Arch Environ Contam Toxicol (2008) 54:466–472
DOI 10.1007/s00244-007-9043-z

Acute Toxicity of Aqueous Copper, Cadmium, and Zinc to the Mayfly *Rhithrogena hageni*

Stephen F. Brinkman · Walter D. Johnston

Received: 28 August 2007 / Accepted: 31 August 2007 / Published online: 5 October 2007

Abstract Heptageniid mayfly nymphs have been suggested as sensitive indicators of metal contamination in streams based on biomonitoring studies, experimentation in situ, and experimentation in microcosm. Laboratory tests were conducted to evaluate the sensitivity of *Rhithrogena hageni*, a heptageniid mayfly, to waterborne copper, cadmium, and zinc. Tests were conducted with soft water (hardness = 40–50 mg/L) at about 12°C. Toxicity endpoints were survival and moulting (%/day). Median 96 hr lethal concentrations were 0.137, 10.5, and 50.5 mg/L for copper, cadmium and zinc, respectively. The average daily moulting rate of survivors significantly decreased after exposure to these metals in solution.

Introduction

Aquatic insects are often used to assess biological impacts of trace metal pollution. Case studies of contaminated rivers in the Rocky Mountain region have found reduced mayfly abundances immediately downstream of point-source inputs of metals (Winner et al. 1980; Cain et al. 1992; Clements et al. 2000). Heptageniid mayflies have been recognized as especially sensitive to metals in streams (Leland et al. 1989; Peckarsky and Cook 1981; Clements 1994; Clements and Kiffney 1995; Clements et al. 2002) and in microcosm experiments (Clements 2004). *Rhithrogena hageni* has been identified as a strong indicator of metal contamination (Nelson and Roline 1993; Kiffney and Clements 1994; Clements et al. 2000). Kiffney and Clements (1994) found that the concentration of zinc correlated with reduced heptageniid abundances in stream microcosms was well below the hardness-based ambient aquatic life criterion for zinc (United States Environmental Protection Agency 1996).

Ambient aquatic life water quality criteria and standards are generally derived from results of laboratory toxicity tests conducted with only one species and one toxicant (United States Environmental Protection Agency 1985a). However, acceptable data from such tests are lacking for mayflies. Results of a single study were used for the development of US aquatic life water quality criteria for cadmium (United States Environmental Protection Agency 2001). No mayfly toxicity data were used for the development of water quality criteria for copper or zinc (United States Environmental Protection Agency 1985b; 1996). The objective of this study was to provide toxicity data for use in developing copper, cadmium and zinc criteria and state standards. We conducted a series of experiments to determine the lethality of each metal to *R. hageni* in a controlled laboratory setting.

Materials and Methods

Collection and Handling

Rhithrogena hageni nymphs were collected by hand from shallow riffles on the Cache la Poudre River (Larimer County, CO, USA), which has no history of metal contamination. Nymphs were collected from the same site in

S. F. Brinkman · W. D. Johnston
Aquatic Toxicology Laboratory, Colorado Division of Wildlife, Fort Collins, Colorado, USA

S. F. Brinkman (✉)
Colorado Division of Wildlife, 317 West Prospect Rd., Fort Collins, CO, USA
e-mail: steve.brinkman@state.co.us

October, November, and December of 2005 for the zinc, copper, and cadmium tests, respectively. Nymphs were gathered at least seven days prior to each test. Individuals were identified to genus in the field using a taxonomic key from Ward and Kondratieff (1992). Species identity was verified by two independent experts at Colorado State University.

Nymphs were transported in a 30-liter cooler along with cobble substrate from the collection site. The transport unit was aerated with airstones connected by nylon tubing to a battery-operated pump. Temperature was maintained near ambient conditions (4–6°C) during transport to the Colorado Division of Wildlife aquatic toxicology laboratory in Fort Collins, Colorado, USA. Nymphs were carefully transferred with a paint brush to glass holding tanks containing Cache la Poudre River water. Holding tanks were aerated and incubated at 4°C. Water was gradually replaced (50% per day) with test dilution water and the incubator temperature was gradually increased (2°C per day) to test temperature (11–12°C).

