Integrated Environmental Assessment and Management — Volume 6, Number 2—pp. 199–209
© 2009 SETAC

Calibrating Biomonitors to Ecological Disturbance: a New Technique for Explaining Metal Effects in Natural Waters

Samuel N. Luoma,†,‡,§ Daniel J. Cain,§ and Philip S. Rainbow‡*
†John Muir Institute of the Environment, University of California, Davis, California 95616, USA
‡Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK
§US Geological Survey, MS465, 345 Middlefield Road, Menlo Park, California 94025, USA

(Submitted 18 June 2009; Returned for Revision 10 October 2009; Accepted 17 November 2009)

ABSTRACT

Bioaccumulated toxic metals in tolerant biomonitors are indicators of metal bioavailability and can be calibrated against metal-specific responses in sensitive species, thus creating a tool for defining dose–response for metals in a field setting. Dose–response curves that define metal toxicity in natural waters are rare. Demonstrating cause and effect under field conditions and integrated chemical measures of metal bioavailability from food and water is problematic. The total bioaccumulated metal concentration in any organism that is a net accumulator of the metal is informative about metal bioavailability summed across exposure routes. However, there is typically no one universal metal concentration that is indicative of toxicity, especially across species, largely because of interspecies differences in detoxification. Stressed organisms are also only present across a narrow range in the dose–response curve, limiting the use of singles species as both biomonitors and bioindicator of stress. Herein we show, in 3 field settings, that bioaccumulated Cu concentrations in a metal-tolerant, riverine biomonitor (species of the caddisfly genus *Hydropsyche* spp.) can be calibrated against metal-specific ecological responses across very wide ranges of contamination. Using the calibrated dose–response, we show that reduced abundance of species and individuals from particularly sensitive mayfly families (heptageniid mayflies) is more than 2-fold more sensitive to bioavailable Cu than other traditional measures of stress like EPT or total number of benthic macroinvertebrate species. We propose that this field dose-response curve be tested more widely for general application, and that calibrations against other stress responses be developed for biomonitors from lakes, estuaries, and coastal marine ecosystems. Integr Environ Assess Manag 2010;6:199–209. © 2009 SETAC

Keywords: Biomonitor Metals Mayflies Copper Clark Fork River

Review—Global Environmental Issue

INTRODUCTION

Managing ecological risks from metal contamination in aquatic environments remains an important challenge. Metals continue to be ubiquitous in the products used by humans. In-use stocks continue to grow and controlling waste discharges will be a challenge into the foreseeable future. Some legacy contamination remains unremediated and new hotspots of contamination are likely growing where economic development is expanding faster than environmental regulation. New technologies threaten to introduce metals into the aquatic environment in forms with which we have little experience (e.g., nanometallic products; Luoma 2008). The ongoing challenges of managing ecological risks from metal contamination will require improved approaches to risk assessment and to setting environmental guidelines (e.g., Di Toro 2003) or at least more effective validation of the existing approaches. Luoma and Rainbow (2008) have suggested that a new generation of risk-assessment approaches might profit from a greater emphasis on biological and ecological principles, as well as a stronger integration of field observations into decision making. They defined an interdisciplinary and lateral risk assessment and risk-management process as one that gives equal weight to observational data from nature, experimental studies of key processes, and new models, as well as expanded in situ and chronic toxicity testing.

In this study, we propose a methodology that is an example of lateral risk assessment. It bridges 2 established field methodologies that are traditionally used independently. We show that we can (a) measure bioavailable metal exposure in terms of the concentration bioaccumulated into the tissues of a widespread biomonitoring species, (b) calibrate that measure of bioavailable metal against response data from other, more sensitive, species, and (c) thereby derive dose–response curves for metal effects in streams using the combination of biomonitoring data and traditional measures of stress, such as changes in community structure. We test the principle in 3 case studies in which the metal copper (Cu) was shown in earlier studies to be the primary toxicant. We derive the calibration from an extensive data set, collected over 20 y, from the Clark Fork River, Montana (Cain et al. 2004; in prep.). The Clark Fork is a mine-contaminated river undergoing remediation; thus the data set integrates changes in contamination in time and space. Long-term studies of this sort can be used to reduce covariance among important parameters (e.g., Luoma et al. 2001) and point to cause–effect in circumstances in which it is otherwise ambiguous (e.g., Hornberger et al. 2000; Brown et al. 2003). We assess the universality of the calibration by comparing dose–response in the Clark Fork against bioavailable exposure and community

* To whom correspondence may be addressed: snluoma@ucdavis.edu
Published on the Web 11/17/2009.
DOI: 10.1897/IEAM_2009-067.1

change observed in smaller data sets from the Phillipines (David 2003), and Spain (Sola et al. 2004).

For this study, we define a biomonitor as an organism from the field that accumulates trace metals in its tissues. The accumulated metal concentration is a relative measure of the metal taken up by all routes by that organism, integrated over a preceding time period. The accumulated metal concentrations, by definition, reflect external influences that affect bioavailability, such as environmental chemistry. They also reflect the integration of the array of internal factors that define uptake by that species. This may result in a different absolute internal exposure in one species from one that may occur in other species in the same habitat, although changes in exposure as a function of bioavailable concentration are generally correlated among species (Luoma and Rainbow 2008).

In contrast, a bioindicator is an organism whose adverse response to metals in the field is used as an indicator of stress. In some applications, exposure (determined by bioaccumulated metal) and response are determined in the same bioindicator (Salazar and Salazar 1995). A biomarker is a biological response (e.g., a biochemical, cellular, physiological, or behavioral variation) that can be measured at the lower levels of biological organization, in tissue or body fluids or, perhaps, at the level of the whole organism (Luoma and Rainbow 2008). Calibration is defined as quantification of dose–response, and thereby effect concentrations, under field conditions. The effect concentration is defined by the concentration of bioaccumulated Cu in *Hydropsyche* above which simplification of the composition of benthic communities occurs. The successful calibration must be generally consistent across several field sites. It should also be sufficiently quantitative to identify differences in sensitivity among different ecological measures of effects. Causation is addressed, in part, by focusing on responses that are recognized as mechanistically diagnostic of metal effects, and in part by an interpretive approach that recognizes data patterns reflecting the influence of a single stressor in a multistressor environment (Luoma et al. 2001).

JUSTIFICATION

Traditional toxicity-testing methodologies were developed to provide unambiguous dose–response data that regulators and risk assessors could use to establish clear ''effects concentrations'' for natural waters. Toxicity tests carefully control potential confounding factors and can be standardized to derive comparable data among different experiments and experimenters. The existing toxicity-testing databases for metals are dominated by tests of acute toxicity. This is the simplest, most practical, and least ambiguous approach to experimentally defining toxicity. These widely used tools also have widely recognized limitations, however. In particular, substantial uncertainty exists about the accuracy of extrapolations to nature of the dose–responses observed in experimental data (Luoma 1995). Relatively arbitrary application factors are usually considered necessary to avoid underprotecting nature (Cairns and Mount 1990). Validation of the dose–response curve observed in toxicity tests against what actually happens in nature (with or without an application factor) is also difficult (e.g., Luoma and Rainbow 2008).

Despite the uncertainties in toxicity-testing-driven assessments of ecological risk, the common view since the 1950s has been that there are few acceptable alternatives. Formal definitions of toxicity data acceptable for use in the regulatory arena often specify exclusion of field data (US Environmental Protection Agency [USEPA] 2001). Historically, the arguments have been that nature was too complex, too time consuming, and too expensive to understand (Cairns and Mount 1990). It is also argued that predictive tools cannot be developed from observations of nature (Adams and Rowland 2003). More specifically, the challenges in field evaluations of metal toxicity lie in (a) finding a consistent definition of exposure in systems where bioavailability may vary, (b) detecting sensitive effects endpoints, and (c) determining causation where multiple stressors (or influential factors) may be at work. If we are to move to a lateral approach that takes advantage of field data, one challenge is to develop quantitative and robust dose–response relationships from nature that account for these difficulties (e.g., Ohlendorf 2003).

