline water from North Dakota lakes on survival of fathead minnow (*Pimephales promelas*) embryos and sac fry. *J. Fish. Res. Board Can.* **32**:809–812.
14. **Dwyer, F.J., S.A. Burch, C.G. Ingersoll** and **J.B. Hunn.** 1992. Toxicity of trace element and salinity mixtures to striped bass (*Morone saxatilis*) and *Daphnia magna. Environ. Toxicol. Chem.* **11**:513–520.
15. **Mount, D.R., K.R. Drottar, D.D. Gulley, J.P. Fillo** and **P.E. O'Neil.** 1992. Use of laboratory toxicity data for evaluating the environmental acceptability of produced water discharge to surface waters. In J.P. Ray and F.R. Engelhardt, eds., *Produced Water: Technological/Environmental Issues and Solutions.* Plenum, New York, NY, USA, pp. 175–185.
16. **U.S. Environmental Protection Agency.** 1991. Methods for aquatic toxicity identification evaluations. Phase I toxicity characterization procedures, 2nd ed. EPA/600/6-91/003. Washington, DC.
17. **U.S. Environmental Protection Agency.** 1994. Methods for the determination of metals in environmental samples. Supplement 1. EPA/600/R-94/111. Washington, DC.
18. **U.S. Environmental Protection Agency.** 1983. Methods for chemical analysis of water and wastes. EPA/600/4-79/020. Cincinnati, OH.
19. **American Public Health Association.** 1989. *Standard Methods for the Examination of Water and Wastewater.* Washington, DC.
20. **Dixon, W.J.** 1985. *BMDP Statistical Software.* University of California Press, Berkeley, CA, USA.
21. **Hamilton, M.A., R.C. Russo** and **R.V. Thurston.** 1977. Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. *Environ. Sci. Technol.* **11**: 714–719. Correction **12**:417(1978).
22. **Mount, D.R.** and **D.D. Gulley.** 1992. Development of a salinity/toxicity relationship to predict acute toxicity of saline waters to freshwater organisms. GRI-92/0301. Gas Research Institute, Chicago, IL, USA.
23. **Florida Department of Environmental Protection.** 1995. Protocols for determining major-seawater ion toxicity in membrane technology water-treatment concentrate. Bureau of Laboratories, Tallahassee, FL, USA.