Test Methods

Source water for the zinc toxicity test consisted of a mixture of onsite well water and reverse osmosis water. A conductivity controller maintained the diluent source water hardness near 45 mg/L. Dechlorinated municipal tap water (Fort Collins, CO, USA) was used for the cadmium and copper tests, due to logistical constraints. Both diluent sources had similar water quality characteristics (Table 1). Source water supplied a continuous-flow serial diluter (Benoit et al. 1982) constructed of Teflon, polyethylene, and polypropylene components. The diluter delivered five concentrations of metal toxicant with a 50% dilution ratio and a control. A flow splitter allocated each concentration equally among four replicate exposure chambers at a rate of 40 mL/min. Food-grade vinyl tubing delivered test solutions to exposure chambers. Metal stock solutions were prepared by dissolving a calculated amount of metal sulfate salts in deionized water. A concentrated stock solution was delivered to the diluter by peristaltic pump at a rate of 2.0 mL/min.

Table 1 Mean (SD) water quality parameters of exposure water in cadmium, copper, and zinc toxicity tests. ND = not determined

	Cache la Poudre	Copper test	Cadmium test	Zinc test
Hardness (mg/L)	40.2	44.0 (3.5)	48.0 (2.0)	44.4 (1.1)
Alkalinity (mg/L)	36.4	34.5 (1.0)	34.5 (0.6)	39.9 (1.3)
Temperature (°C)	5.0	11.1 (0.4)	12.0 (0.3)	11.9 (0.1)
pH (SU)	7.95	7.72 (0.03)	7.66 (0.10)	7.77 (0.07)
Dissolved oxygen (mg/L)	ND	ND	9.07 (0.15)	ND

Exposure chambers consisted of 1.25 L, cylindrical, polypropylene containers equipped with an air-lift system constructed from half-inch polyvinyl chloride (PVC) pipe. Water collected from the center of the container flowed down through the PVC pipe immersed in a temperature-controlled water bath, then up to the top of the container where an elbow diverted the flow in a circular pattern. The air-lift maintained dissolved oxygen levels at saturation levels and provided continuous, circular flow in the exposure chamber. Two 5 × 5 cm, unglazed, ceramic tiles were placed in each unit to serve as a substrate.

Ten *R. hageni* nymphs were randomly assigned to each of the 24 exposure chambers. Each chamber was assigned to one of six treatment levels with four replicates for each treatment level. Mortality, defined as failure to respond to repeated prodding, and occurrence of exuvia were recorded daily. Nymphs were not fed during the experiments.

Physical and chemical characteristics of exposure water were measured daily for the first 96 hours of the test. Hardness and alkalinity were determined titrimetrically according to standard methods (APHA 1998). A Thermo Orion 635 meter was used to measure pH and temperature. Dissolved oxygen was measured using an Orion 1230 dissolved oxygen meter. Electronic meters were calibrated prior to each use.

Water samples were collected daily for dissolved metal analysis during the first 96 hours of the test. Exposure water was passed through a 0.45 μm filter and immediately preserved with high-purity nitric acid to pH <2. Chambers with no remaining survivors were not sampled. Metal concentrations were measured using an Instrumentation Laboratory Video 22 (Allied Analytical Systems, Franklin, MA) atomic absorption spectrometer with air/acetylene flame and Smith–Hieftje background correction. The spectrometer was calibrated prior to each use and the calibration verified using a National Institute of Standards and Technology (NIST) traceable quality assurance/quality control (QA/QC) standard from an outside source. Sample splits and spikes were collected and prepared during each sampling event to verify reproducibility and to quantify analytical recovery. Mean recovery for the QA/QC standard was 101% (range 97–104%) and for spiked samples was 100% (range 97–102%). The mean percentage difference between sample splits was <3.3%. Detection limits for all three metals were <0.01 mg/L.

Median lethal concentrations (LC_{50}) of cadmium and zinc were estimated using the trimmed Spearman–Karber technique with automatic trim (Hamilton et al. 1977; 1978). Spearman–Karber could not be used to estimate copper LC_{50} due to the nature of the observed concentration–response relationship; therefore probit analysis was

used. Both survival at termination and moulting rate (%/day) were analyzed with one-way analysis of variance (ANOVA). Survival data were arcsine-transformed prior to ANOVA. No transformation was used for moulting data. Assumptions of normal error distribution and homogeneous group variances were tested using the Shipiro–Wilk and Levene tests, respectively.