Understanding the influences of bioavailability in natural waters remains a challenge. Models like the biotic ligand model (BLM; Niyogi and Wood 2003) quite effectively account for speciation and bioavailability in toxicity testing data. The BLM predictions have been validated in toxicity tests with different waters from nature (De Schamphelaere et al. 2005), but are not fully validated in situ. One reason is that dietary exposures are unaccounted for in the BLM, but uptake from food is an important route of exposure and can contribute to toxicity (Hook and Fisher 2001; Bielmyer et al. 2006). Biodynamic models (Luoma and Rainbow 2005) are an alternative to the BLM. Biodynamics accounts for uptake from all pathways and takes into account physiological and autecological factors that drive bioaccumulation differences among species. To account for speciation, the biodynamic parameters must be derived for particular geochemical conditions, although linkage of biodynamic and biotic ligand models for that purpose is not inconceivable (Croteau and Luoma 2007). But biodynamic models do not yet directly address toxicity, although that is an obvious next step (Martin et al. 2007). Although ecological risk assessments typically call for characterizing exposure and effects, no modeling approach has yet been developed that quantitatively links exposure to bioavailable metal from multiple routes to adverse effects in a natural setting.

The ''tissue residue'' approach was originally proposed as a solution to the lack of explanatory models, particularly for organic contaminants. One critical body concentration of each contaminant was thought to define the onset of adverse effects in all organisms. The critical-body-concentration concept has some serious limitations when applied to metals (e.g., Borgmann et al. 1991). First, for most metals, toxicity occurs at different tissue concentrations in different species. The reason for the differences among species is the tendency for metals to accumulate in a form that has been immobilized so that it cannot participate with or interact with normal biological functions (i.e., metal that is detoxified). Other fractions of metal occur that are available metabolically (bioactive) and play an essential role or a toxic role in biochemistry (Rainbow 2002). For example, trace metals that behave as cations associate with glutathione or metallothionein-like proteins and are sequestered into lysosomes to prevent toxicity. Phosphate (Zn, Pb) and sulfide (Cu, Ag) granules can also be sequestered inside or outside cells, and accumulate, but away from the cell machinery where they may cause harm. The mechanisms of detoxification differ among metals and metalloids, and the degree of detoxification can differ widely among species. Thus, different species accumulate detoxified metal forms to a widely different degree (e.g., Buchwalter et al. 2007) and the differences among species vary among metals and metalloids.

In addition to detoxification, uptake rate and route can affect the concentration of metal in tissues at the onset of signs of toxicity. For example, Ag adsorbs to the surface of organisms when exposure is by means of dissolved metal but is taken up internally when exposure is by means of food (e.g., Hook and Fisher 2001). Toxicity to reproduction occurs at a much lower metal concentration in tissues when Ag is accumulated from food, as compared with when it is accumulated from water. The occurrence of metal toxicity is also rate dependent, not burden dependent. Organisms that detoxify metal as fast as it is accumulated will not suffer toxic effects at a total body concentration as low as organisms that allow biochemically active metal to build up (Rainbow 2002; Luoma and Rainbow 2005). One result is that the tissue concentration that correlates to the onset of toxicity may be different in laboratory exposures compared with the field, even in the same species. This is because most toxicity tests take advantage of the high-exposure-concentration and low-exposure-duration approach for practical reasons. But, at high concentrations, toxicity occurs when excretion and detoxification cannot keep up with the extremely rapid uptake rate associated with an extreme dose. In these instances, toxicity can be manifested at lower concentrations of metal in tissues (little metal in the detoxified fraction) than in a chronic exposure situation (Croteau and Luoma 2009).

Another limitation when applying the traditional bioindicator approach under field conditions is that populations that express biomarker responses to metals in nature will be extirpated if that stress becomes too severe (the population disappears). Thus the expectedly most useful organisms from which to determine if local bioavailability has reached a physiologically critical threshold are those that are no longer present in the community. In streams, for example, some species of mayflies have a tendency to bioaccumulate the highest concentration of bioactive metal but also to disappear first in metal-contaminated situations (Cain et al. 2004; Buchwalter et al. 2007).

Examples do exist where bioaccumulated metal concentrations in a *bioindicator* species can be correlated to the onset of adverse effects in that species in the field. In general, the bioindicator approach may be well suited for in situ bioassays. For example, metal bioaccumulation in carefully managed transplantations to contaminated natural waters of mussel larvae can be correlated to effects on sublethal processes such as growth (Salazar and Salazar 1995). Bioaccumulation determined in one surviving life stage (the biomonitor) may also be related to toxicity to a more sensitive life stage (the bioindicator). An example is the correlation of selenium concentrations in the tissues of an adult organism with the onset of reproductive damage such as failure of eggs to hatch or teratogenesis (e.g., Ohlendorf 2003). Bioaccumulated tissue concentrations of Se, Cu, and Ag in adult survivors also have been related to sublethal reproductive effects (failure of gonads to mature) when metal-tolerant individuals immigrate to a site, and thereby made up for the lack of local recruitment (e.g., Palace et al. 2007; Hornberger et al. 2000). If the bioindicator approach is applied to species able to tolerate a wide range of exposures, however, the concentration that might cause stress to other, more sensitive, species will be underestimated. Thus, in general, the window of opportunity to apply the bioindicator approach, especially to metal-sensitive species or processes, is narrow.

THE CALIBRATED BIOMONITOR

The less explored alternative is to calibrate bioaccumulated metal concentrations in a widespread, metal-tolerant species to effects measured in more sensitive species or processes from the same area. Once a dose–response relationship is established from these 2 data sets, then critical thresholds can be defined and perhaps extrapolated to other environments. The concept is to use the widespread biomonitor species as a chemical probe for bioavailable metal. In theory, then, bioavailable metal can be related to any measure of effect.

Both freshwater and marine or estuarine species have been identified that have the requisite, long-known characteristics of a useful biomonitor and can be used to monitor bioavailable metal (e.g., Bryan et al. 1980; Phillips and Rainbow 1994; Hare 1992). A single, cosmopolitan (widespread) species makes the most useful biomonitor. The species must be a net bioaccumulator of metals, as defined by Rainbow (2002). It must be tolerant to a range of conditions and to the metal contaminants of interest, so it is present at metal bioavailabilities that cause sensitive species to disappear. And, like a good chemical probe, it should be feasible to use. That means the species should be widespread in sufficient numbers to be feasible to collect with the proper taxonomic specificity. Rigorous application of robust collecting, handling, and sample preparation protocols is as important in using a biomonitor as it is with any chemical probe (e.g., collecting, filtering, and analyzing an ultraclean water sample). Inexperienced practitioners must learn proper technique, but the techniques are feasible to learn and use routinely.

Metal bioaccumulation by a biomonitor can be affected by animal size, undigested gut content, gender, reproductive stage, seasonal growth cycles, and other attributes causing seemingly stochastic variability among individuals (Brown and Luoma 1995). For the data collected in this study, protocols were used that were developed over the years to minimize each of these sources of variability (e.g., Bryan et al. 1980; Strong and Luoma 1981; Cain et al. 1992; Phillips and Rainbow 1994). For example, samples can be corrected for size by collecting individuals from a range of sizes and interpolating to a commonly occurring size (Cain et al. 1992; Brown and Luoma 1995). Gut content, which is a bias in some cases but not others, can be countered by depuration (Brown and Luoma 1995) and/or by quantifying its influence in a specific environment (Cain et al. 1995). Brown and Luoma (1995) examined sources of variability in use of the bivalve *Corbula amurensis* as a biomonitor in San Francisco Bay. They found that temporal variability in monthly collections had a coefficient of variation of 10–12%; small scale spatial variability (within a 3-km grid) showed variability of 10–25% and large scale spatial variability (where exposures were similar) varied 3–12%. Precision and statistical power improve with the number of individuals analyzed. The critical threshold for statistical power occurs between 15 and 20 individuals of the same species from the same site (Gordon et al. 1980). Brown and Luoma (1995) and Cain et al. (1992) greatly reduced variability by analyzing even larger numbers of individuals (30–120) per sample. They reduced analytical cost and interpreted size effects by compositing animals of similar size. The important point is that attention to robust biomonitoring protocols can result in sufficient statistical power to differentiate concentrations at which effects occur.

CASE STUDY: THE CLARK FORK RIVER, MONTANA

The metal-contaminated Clark Fork River is an intensively studied river affected by a long legacy (since circa 1850) of mining-related metal contamination. Mine wastes have created an extensive longitudinal gradient in metal concentrations in water, bed sediments, and organisms, extending from Silver Bow Creek to the terminus of the Clark Fork in Lake Pend Orielle (Moore and Luoma 1990). Tailings ponds were completed at the mouth of Silver Bow, Mill, and Willow Creeks in the 1950s, approximately 100 y after mining began. Further remediation of floodplain contamination below the tailings ponds began in 1995 and is ongoing (Hornberger et al. 2009). The historic metal contamination was accompanied by an extreme degradation of the fish and benthic invertebrate communities in the river before tailings ponds were constructed in the 1950s (Moore and Luoma 1990). Communities have been progressively recovering since the 1950s with particular progress in the most affected upstream reaches in the last decade (Cain et al. in preparation). Like many mining sites, several metals characterized the contamination in Clark Fork sediments, including Cu, Cd, As, Pb, and Zn. A USEPA ecological risk assessment for the lower Clark Fork (USEPA 1999) concluded Cu was the metal most likely to cause adverse effects. The calibration presented here emphasizes Cu, because preliminary analyses showed that bioaccumulated Cd and Zn do not correlate in a simple manner with community change. If effects occur from Pb or As (Hansen et al. 2004), they are probably more localized than for Cu, because of the more restricted occurrence of these contaminants (e.g., Hornberger et al. 2009).

METHODS

Detailed methods for collection and analysis of bioavailable Cu concentrations are described in detail by Cain et al. (1992, 2004) and Hornberger et al. (2009). The cosmopolitan biomonitors *Hydropsyche cockerelli* and *H. occidentalis* (caddisfly larvae) were present throughout the river as well as in tributaries. *Hydropsyche* spp. were well suited as biomonitor species in the Clark Fork River. They occur in riffle habitat in sufficient abundance for collection across the contamination gradient in the Clark Fork system. Most years, they could be found at a site in the most contaminated of the tributaries that form the Clark Fork, and they were in evidence every year in other tributaries through the upper Clark Fork to its confluence with the Blackfoot River (and even further downstream, see Cain et al. 1992). At each site, animals were sufficiently abundant for collection of at least 20 and usually several hundred individuals. Relationships between metal uptake and animal size as well as gut content have been explored elsewhere (e.g., Cain et al. 1995), and, as in the case of any interspecific differences (Hornberger et al. 2009), these factors had little influence on interpretations.

Bioaccumulation was determined in *Hydropsyche* spp. once per year in August from 1986 through 2003 (the latest ecological data available). Early studies confirmed that interspecific variation in bioaccumulated metal concentrations between the 2 dominant species (*H. occidentalis* and *H. cockerelli*) was insignificant. Therefore, data were combined for the 2 species (for exact details, see Hornberger et al. 2009; Cain et al. 1992; Cain et al. manuscript in preparation). These data are hereafter referred to as biomonitoring data, as defined above (our definition should not be confused with a different use of the same term in the context of bioassessment).

The benthic macroinvertebrate community has been monitored since 1986 with samples collected in August of each year. Data were issued in annual reports prepared by McGuire (2004) for the State of Montana (and now for the USEPA). We compiled these data and the bioaccumulation data into a common database. Cain et al. (2004; in preparation) interpreted the changes in space and time that have occurred in both metal contamination and community ecology in this complex, recovering river system. In this study, we use indices of community structure as calculated in these 3 papers (Cain et al. 1992, 2004; Hornberger et al. 2009). Four samples of invertebrates were collected at each riffle with a modified Hess sampler ($0.1\,m^2$) fitted with a 1-mm mesh net, as reported by McGuire (2004). All Ephemeroptera, Plecoptera, and Trichoptera were separated and enumerated at the species level, although some species were listed as *Genus* sp. Unless otherwise noted, metric values were the mean of the 4 replicate samples. Metrics evaluated in the study included total number of macroinvertebrate species (species richness), the number of species present in the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT), the number of species present in the order Ephemeroptera (mayfly richness), the number of species present in the mayfly family Heptageniidae, and the total number of individual Heptageniidae (abundance). Several studies show that some species of larval mayflies are particularly vulnerable to adverse effects from metal contamination (e.g., Clements 2000; Cain et al. 2004). More recently, the mechanistic basis of that vulnerability has emerged (Buchwalter et al. 2007). Thus, the number of larval mayfly species is thought to be a sensitive measure of metal stress (Winner et al. 1980). In particular, heptageniid mayflies are in a family well known to be especially vulnerable to metals and, therefore, diversity and abundance within this group are indicators of metal stress (Clements 2004; Cain et al. 2004; Buchwalter et al. 2007). The index of biological integrity (IBI) was also calculated by McGuire (2004). It is designed to integrate effects of different environmental stressors, and to provide a measure of the overall ecological condition of a site. The index is based on the composite score of 10 biological metrics and expressed as a percentage of the maximum possible score (McGuire 2004).

The data for the present analysis were restricted to 5 sites on the Clark Fork River where both long-term benthic community and long-term biomonitoring data were collected in the same year, in proximity to one another, and in similar types of habitat (Figure 1). Similar data were also used from 3 relatively uncontaminated tributaries, Rock Creek, the Little Blackfoot, and the Blackfoot Rivers, and 1 highly contaminated site on Silver Bow Creek. The Clark Fork is formed at the confluence of creeks: Silver Bow, Mill, Willow, and Warm Springs Creeks. Data from these sites were available from most years between 1986 and 2003. Thus both the ecological data and the biomonitoring data represent both spatial trends in each year and temporal variability among years across 9 sites on the river. At the extremes (which included Silver Bow data), metal contamination followed an upstream to downstream trend in every year. But within the intervening reach of the Clark Fork itself, the spatial gradient changed from year to year. The temporal variability was often different

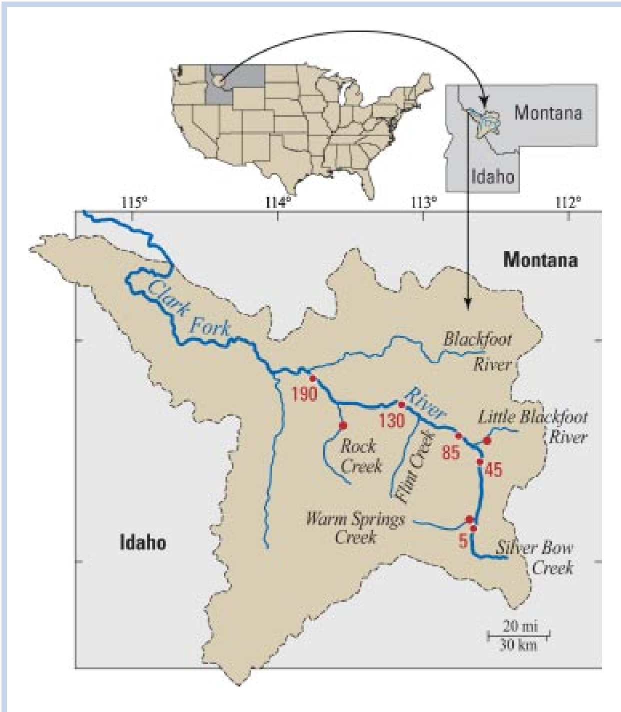

Figure 1. Map of the Clark Fork River, Montana. Numbers are km from the head of the Clark Fork River.

among sites (Cain et al. in preparation). Because site-specific changes in time were large and occurred differently at different locations, covariances in the data set were reduced.

RESULTS

Copper concentrations in Hydropsyche spp.