Treatment means were compared to the control using Williams' one-tailed test (Williams 1971; 1972) with the type I error rate fixed at 0.05 to determine no-observed-effect concentrations (NOEC) and lowest-observed-effect concentrations (LOEC). Survival data of the cadmium and copper tests did not meet assumptions of normality or homogeneity of variance and were analyzed using Steel's nonparametric many–one rank test.

Results

Water quality parameters were reasonably consistent among the different metal tests (Table 1). Standard deviations of each parameter indicated constant water quality within each experiment. Dissolved oxygen was at saturation in the cadmium test (Fort Collins elevation 1520 m) but was not measured in the copper or zinc tests. Nymph survival in the control treatments was 100% in the cadmium and copper tests and 97.5% in the zinc test. Survival decreased with increasing metal concentrations (Fig. 1). Copper, cadmium, and zinc LC_{50}s after 96 hours were 0.137, 10.5, and 50.5 mg/L, respectively. Survival continued to decline after 96 hours in most metal treatments. No survivors remained after seven days of exposure to copper concentrations ≥0.138 mg/L, the lowest concentration tested. Nymph survival declined at cadmium concentrations ≥3.52 mg/L (LOEC) but remained high at concentrations ≤1.88 mg/L (NOEC) (Fig. 1). In the zinc test, the LOEC and NOEC after 10 days were 10.8 and 5.3 mg/L, respectively.

The daily moulting rate (%/day) was significantly reduced by exposure to high concentrations of copper, cadmium and zinc (Fig. 2). Control moulting rate was similar in each test and ranged from 9.3 to 11.7% per day. Moulting was significantly reduced at copper concentrations ≥0.483 (LOEC), but not ≤0.256 (NOEC). The cadmium LOEC and NOEC were 3.52 and 1.88 mg/L, respectively. The LOEC and NOEC for zinc were 10.8 and 5.33 mg/L, respectively.

Discussion

Test conditions and apparatus appeared suitable for maintaining and exposing *Rhithrogena hageni*. The air-lift system provided constant circular flow and maintained

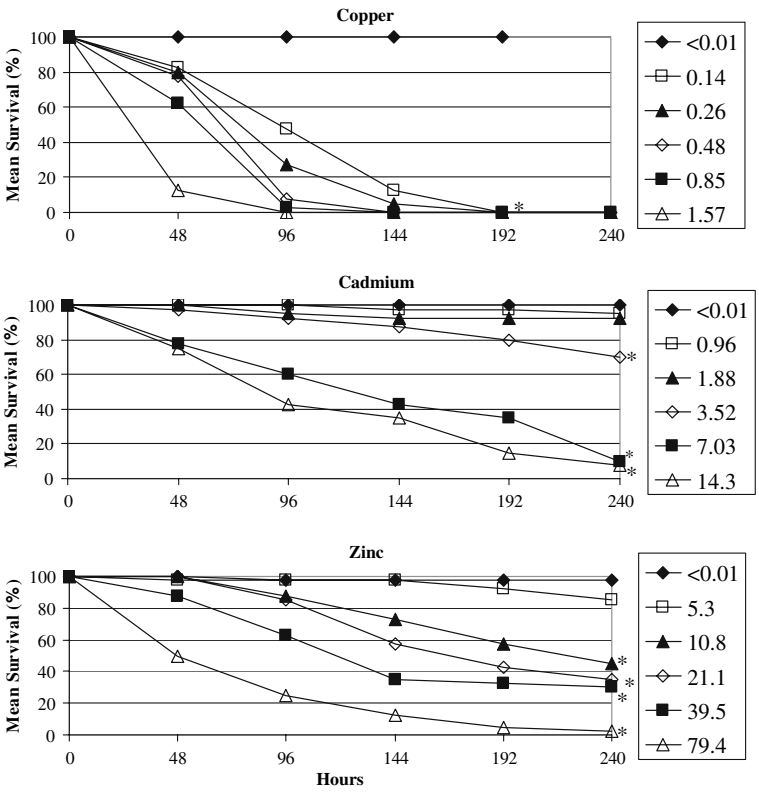

Fig. 1 Mean survival (%) of *R. hageni* versus duration of exposure to copper, cadmium, and zinc (mg/L). Asterisks indicate significantly less than control ($p < 0.05$)