Hydropsyche spp. are strong bioaccumulators of metals, as evidenced by the range of concentrations in their tissues in the Clark Fork watershed. Copper concentrations in *Hydropsyche* spp. varied from 11 to 900 μg/g dw across the watershed. This is the range of concentrations that is observed in the global literature for hydropsychid caddisflies (see Luoma and Rainbow 2008).

The lowest bioaccumulated Cu concentrations occurred in tributaries unaffected by mining. Copper concentrations in *Hydropsyche* spp. ranged from 11–21 μg/g dw in 17 collections from Rock Creek, the Little Blackfoot, and the Blackfoot River. Concentrations in the mainstem of the Clark Fork varied from 34–158 μg/g dw among 93 collections from the 5 stations between the 1986 and 2003. In general there was an upstream to downstream gradient in most years. But there was nearly as much year to year variation at individual stations as there was variation among stations. For example, at 4.7 km from the head of the river, bioaccumulated Cu varied from 51 ± 2 μg Cu/g dw in 1998 to 148 ± 9 μg Cu/g dw in 1987. At the site 85 km from the head of the river, the range of concentrations in 16 different years was from 23 ± 9 μg/g dw in 2001 to 158 ± 27 μg/g dw in 1997. A trend of declining concentrations in time was noted at the stations 4.7 km and 45 km from the head of the river. Temporal trends at stations further downstream were more complex (Cain et al. in preparation; Hornberger et al 2009).

The highest Cu concentrations in *Hydropsyche* spp. were found from Silver Bow Creek. Mean Cu concentrations ranged from 295–986 μg/g dw in 7 collections between 1992 and 2003. *Hydropsyche* species were not present at this site every year and were in relatively low abundance when they were present. In 4 collections from Warm Springs Creek, mean bioaccumulated Cu concentrations varied from 95 to 130 μg/g dw.

Benthic community structure

The structure of the benthic community in the Clark Fork and its tributaries also varied widely over the course of the study. The number of EPT species varied from 20–24 in Rock Creek, and 18–24 in the Blackfoot River and Little Blackfoot Rivers (streams with the lowest bioavailable Cu concentrations). As few as 2.5 EPT species (on average) were found in Cu-contaminated Silverbow Creek and 11–26 in the Clark Fork. The number of macrobenthic species varied from 30–48 in the uncontaminated streams during the 18 y of study. From 5–19 invertebrate species were found in Silver Bow Creek depending upon the year. From 25–50 species were found in the Clark Fork among all stations and times. Variation in macrobenthic species richness from year to year at each station in the Clark Fork was as great as variation from station to station.

The number of mayfly species in the uncontaminated tributaries varied from 5–10. In Silverbow Creek, there were no mayfly species in some years and 1 in others. In the Clark Fork, the number ranged from 1–10 over time and space. Multiple collections within each site yielded a maximum of 1–4 heptageniid mayfly species in each net at the uncontaminated tributaries over the 18 years of study. For example, 3 or 4 heptageniid species were found every year in Rock Creek. Only twice was 1 individual of a species of heptageniid found in highly contaminated Silverbow Creek, by contrast. In the Clark Fork, the range was 0–4 species over time and space. The abundance of heptageniids ranged from 2–38 individuals per year in the uncontaminated environments. The range in the Clark Fork was 0–45.

Calibrating the biomonitor

Copper concentrations in *Hydropsyche* spp. did not correlate significantly with the variability in any measure of community structure in the uncontaminated tributaries alone ($p < 0.05$). For example, 8–10 species of mayflies, and 5–38 individuals from the mayfly family Heptageniidae were found in Rock Creek in 5 different collections (Figure 2a,b). There was no relationship within this stream between bioavailable Cu and either measure of community structure. Of course, Cu would not be expected to affect heptageniids at these low concentrations. But the lack of a correlation with bioavailable Cu is important in that it indicates that variation in heptageniid abundance (for example) did not correlate with basinwide environmental conditions that might covary with Cu bioavailability in an uncontaminated situation (e.g., annual variability in rainfall and runoff, interannual differences in growth or instar stage of *Hydropsyche* spp.).

When all the data were considered, bioaccumulated Cu in *Hydropsyche* spp. was strongly related to every measure of benthic invertebrate community structure ($p < 0.01$; Table 1). Bioavailable Cu explained 48–61% of the variation in the community. Although we do not address the question

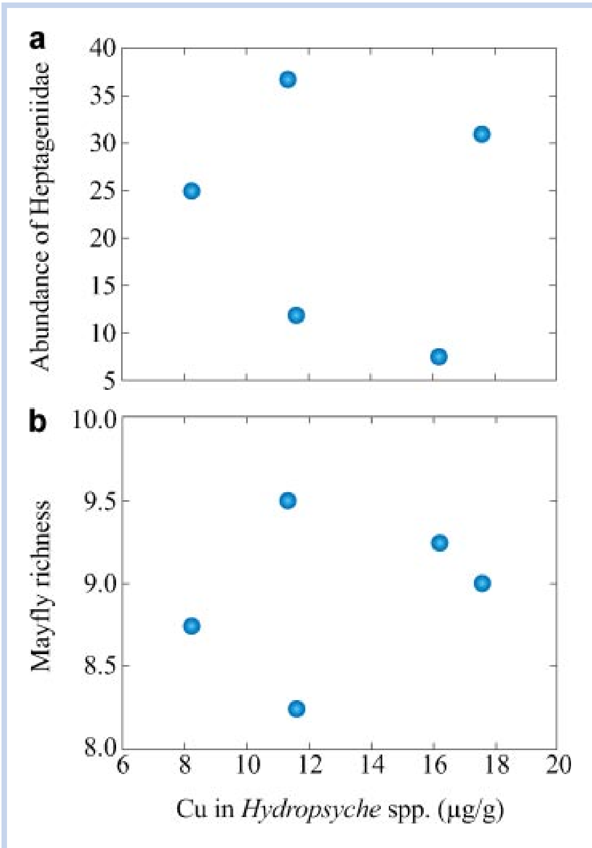

Figure 2. Abundance of heptageniid mayflies (number of individuals per 0.1 square meter) (**a**) and the total number of mayfly species (mayfly richness) (**B**) in August of 1993–95 and 1997–98, as observed in 4 replicate Hess samples, as a function of copper concentrations in the biomonitor *Hydropsyche* spp. from the uncontaminated tributary Rock Creek, in the Clark Fork watershed.

in this study, other possible causes of the changes in community structure (temperature, hydrology, stream gradient, Cd, Zn) could not explain the variance in this data set to the degree that metals did (Cain et al. in preparation). The distribution of data in the relationship with bioavailable Cu also followed a pattern more indicative of a dose–response curve than of a simple linear regression (Figure 3). The total number of benthic macroinvertebrate species (species richness; Figure 3c), the total number of mayfly species (mayfly richness; Figure 3b) and the number of individual heptageniids per square meter (Figure 3a), for example, were always very low in Silverbow Creek, where bioavailable Cu ranged from 295 μg/g to 986 μg/g. The benthic community was always extremely disturbed, by any measure, when bioavailable contamination reached extreme concentrations.

When the data from the highly contaminated Silverbow Creek were excluded, correlations with community structure remained statistically significant ($p < 0.05$) (Table 1). The data distribution was complex for the 2 variables most diagnostic of metal effects: number of heptageniid species (Figure 4a), and the number of individual heptageniids (Figure 4b). But the relationship was what might be expected from a regression against a single influential variable in an environment where multiple variables are important. Data distributions such as these are common where one environmental stressor imposes a cap on a response, but other variables are influential below the threshold of effect (Luoma 1996; Cade and Noon 2003; Ramsey et al. 2005; Luoma and Rainbow 2008). Heptageniid abundance, for example, ranged from 48–0 in environments where bioaccumulated Cu was less than about 60 μg/g dw in *Hydropsyche* spp., indicating that factors other than Cu drove abundance of these organisms in this exposure range. But never more than 10 heptageniids were found in environments where Cu bioaccumulation exceeded about 80 μg/g dw. Copper appeared to cap the number of heptageniids that could survive. The latter results were not biased by any individual site. At least 1 collection from every site on the Clark Fork River was represented within the data set where Cu bioaccumulation was in excess of 80 μg/g dw. The important point in this case is that the biomonitor's measure of bioavailable metal allowed characterization of the complex relationship with Cu in one of the most Cu-sensitive groups of organisms in the river.