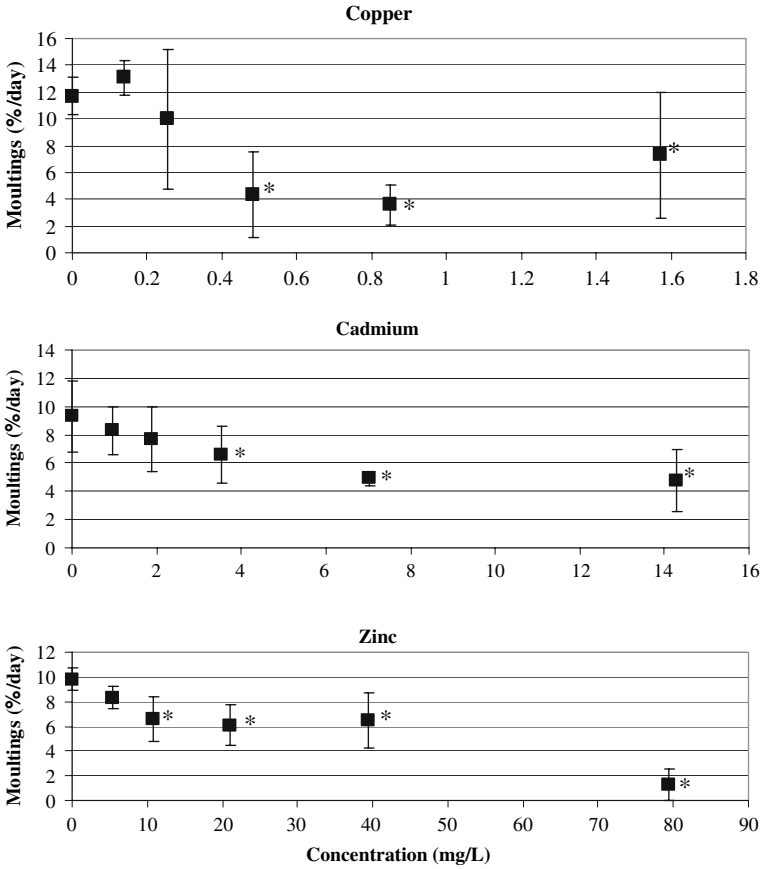

Fig. 2 Daily moulting rate (%/day) versus concentrations of copper, cadmium, and zinc. Asterisks indicate significantly less than control ($p < 0.05$)

saturated levels of dissolved oxygen. The use of a continuous flow-through diluter to deliver exposure solutions minimized variation in exposure concentrations. Survival in control treatments was at least 97% for up to 10 days. Test organisms moulted regularly in the control treatments. Thus we conclude that our test apparatus provided the conditions necessary for survival of *R. hageni* nymphs. The same exposure apparatus also proved suitable for toxicity tests with nymphs of other mayfly and stonefly species (Colorado Division of Wildlife, unpublished data).

R. hageni nymphs were acutely sensitive to metals in the order: Cu > Cd > Zn. Median lethal cadmium and zinc concentrations were 77 and 379 times greater, respectively, than the copper LC_{50}. The relative sensitivity was consistent with limited metal toxicity data available for mayflies. *Ephemerella subvaria* was at least six times more sensitive to copper than cadmium (Warnick and Bell 1969). *Epeorus latifolium* was more sensitive to waterborne copper than zinc (Hatakeyama 1989). Clements (2004) found heptageniid abundance in colonized trays was unaffected by zinc concentrations up to 30 times the United States Environmental Protection Agency criterion. Abundance was only slightly reduced when cadmium was added to zinc. However, the addition of copper to the mixture caused sharp decreases of heptageniid abundance.

A sublethal endpoint, daily average moultings (%/day), was significantly reduced by high concentrations of copper, cadmium, and zinc. The moulting rate declined as cadmium and zinc exposure concentrations increased and was reduced at cadmium and zinc concentrations that also reduced survival. In contrast, copper reduced the moulting rate at much higher than lethal concentrations. Reduction of the moulting rate (relative to control) varied among the metals. Moulting was affected most by zinc and was reduced to about 13% of control at 79 mg/L. Cadmium and copper reduced moulting by about one-half to one-third of control. Reduced moulting was probably the result of impaired growth and/or development. Increased moulting interval and reduced growth has been previously reported for the mayfly, *Epeorus latifolium*, when exposed to copper and zinc (Hatakeyama 1989). The long-term consequences of increased moulting interval are unknown but it might reduce the chance of survival to the adult reproductive stage.

Mayflies are poorly represented in databases used to derive ambient aquatic life water quality criteria for metals. Our results indicate that current acute Cd, Cu, and Zn criteria adequately protect *R. hageni* nymphs. LC_{50} values for Cd, Cu, and Zn were 10,500, 16.8, and 830 times higher than United States Environmental Protection Agency's

acute criteria, respectively, when adjusted for test water hardness. Nymphs exposed to the higher concentrations of Cd and Zn continued to experience mortality after the 96 hour acute exposure period. All nymphs exposed to copper ≥0.138 mg/L died within eight days of exposure. These results suggest that chronic toxicity values may be much lower than acute thresholds. Reported acute/chronic ratios are ≤434 for Cd and ≤41 for Zn (United States Environmental Protection Agency 1996; 2001). If we apply the highest reported acute-chronic ratios, it appears that *R. hageni* should be protected by chronic criteria. Acute-chronic ratios for copper are as high as 152, though most are less than 40 (United States Environmental Protection Agency 1985b). Additional tests are necessary to determine whether *R. hageni* is protected by chronic criteria.

Comparison of Laboratory Results to Field Observations

Based on our laboratory exposures, *R. hageni* appears to be tolerant of short-term exposure to waterborne cadmium and zinc. No significant decrease in survival was detected at concentrations as high as 1.88 mg/L Cd or 5.33 mg/L Zn. Tolerance of *R. hageni* to acute Cd and Zn exposures may be due to slow biological uptake of these metals. Uptake of aqueous cadmium and zinc was extremely slow in a congeneric species, *R. morrisonii*. (Buchwalter and Luoma 2005). Our observed tolerance of *R. hageni* to zinc contrasts with numerous biomonitoring studies that have attributed reduced heptageniid abundance to much lower concentrations of zinc. For example, abundances of *R. hageni* and other heptageniid mayfly species in the east fork of the Arkansas River were reduced downstream of the Leadville Mine Drainage Tunnel where zinc concentrations ranged from 0.1 to 1.0 mg/L (Clements 1994; Nelson and Roline 1996; Clements 2004). Following the installation of a treatment plant, zinc concentrations dropped below criteria levels and abundances of *R. hageni* and other heptageniids at downstream sites increased (Nelson and Roline 1996; Clements 2004).

The apparent discrepancy between the laboratory-derived lethal concentrations and concentrations found to decrease abundance in biomonitoring studies could arise from several causes. Synergism may occur in metal mixtures typically found in streams thus increasing toxicity in situ (Clements 2004). Also, sensitive taxa may be eliminated or reduced by pulses of metals undetected by grab samples collected during biomonitoring studies. However, it is unlikely that a pulse of metal as high as the concentrations used in our laboratory tests would ever occur in places such as the Arkansas River. Field observations may also be confounded by other environmental variables unrelated to metals such as stream size, temperature, flow, and nonmetal inputs.

Differences between effect concentrations observed in the laboratory and in situ may have been due to experimental factors. Insufficient test duration or the use of a tolerant life stage could have resulted in toxicity thresholds much greater than those that have been observed in streams. Mortality in our test did not cease at 96 hours but continued to increase until test termination. Thus, longer exposures might have resulted in significantly lower lethal thresholds. In addition, late-instar nymphs may be more tolerant of metals than earlier instars. Use of late-instar nymphs was necessary due to the difficulty of identifying and handling early instars. Smaller individuals were more sensitive to a metal mixture for several mayfly species including *R. hageni* (Kiffney and Clements 1996; Clark and Clements 2006). In metal-impacted streams, the reduction or elimination of metal-sensitive early instars may be offset by recolonization of tolerant late instars from clean tributaries or from upstream of the metal source. However, heptageniid mayflies drift only rarely (Rader 1997), therefore recolonization by metal-tolerant instars would be slow.

Indirect effects of metals could also have contributed to differences between metal concentrations that caused lethality in the laboratory and decreased abundance in streams. Increased drift of mayflies (Leland et al. 1989; Clements 1999) and increased risk of predation (Clements 1999) in response to metal exposure could reduce mayfly abundance in metal-impacted streams. Metal-caused shifts in periphyton communities (Hatakeyama 1989; Medley and Clements 1998; Hill et al. 2000), which are a primary food source for scrapers such as heptageniid mayflies (Merritt and Cummins 1996), could influence their abundance as well.