The consistent measure of bioavailable exposure also allowed us to test whether differences in sensitivity existed among the different measures of community response. We chose the 80th percentile of all data sets as typical of the value at no effect, then calculated, from a linear regression, the metal concentration in *Hydropsyche* spp. that was present when the community measure was half of that 80th percentile value (Table 1). This relatively crude approach suggested that mayfly richness was approximately twice as sensitive to disturbance by Cu as were benthic macroinvertebrate species richness, the IBI or EPT. Measures of the heptageniid mayfly community may have been more sensitive than was total mayfly richness (Table 1).

Applying the calibration to other environments

An important question is whether there is some universality to the metal concentrations in a biomonitor at which adverse effects begin. Any effects of metals, of course, will be superimposed upon a site-specific baseline of species richness (or EPT, IBI, mayfly richness, etc.) established by local conditions (habitat, availability of recruits, etc.). But there might be some universality in the bioavailable metal concentration (as defined by the biomonitor) at which conditions deviate from the baseline. There is no data set comparable to the Clark Fork in terms of the density of data across a range of conditions, but there are indications from other data sets that the thresholds in the Clark Fork have at least some applicability elsewhere.

Sola et al. (2004) investigated the recovery of the Guadiamar River in Spain after a large tailings spill. Their published data included determination of metal concentrations in species of *Hydropsyche* (no species name given) and benthic community data. Using PCA analysis they divided the river into upstream control stations not affected by current mining activities (control group 1), a severely polluted section close to the mine (group 2), and a more distant recovering section (group 3). The ecological data published from the Guadiamar are not directly comparable to the Clark Fork. But it was clear that there was a relationship between impairment and Cu concentrations in *Hydropsyche* sp. (Table 2). Mayflies were well represented where Cu concentrations averaged 22.6 μg/g dw in *Hydropsyche* (Control Group 1). Four families of Ephemeroptera and a mean of 184 individuals per family occurred among these 3 control sites. Where Cu concentrations in *Hydropsyche* averaged 95 μg/g dw Ephemeroptera were represented by only 1 family and a

Table 1. Coefficients of determination and the slope for linear regressions of bioaccumulated copper concentrations (μg/g dw) in *Hydropsyche* spp. versus different measures of community structure in the Clark Fork River, MT[a]

Cu concentration in *Hydropsyche* versus:	R	R^2	Slope	80th Percentile	Cu concentration at 1/2 80th percentile
IBI	−0.69	0.48	−0.06	94.0	634
without Silverbow	*−0.55*	*0.30*	*−0.24*	*94.0*	*202*
Invertebrate species richness	−0.76	0.57	−0.04	43.5	432
without Silverbow	*−0.39*	*0.15*	*−0.07*	*44.0*	*298*
EPT	−0.78	0.61	−0.03	22.0	386
without Silverbow	*−0.55*	*0.30*	*−0.05*	*22.0*	*219*
Mayfly species richness	−0.69	0.48	−0.01	8.00	278
without Silverbow	*−0.65*	*0.42*	*−0.04*	*8.00*	*128*
Heptageneiid mayfly species richness	−0.44	0.20	−0.0023	2.00	124
without Silverbow	*−0.67*	*0.45*	*−0.02*	*2.00*	*73*
Heptageneiid mayfly abundance	−0.3160	0.0999	−0.0158	11.3	175
without Silverbow	*−0.52*	*0.27*	*−0.13*	*11.3*	*77.9*

[a]The concentration of copper in *Hydropsyche* at the 80th percentile value in the community data is also presented as a crude approximation of the baseline Cu concentration. Copper concentrations at one-half the 80th percentile are presented as approximations of an effect concentration (EC50) that can be compared among measures of community structure. IBI: 10 is a 10-measure index of biological integrity; Invertebrate species richness is the average number of benthic macroinvertebrate species in 3 replicate 1-square meter samples; EPT is the average total number of Ephemeroptera, Plecoptera, and Trichoptera species per square meter among 3 replicate square meter samples; Mayfly species richness is the average number of mayfly species per square meter; Heptageneiid mayfly species richness is the average number of heptageneiid mayfly species per square meter; Heptageneiid mayfly abundance is the number of individual heptageniid mayflies found in 3 replicate 1-square meter samples). The first line for each measure uses data from the Clark Fork plus data from Silverbow Creek, Warm Springs Creek, the Little Blackfoot River, the Blackfoot River, and Rock Creek. The second line for each measure excludes the highly contaminated Silverbow Creek.

mean of 17 individuals per family (Group 3). No Ephemeroptera were found at the most contaminated site (Group 2). *Hydropsyche* were also absent from that site. The observation from the Clark Fork was that fewer mayfly species occurred as Cu concentrations in *Hydropsyche* spp. increased beyond 100 μg/g dw. This was generally supported in the Guadiamar.

Cadmium concentrations were also more elevated in *Hydropsyche* spp. from the Guadiamar than they are in the Clark Fork. There was no detectable correlation between Cd in *Hydropsyche* spp. and community structure in the Clark Fork data. But Cd in *Hydropsyche* spp. averaged 6.0 μg/g dw with a range of 4.5–6.7 μg/g dw in the recovering zone of the Guadiamar. The highest Cd concentration in the Clark Fork system, excluding Silverbow Creek, was 3 μg/g dw. Additive effects of elevated Cd and Cu could have influenced the threshold of effects in the Guadiamar, driving it down when calibrated against Cu.

David (2003) investigated Cu contamination in the 30-km-long Boac-Makulapnit River on Marinduque Island, the Philippines. This river drains a large porphyry copper mine, and Cu is the main contaminant in the river. A qualitative assessment of riffle zone invertebrate communities based on a rapid bioassessment approach was conducted to provide some indication of the relative occurrence of major aquatic insect groups. David (2003) compared Cu concentrations in *Hydropsyche* spp. (identification was possible only to the genus level for this locality) with benthic community structure in tributaries to the river and in the contaminated mainstem. Two tributary stations were sampled in each of the years 2001, 2002, and 2003. In the uncontaminated upstream Boac River, concentrations of Cu in the biomonitors ranged from 19–23 μg/g dw and averaged 20.5 μg/g dw. In the upstream Makluapnit River, above the mining activity, Cu concentrations in the *Hydropsyche* ranged from 23–39 μg/g dw among the 3 years, averaging 32.8 μg/g dw. In both of these streams, mayflies from 4 families were found: Baetidae, Ephemerellidae, Leptophlebiidae, and Heptageniidae.

No macrobenthic fauna were found closest to the inputs from mining. Copper concentrations in *Hydropsyche* spp. at the locations where they were first found were 980–1361 μg/g dw in different years. This is similar to the maximum concentration tolerated by *Hydropsyche* spp. in Silverbow Creek. No mayflies were found where this much Cu occurred in *Hydropsyche* spp. in the Boac-Makulapnit River, similar to Silverbow Creek. Further downstream from the mine, Cu concentrations in *Hydropsyche* spp. ranged from 187–600 μg/g dw in the Boac-Makulapnit system. Only mayflies from the family Baetidae were found at these sites. Baetidae were also the 1 family of mayflies that occasionally was observed in the Clark Fork, where Cu concentrations exceeded 100 μg/g dw in *Hydropsyche* spp. Other families, including the Heptageniidae, were not present in either system when concentrations in the biomonitors were in excess of 100 μg/g dw.

It is premature to suggest precise thresholds for Cu concentrations in hydropsychids that are indicative of effects elsewhere in benthic communities from mine-impacted rivers. But in all 3 Cu-rich rivers from which the appropriate data is available, a Cu bioavailability represented by about 100 μg/g dw bioaccumulated in *Hydropsyche*, perhaps combined with a Cd bioavailability represented by about 6 μg/g dw bioaccumulated Cd, is associated with a substantial degradation in the number and types of mayfly species.