Perhaps most important is the possible influence of dietary rather than waterborne metal exposure affecting mayfly abundance in metal impacted streams. *Hexagenia rigida* accumulated Cd and Zn primarily from the diet and not from the water (Hare et al. 1991). Copper, Cd, and Zn concentrations in invertebrates have been found to be more strongly correlated with metal content of *aufwuchs* (periphyton and associated embedded abiotic material that serves as food for grazing benthic invertebrates) than with water or sediment concentrations (Kiffney and Clements 1993; Beltman et al. 1999). Several experiments have documented toxicity of dietary metals to mayflies. Growth and emergence were reduced in *Epeorus* mayflies fed diatoms with elevated levels of copper or zinc (Hatakeyama 1989). *Baetis tricaudatus* mayflies experienced reduced growth when fed metal-contaminated biofilm (Courtney and Clements 2002; Carlisle and Clements 2003) and cadmium-dosed diatom mats (Irving et al. 2003). Reduced

growth from dietary exposure to metals may be due to food avoidance (Hatakeyama 1989; Irving et al. 2003; Wilding and Maltby 2006) or reduced food quality (Courtney and Clements 2002; Carlisle and Clements 2003).

Metal toxicity is generally assumed to occur through waterborne exposure and environmental regulations do no take into account the potential impact of dietary sources of metals to aquatic organisms. In instances where dietary exposures exert their own toxicity or interact with waterborne exposures, water quality criteria and standards may underprotect organisms in aquatic environments. Future studies on the effects of dietary versus aqueous exposure, for both early and late instars of aquatic invertebrates, may help explain differences between laboratory toxicity tests and field studies.

Acknowledgments This study was supported by EPA region 8 and by US Fish and Wildlife Service Federal Aid Grant F-243. The authors wish to thank Daria Hansen for her assistance with the tests, and Dr. William Clements whose comments greatly improved this manuscript.

References

APHA (1998) Standard Methods for the Examination of Water and Wastewater, 16th edn. American Public Health Association, American Water Works Association, and Water Pollution Control Federation. Washington, D.C

Beltman DJ, Clements WH, Lipton J, Cacela D (1999) Benthic invertebrate metals exposure, accumulation, and community-level effects downstream from a hard-rock mine site. Environ Toxicol Chem 18:299–307

Benoit DA, Mattson VR, Olsen DC (1982) A Continuous Flow Mini-diluter System for Toxicity Testing. Water Res 16:457–464

Buchwalter DB, Luoma SN (2005) Differences in dissolved cadmium and zinc uptake in stream insects: mechanistic explanations. Environ Sci Technol 39:498–504

Cain DJ, Luoma SN, Carter JL (1992) Aquatic insects as bioindicators of trace-element contamination in cobble-bottom rivers and streams. Can J Fish Aquat Sci 49(10):2141–54

Carlisle DW, Clements WH (2003) Growth and secondary production of aquatic insects along a gradient of Zn contamination in Rocky Mountain streams. J North Am Benthol Soc 22(4):582–597

Clark JL, Clements WH (2006) The use of in situ and stream microcosms experiments to assess population and community level responses to metals. Environ Toxicol Chem 25:2306–2312

Clements WH (1994) Benthic invertebrate community responses to heavy-metals in the upper Arkansas River basin, Colorado. J North Am Benthol Soc 13(1):30–44

Clements WH (1999) Metal tolerance and predator-prey interactions in benthic macroinvertebrate stream communities. Ecol Appl 9(3):1073–1084

Clements WH (2004) Small-scale experiments support causal relationships between metal contamination and macroinvertebrate community responses. Ecol Appl 14(3):954–967

Clements WH, Carlisle DM, Lazorchak JM, Johnson PC (2000) Heavy metals structure benthic communities in Colorado mountain streams. Ecol Appl 10:626–638

Clements WH, Kiffney PM (1995) The influence on benthic community responses to heavy-metals in Rocky Mountain streams. Can J Fish Aquat Sci 52(9):1966–1977

Clements WH, Carlisle DM, Courtney LA, Harrahy EA (2002) Integrating observational and experimental approaches to demonstrating causation in stream biomonitoring studies. Environ Toxicol Chem 21(6):1138–1146

Clements WH, Newman MC (2002) Community Ecotoxicology. Wiley, Chichester UK

Courtney LA, Clements WH (2002) Assessing the influence of water and substratum quality on benthic macroinvertebrate communities in a metal-polluted stream: an experimental approach. Freshwater Biol 47:1766–1778

Hamilton MA, Russo RC, Thurston RV (1977) Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol 11(7):714–719

Hamilton MA, Russo RC, Thurston RV (1978) Correction. Environ Sci Technol 12:417

Hare L, Saouter E, Campbell PGC, Tessier A, Ribeyre F, Boudou A (1991) Dynamics of cadmium, lead, and zinc exchange between nymphs of the burrowing mayfly *Hexagenia rigada* (Ephemeroptera) and the environment. Can J Fish Aquat Sci. 48:39–47

Hatakeyama S (1989) Effect of copper and zinc on the growth and emergence of *Epeorus latifolium* (Ephemeroptera) in an indoor model stream. Hydrobiologia 174:17–27

Hill BH, Willingham WT, Parrish LP, McFarland BH (2000) Periphyton community responses to elevated metal concentrations in a Rocky Mountain stream. Hydrobiologia 428(1–3):161–169

Irving EC, Baird DJ, Culp JM (2003) Ecotoxicological responses of the mayfly *Baetis tricaudatus* to dietary and waterborne cadmium: implications for toxicity testing. Environ Toxicol Chem 22:1058–1064

Kiffney PM, Clements WH (1994) Structural responses of benthic macroinvertebrate communities from different stream orders to zinc. Environ Toxicol Chem 13(3):389–395

Kiffney PM, Clements WH (1996) Size-dependent response of macroinvertebrates to metals in experimental streams. Environ Toxicol Chem 15(8):1352–1353

Leland HV, Fend SV, Dudley TL, Carter JL (1989) Effects of copper on species composition of benthic insects in a Sierra-Nevada, California stream. Freshwater Biol 21(2):163–179

Merritt RW, Cummins KW (1996) An introduction to the aquatic insects of North America, Kendall/Hunt, Dubuque, IA, USA

Nelson SM, Roline RA (1993) Selection of the mayfly *Rithrogena* [sic] *hageni* as an indicator of metal pollution in the upper Arkansas River. J Freshwater Ecol 8(2):111–119

Peckarsky BL, Cook KZ (1981) Effect of Keystone mine effluent on colonization of stream benthos. Environ Entomol 10:864–871

Rader RB (1997) A functional classification of the drift: traits that influence invertebrate availability to salmonids. Can J Aquat Sci 54:1211–1234

United States Environmental Protection Agency (1985a) Guidelines for deriving numerical standards for the protection of aquatic organisms and their uses. PB85–227049. Washington, DC USA

United States Environmental Protection Agency (1985b) Ambient water quality criteria for copper – 1984. EPA 440/5-84-031. Washington, DC, USA

United States Environmental Protection Agency (1996) 1995 Updates: Water quality criteria documents for the protection of aquatic life in ambient water. EPA-820-B-96-001. Washington, DC, USA

United States Environmental Protection Agency (2001) 2001 Update of ambient water quality criteria for cadmium. EPA 822-R-01-001. Washington, DC, USA

Ward JV, Kondratieff BC (1992) An illustrated guide to the mountain stream insects of Colorado. University Press of Colorado, Niwot Colorado

Warnick SL, Bell HL (1969) The acute toxicity of some heavy metals to different species of aquatic insects. J Water Pollut Control Fed 41:280–284

Wilding J, Maltby L (2006) Relative toxicological importance of aqueous and dietary metal exposure to a freshwater crustacean: implication for risk assessment. Environ Toxicol Chem 25(7):1795–1801

Williams DA (1971) A test for differences between treatment means when several dose levels are compared with a zero dose control. Biometrics 27:103–117

Williams DA (1972) The comparison of several dose levels with a zero dose control. Biometrics 27:103–117

Winner RW, Boesel WM, Farrel MP (1980) Insect community structure as an index of heavy-metal pollution in lotic ecosystems. Can J Aquat Sci 37:647–55