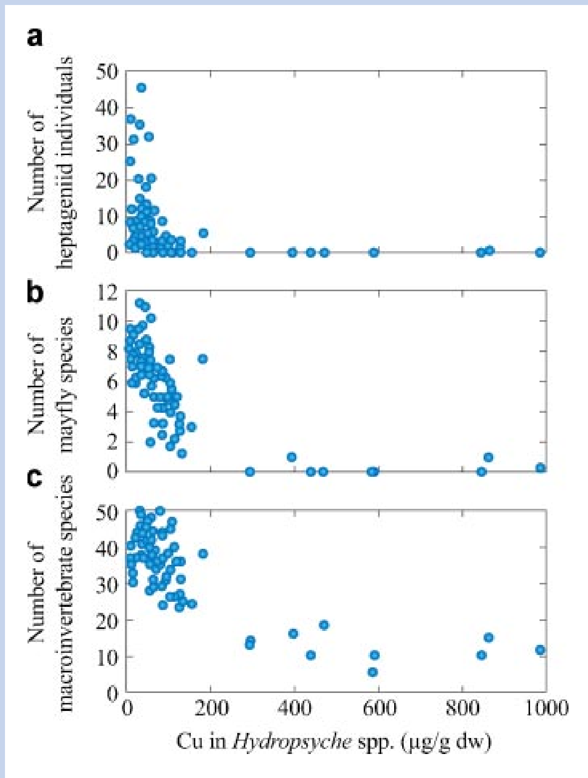

Figure 3. Three measures of ecological community structure from the Clark Fork River, Silverbow Creek, and 3 tributaries of the Clark Fork, as a function of copper concentrations in the biomonitor *Hydropsyche* spp. The ecological measures are **a**) average among 3 replicates of the abundance of individual heptageniid mayflies (number found in 0.1 square meter), **b**) average number of mayfly species (mayfly richness) found in 4 replicate 0.1 square meter Hess samples, and **c**) average number of benthic macroinvertebrate species (invertebrate species richness) found in 4 replicate 0.1 square meter samples. Samples were collected from Clark Fork, Silverbow and other tributary sites repeatedly over a period of 15 years in August of each year.

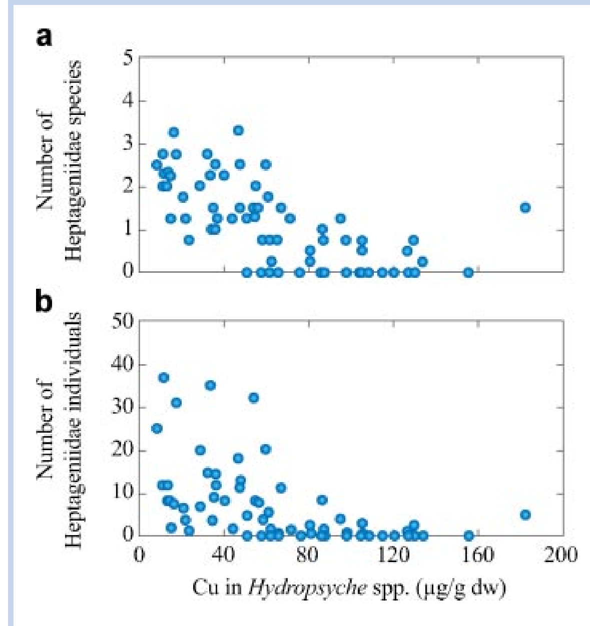

Figure 4. The average number of heptageniid mayfly species (**a**) and number of heptageniid individuals per 0.1 square meter (**b**) as observed in 4 replicate Hess samples from each site, as a function of copper concentrations in the biomonitor *Hydropsyche* spp. at that site. Data from Silverbow Creek were excluded from this figure. Samples were collected from the same sites repeatedly over a period of 15 years in August of each year.

DISCUSSION

Understanding of the biological, ecological, and geochemical processes that influence metal fate and effects in natural waters has developed rapidly in recent years. New tools are beginning to explain bioaccumulation in either mechanistic or operational ways (Luoma and Rainbow 2008). Better chemical models of speciation are also available, as are better operational methods for addressing bioavailable metal from dissolved phases (Buck and Bruland 2005). Biodynamic models allow integration of geochemical data with physiological traits of each species and species-specific responses to metal exposure from diet and water in typical situations (Luoma and Rainbow 2005; Buchwalter et al. 2007). Nevertheless, few of those tools are yet fully calibrated against apparent metal toxicity in nature. Metal bioaccumulation in biomonitors is a fully operational methodology. Moreover, it appears that calibration against metal stress responses in nature is feasible.

Clements (2004) stated that poor mechanistic understanding of ecological responses limited incorporating ecological observations into assessing risks from metals. In recent years, that understanding has advanced in important ways. It is now clear that:

1) Metals manifest their effects ecologically by specifically eliminating metal-sensitive species.
2) Metal sensitivity is partly defined by physiological traits of the species. Those animals for which uptake rates of metal from food or water are high and either rate constants of loss or detoxification capabilities are low, are most likely to both bioaccumulate the highest concentrations of bioactive metals (that fraction most likely to induce

Table 2. Bioaccumulated Cu concentrations (µg/g dw) in *Hydropsyche* spp. and measures of benthic invertebrate community structure in the Guadiamar River, Spain (Sola et al. 2004)

	Inverts		Ephemeroptera	
	Cu in *Hydropsyche* (µg/g dw)	Number of families	Number of Families	Mean number individuals/family/station
Group 1	17–30	19	8	184
Group 3	68–125	8	1	17
Group 2	Not present	3	0	0

adverse effects, see Martin et al. 2007) and bioaccumulate that concentration fastest.

3) The latter is important because toxicity occurs when the rate of uptake, summed across all exposure routes, exceeds the rate of loss plus the rate of detoxification (Rainbow 2002). The species that have a tendency to bioaccumulate the highest concentration of bioactive metal in streams are the same species that disappear first in metal contaminated situations (Cain et al. 2004; Buchwalter et al. 2007).
4) This explains why some mayfly species have long been considered good indicators of metal effects in streams (e.g., Winner et al. 1980; Clements 2000, 2004). Many mayflies have the physiological traits that favor rapid bioaccumulation of biologically active metal, and several of them have reduced detoxification capabilities (Buchwalter et al. 2007). Either the number of species or the abundance of individuals of the susceptible mayflies (e.g., the heptageniids) can be considered both diagnostic and sensitive measures for defining effects of metals in an ecosystem (Buchwalter et al. 2007).
5) Large bodies of data are also developing that make it more practical to consider confounding issues in natural waters, allowing us to address cause and effect in increasingly effective ways with regard to metal contamination (Luoma and Rainbow 2008).

In systems where a body of knowledge suggests that Cu has a strong influence on the benthic invertebrate community, Cu concentrations in biomonitors (hydropsychid caddisflies) appear to yield a typical dose–response curve when calibrated against several measures of community structure. The data set for the Clark Fork River is especially well suited for such a calibration. Data sets that are this complete are rare. However, once well calibrated, data from a biomonitor may then be applied to other environments where that biomonitor is present. For example, the very coarse thresholds of effect for the Clark Fork that were determined here were generally consistent with apparent effect levels in contaminated rivers from both Spain and the Philippines.

Approaches to reduce bias and variability have been developed for several biomonitoring species, and metal concentrations in many such species are well known (Table 3). Comparable biomonitoring data are available from nearly every kind of aquatic environment (e.g., Luoma and Rainbow 2008). Such data facilitate, at a minimum, comparative interpretations of the degree of bioavailable contamination from the different environments. Notable biomonitor species from which such comparisons are possible from estuarine and coastal environments include seaweeds (e.g., *Fucus vesiculosus*), tellinid bivalves (*Macoma* spp., *Scrobicularia plana*), barnacles of various species, and talitrid amphipod crustaceans (e.g., *Platorchestia platensis*) (Luoma and Rainbow 2008). Particularly large data sets for mussels (e.g., *Mytilus edulis*) and oysters are available from coastal waters of the United States (O'Connor 1996). *Chaoborus* sp. is an insect larva that occurs widely in the water column of metal contaminated (and uncontaminated) lakes and is used as a biomonitor (Hare 1992; Croteau et al. 1998), as are yellow perch (Campbell et al. 2003). Among the benthos of lakes, species of the alderfly genus *Sialis* (Roy and Hare 1999) have been used. Understanding of the metal bioaccumulation in these species is well developed (Hare 1992). Like the caddisflies from the genera *Hydropsyche* or *Arctopsyche* (e.g., Kiffney and Clements 1994; Cain et al. 1992; Maret et al. 2003), the (nearly) full range of possible bioaccumulated concentrations from such species is known, from the most contaminated to uncontaminated environments. The range of potential bioaccumulation within biomonitoring species is also large (see examples in Table 3), allowing a wide dynamic range for interpretation of the degree of contamination and effects. It seems possible that every one of these species could be calibrated, like any other probe, against a stress response, where a sufficient range of contamination exists.

Table 3. Ranges of metal concentrations (μg/g dw) in some examples of cosmopolitan biomonitors from field sites with different levels of contamination (from Luoma and Bryan 1982; Luoma and Rainbow 2008)

	Silver		Copper	
Biomonitors	**Typical**	**Highest**	**Typical**	**Highest**
Mussels				
Mytilus edulis	0.1–1	17	3–20	50–60
Perna viridis			10–30	220
Oysters				
Crassostrea virginica	0.2	8	6–55	1,000+
Crassostrea gigas	1.6	46	80	5,110
Barnacle				
Balanus Amphitrite	0.7	23	50–60	3,500
Tellinid bivalves				
Macoma balthica	0.2–1	120	15–50	220
Scrobicularia plana	0.1–5	260	10–60	750
Polychaete				
Nereis diversicolor	0.3–0.7	4	12–40	830
Caddisfly				
Hydropsyche spp.			<20	>900

More investigation is necessary to clarify the precision of thresholds defined by biomonitor species. But in environments where hydropsychid caddisflies are abundant, the existing literature suggests it is possible to pose the following null hypotheses:

1) The vast majority of mayfly species will not survive conditions in which Cu bioavailabilities cause bioaccumulated Cu concentrations in hydropsychid caddisflies to exceed 200 μg/g dw.
2) Sensitive mayflies, such as those in families Heptageniidae, will be greatly reduced in abundance, if not extirpated, where Cu concentrations in hydropsychids approach 100 μg/g dw.
3) The total number of benthic macroinvertebrate species (species richness), EPT, and probably other general indices will show indications of dramatic community simplification when Cu concentrations in hydropsychids exceed 200 μg/g dw, but these are less sensitive measures

than those restricted to metal-susceptible taxa. When calibrated, broad measures of community structure like species richness and EPT appear to be approximately 2-fold less sensitive than more metal-specific measures of potential effects.

4) Where metal concentrations are extreme, and hydropsychids are absent, it is likely that metals can be implicated if the community structure is also extremely simplified.
5) While the measures used in this study are diagnostic of metal toxicity, in that they focus on metal-sensitive taxa, we cannot discount secondary processes (e.g., loss of predators) as contributing to the community changes.

It is surprising that so few data sets are published with simultaneous collection of stream community structure and biomonitoring data. The advantages of both approaches have been known for decades. Used together in appropriate ways, these 2 types of data help address 2 of the most important challenges in assessing risks from metal contamination. Sensitive cause and effect interpretations can be narrowed by focusing on aspects of community structure known to be especially sensitive to metals. Questions about bioavailability in different conditions can be addressed by using a widespread biomonitoring species. Here we have shown that bioaccumulation of Cu by the biomonitor can be calibrated to metal-specific adverse responses in the community. Moreover, it appears that the calibration works at least in a general sense across widely different stream communities, as long as hydropsychid caddisflies are present. Such comparisons are only possible where bioavailability is accounted for; hence the advantage of the biomonitor. Specifically for hydropsychid biomonitors, we have posed refutable hypotheses that others might test in other environments, to assess the degree to which the thresholds proposed here are generic.

Acknowledgment—Partial support for this work was provided by the John Muir Institute of the Environment, University of California at Davis; The Natural History Museum, London; and the National Research Program, Water Resources Discipline, US Geological Survey. Some of the data were from monitoring programs supported by the US Environmental Protection Agency, Helena, Montana, and the Department of Fish, Wildlife, and Parks, State of Montana. We also thank Dr. CP David for sharing his data from the Philippines.

REFERENCES

Adams WJ, Rowland CD. 2003. Aquatic toxicology test methods. In: Hoffman DJ, Rattner BA, Burton GA, Cairns J, editors. Handbook of Ecotoxicology. Boca Raton (FL): CRC. p 19–45.

Bielmyer GK, Grosell M, Brix KV. 2006. Toxicity of silver, zinc, copper, and nickel to the copepod *Acartia tonsa* exposed via a phytoplankton diet. *Environ Sci Technol* 40:2063–2068.

Borgmann U, Norwood WP, Babirad IM. 1991. Relationship between chronic toxicity and bioaccumulation of cadmium in *Hyalella azteca*. *Can J Fish Aquat Sci* 48:1055–1060.

Brown CL, Luoma SN. 1995. Use of the euryhaline bivalve Potamocorbula amurensis as a biosentinel species to assess trace metal contamination in San Francisco Bay. *Mar Ecol Prog Ser* 124:129–142.

Brown CL, Parchaso F, Thompson JK, Luoma SN. 2003. Assessing toxicant effects in a complex estuary: A case study of effects of silver on reproduction in the bivalve, *Potamocorbula amurensis*, in San Francisco Bay. *Int J Hum Ecol Risk Assess* 9:96–119.

Bryan GW, Langston WJ, Hummerstone LG. 1980. The use of biological indicators of heavy metal contamination in estuaries. *Occasional Publ Mar Biol Assoc* 1:1–73.

Buchwalter DB, Cain DJ, Clements WH, Luoma SN. 2007. Using biodynamic models to reconcile differences between laboratory toxicity and field biomonitoring with aquatic insects. *Environ Sci Technol* 41:4821–4828.

Buck KN, Bruland KW. 2005. Copper speciation in San Francisco Bay: A novel approach using multiple analytical windows. *Mar Chem* 96:185–198.

Cade BS, Noon BR. 2003. A gentle introduction to quantile regression for ecologists. *Front Ecol Environ* 1:412–421.

Cain DJ, Luoma SN, Carter JL, Fend SV. 1992. Aquatic insects as bioindicators of trace element contamination in cobble-bottom rivers and streams. *Can Fish Aquat Sci* 49:2141–2154.

Cain DJ, Luoma SN, Axtmann EV. 1995. Influence of gut content in immature aquatic insects on assessments of environmental metal contamination. *Can J Fish Aquat Sci* 52:2736–2746.

Cain DJ, Luoma SN, Wallace WG. 2004. Linking metal bioaccumulation of aquatic insects to their distribution patterns in a mining-impacted river. *Environ Toxicol Chem* 23:1463–1473.

Cain DJ, Luoma SN. in preparation The response of stream benthic invertebrate assemblages to metal exposure in the Clark Fork River, USA.

Cairns J, Mount DI. 1990. Aquatic toxicology. *Environ Sci Technol* 24:154–161.

Campbell PGC, Hontela A, Rasmussen JB, Giguère A, Gravel A, Kraemer L, Kovesces J, Lacroix A, Levesque H, Sherwood G. 2003. Differentiating between direct (physiological) and food-chain mediated (bioenergetic) effects on fish in metal-impacted lakes. *Hum Ecol Risk Assess* 9*:* 847–866.

Clements WH. 2000. Integrating effects of contaminants across levels of biological organization: An overview. *J Aquat Ecosyst Stress Recovery* 7:113–116.

Clements WH. 2004. Small-scale experiments support causal relationships between metal contamination and macroinvertebrate community responses. *Ecol Appl* 14:954–957.

Croteau MN, Hare L, Tessier A. 1998. Refining and testing a trace metal biomonitor (*Chaoborus*) in highly acidic lakes. *Environ Sci Technol* 32:1348–1353.

Croteau MN, Luoma SN. 2007. Characterizing dissolved Cu and Cd uptake in terms of the biotic ligand model and biodynamics using enriched stable isotopes. *Environ Sci Technol* 41:3140–3415.

Croteau MN, Luoma SN. 2009. Predicting dietborne metal toxicity from metal influxes. *Environ Sci Technol* 43:4915–4921.

David CP. 2003. Establishing the impact of acid mine drainage through metal bioaccumulation and taxa richness of benthic insects in a tropical Asian stream (The Philippines). *Environ Toxicol Chem* 22:2952–2995.

De Schamphelaere KAC, Lofts S, Janssen CR. 2005. Bioavailability models for predicting acute and chronic toxicity of zinc to algae, daphnids, and fish in natural surface waters. *Environ Toxicol Chem* 24:1190–1197.

Di Toro DM. 2003. Executive summary. In: Reiley MC, Stubblefield WA, Adams WJ, Di Toro DM, Hodson PV, Erickson RJ, Keating FJ, editors. Reevaluation of the state of the science for water-quality criteria development. Pensacola (FL): SETAC. p xxi–xxv.

Gordon M, Knauer GA, Martin JH. 1980. *Mytilus californianus as a bioindicator of trace metal pollution: Variability and statistical considerations. Mar Poll Bull* 11:195–198.

Hansen JA, Lipton J, Welsh PG, Cacela D, MacConnell B. 2004. Reduced growth of rainbow trout (*Oncorhynchus mykiss*) fed a live invertebrate diet pre-exposed to metal-contaminated sediments. *Enviorn Toxicol Chem* 23:1902–1911.

Hare LA. 1992. Aquatic insects and trace metals: Bioavailability, bioaccumulation and toxicity. *Crit Rev Toxicol* 22:327–369.

Hook SE, Fisher NS. 2001. Reproductive toxicity of metals in calanoid copepods. *Mar Biol* 138:1131–1140.

Hornberger MI, Luoma SN, Cain DJ, Parchaso F, Brown CL, Bouse RM, Wellise C, Thompson JK. 2000. Linkage of bioaccumulation and biological effects to changes in pollutant loads in South San Francisco Bay. *Environ Sci Technol* 34:2401–2409.

Hornberger MI, Luoma SN, Johnson MJ, Holyoak M. 2009. The influence of remediation in a mine-impacted river: Do improvements upstream impact metal trends over large spatial and temporal scales? *Ecol Appl* 19:1522–1535.

Kiffney PM, Clements WH. 1994. Effects of heavy metals on a macroinvertebrate assemblage from a Rocky Mountain stream in experimental microcosms. *J N Am Benth Soc* 13:511–523.

Luoma SN. 1995. Prediction of metal toxicity in nature from bioassays: Limitations and research needs. In Tessier A, Turner D, editors. Metal speciation and bioavailability in aquatic systems. London (UK): John Wiley and Sons. p 609–646.

Luoma SN. 1995. Prediction of metal toxicity in nature from bioassays: Limitations and research needs. In Tessier A, Turner D, editors. Metal speciation and bioavailability in aquatic systems. London (UK): John Wiley and Sons. p 609–646.

Luoma SN. 1996. The developing framework of marine ecotoxicology: Pollutants as a variable in marine ecosystems? *J Exp Mar Biol Ecol* 200:29–55.

Luoma SN. 2008. Silver nanotechnologies and the environment: Old problems or new challenges. Publication #15, Project for Emerging Nanotechnologies, Woodrow Wilson International Center for Scholars and PEW Charitable Trusts, Washington DC. 71 p.

Luoma SN, Rainbow PS. 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. *Environ Sci Technol* 39:1921–1931.

Luoma SN, Rainbow PS. 2008. Metal contamination in aquatic environments: Science and lateral management. Cambridge (UK): Cambridge Univ. 573 p.

Luoma SN, Clements WH, DeWitt T, Gerritsen J, Hatch A, Jepson P, Reynoldson T, Thom R. 2001. Role of environmental variability in evaluating stressor effects (Chapter 5). In: Baird DJ, Burton GA Jr, editors. Ecological variability: Separating natural from anthropogenic causes of ecosystem impairment. Pensacola (FL): SETAC.

Maret TR, Cain DJ, MacCoy DE, Short TM. 2003. Response of benthic invertebrate assemblages to metal exposure and bioaccumulation associated with hard-rock mining in northwestern streams, USA. *J No Am Bentholog Soc* 22:598–620.

Martin C, Luoma SN, Cain DJ, Buchwalter DB. 2007. Cadmium ecophysiology in seven stonefly (Plecoptera) species: Delineating sources and estimating susceptibility. *Environ Sci Techol* 41:7171–7777.

McGuire DL. 2004. Clark Fork River macroinvertebrate community biointegrity: 2003 assessments. Helena, (MT): State of Montana. 42 p and appendices.

Moore JN, Luoma SN. 1990. Hazardous wastes from large-scale metal extraction. *Environ Sci Technol* 24:1279–1285.

Niyogi S, Wood CM. 2003. Effects of chronic waterborne and dietary metal exposure on gill metal-binding: Implications for the biotic ligand model. *Hum Ecol Risk Assess* 9:813–846.

O'Connor TP. 1996. Trends in chemical contamination in mussels and oysters collected along the U.S. coast 1986–1993. *Mar Environ Res* 41:183–200.

Ohlendorf HM. 2003. Ecotoxicology of selenium. In: Hoffman DJ, Rattner BA, Burton GA, Cairns J Jr, editors. Handbook of ecotoxicology 2nd ed. New York (NY): Lewis. p 465–500.

Palace VP, Halden NM, Yang P, Evans RE, Sterling G. 2007. Determining residence patterns of rainbow trout using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) analysis of selenium in otoliths. *Environ Sci Technol* 41:3679–3683.

Phillips DJH, Rainbow PS. 1994. Biomonitoring of trace aquatic contaminants 2nd ed. London: Chapman and Hall.

Rainbow PS. 2002. Trace metal concentrations in aquatic invertebrates: Why and so what? *Environ Pollut* 120:497–507.

Ramsey PW, Rillig MC, Feris KP, Moore JN, Gannon JE. 2005. Mine waste contamination limits soil respiration rates: A case study using quantile regression. *Soil Biol Biochem* 37:1177–1183.

Roy I, Hare L. 1999. Relative importance of water and food as cadmium sources to the predatory insect *Sialis velata*. *Can J Fish Aquat Sci* 56:1143–1149.

Salazar MH, Salazar SM. 1995. In situ bioassays using transplanted mussels: Estimating chemical exposures and bioeffects using bioaccumulation and growth. In: Stavely JS, Biddinger GR, Mones E, editors. Environmental toxicology and risk assessment. Philadelphia: American Society for Testing and Materials. Publication 040-01280-16L. p 216–242.

Solà C, Burgos M, Plazuelo A, Toja J, Plans M, Prat N. 2004. Heavy metal bioaccumulation and macroinvertebrate community changes in a Mediterranean stream affected by acid mine drainage and an accidental spill (Guadiamar River, SW Spain). *Sci Total Environ* 333:109–126.

Strong CR, Luoma SN. 1981. Variations in correlation of body size with concentrations of Cu and Ag in the bivalve *Macoma balthica*. *Can J Fish Aquat Sci* 38:1059–1064.

[USEPA]. US Environmental Protection Agency. 2001. Update of ambient water quality criteria for cadmium. Washington DC: US Environmental Protection Agency. EPA 822-R-01-001. p. 166.

[USEPA]. US Environmental Protection Agency. 1999. Clark Fork River ecological risk assessment. Helena (MT): USEPA. Available at http://epa.gov/region8/superfund/sites/mt/milltowncfr/millterap.html. 851 p.

Winner RW, Boesel MW, Farrel MP. 1980. Insect community structure as an index of heavy-metal pollution in lotic ecosystems. *Can J Fish Aquat Sci* 37:647–655.