Bull. Environ. Contam. Toxicol. (2004) 72:409–414

DOI: 10.1007/s00128-003-9096-2

Environmental Contamination and Toxicology

Caged Pearl Dace (*Semotilus margarita*) as Sentinels for Gold Mining Wastes in Environmental Effects Monitoring

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Received: 16 June 2003/Accepted: 28 November 2003

Environmental Effects Monitoring (EEM) for Canadian metal mines was initiated in 1993 to assess the health of fish and their habitats potentially impacted by mining effluents (Dumaresq et al. 2002). The EEM program has been designed so that effects are measured in terms of fish, fish habitat and the usability of fisheries resources (Dumaresq et al. 2002). Although these general criteria have been established, consensus on which methods provide the most practical and reliable results for a given exposure scenario is a subject of discussion.

Specifically, considerable debate has occurred with respect to appropriate fish sampling methods for the analysis of effects. Designing a sampling method minimizing bias, representative of existing fish populations, and sensitive enough to detect potential effects can be challenging. At present, holding fish in effluents in cages is not considered as a valid option. However, caging studies could be ideal for evaluating contaminant uptake by fish and for delineating the geographic extent of such accumulations. By caging fish at specific locations, questions concerning residency and exposure also can be eliminated (Fenet et al. 1996). Furthermore, caged fish methods reduce the likelihood of confounded results that may otherwise occur in areas impacted by multiple effluents. The ability to clearly delineate the type of effluent(s) that a given fish is exposed to is an important feature of this approach. For example, fish can be caged at locations where they are either exposed to a single effluent or to a combination of effluents. This is an important concept, as exposure to multiple effluents is a scenario common to many northern Canadian mining communities.

MATERIALS AND METHODS

To examine the utility of caged fish deployments, adult pearl dace (*Semotilus margarita*), a small bodied forage fish species, were caged for 14 days in a freshwater system of northern Ontario, Canada, that receives effluents from two gold mines. Fish were captured from the BCU reference site (Fig. 1) using hoop nets set overnight at a depth of 1 m. Minnows were sorted and a uniform size (mean length: 5.95 ± 0.13 cm) was retained for caging purposes. Twenty minnows (10 males:10 females) were deployed into sinking cages (81 X 81 X 46 cm and

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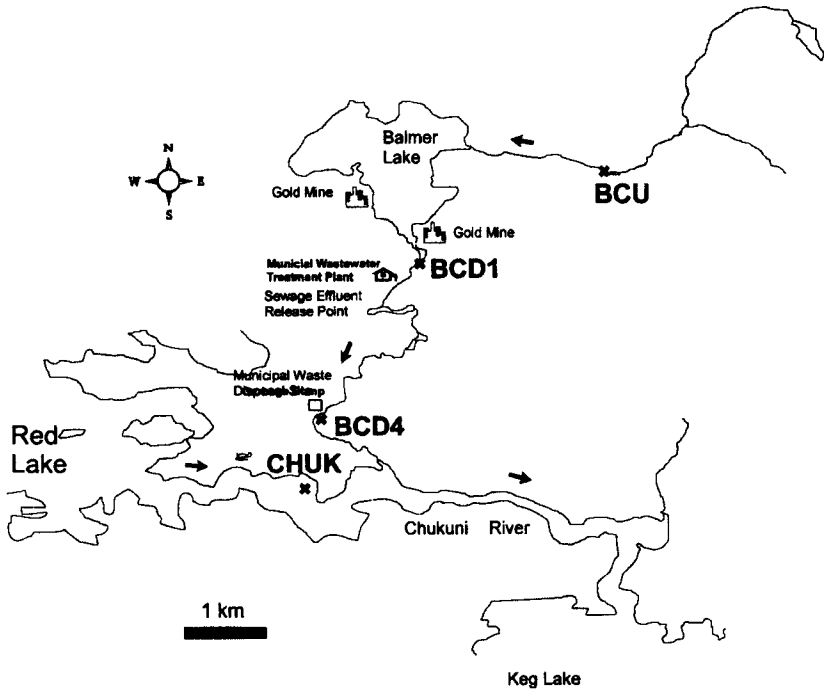

Figure 1. Study site in northwestern Ontario. Arrows show the direction of water flow in the system.

covered with 0.3 cm mesh nylon netting) positioned at a depth of 1.5 m for 14 days. Fish were caged: 1) several meters downstream of Balmer Lake, a tailings pond (BCD1); 2) downstream of Balmer Lake, municipal waste disposal site, and sewage effluent release point and (BCD4); 3) at two reference sites removed from the above listed point sources (CHUK and BCU). After 14 days, minnows were removed from the cages and frozen individually in sterile plastic bags between slabs of dry ice. Upon return to the laboratory, minnows were stored at -90 °C until processing.

Fish were removed from -90 °C storage and thawed on ice to prepare for analysis. After length and weight measurements were recorded, whole body homogenates of each fish were prepared in two volumes of de-ionized distilled water using a Brinkmann PT 10/35 homogenizer (Kinematica AG, Littau-Lucerne, Switzerland).

The cost of analysis dictated that only fourteen randomly selected fish from each cage were analysed for Cd, Pb, Cu, Ni, Zn, As, Se, and metallothionein. Arsenic and Se were determined via hydride generation with atomic fluorescence detection (PS Analytical, Millenium Excalibur, Kent, United Kingdom) and metals were measured using the methods of Harrison and Klaverkamp (1990). Instruments were calibrated against commercial standard solutions (SCP Science,

St. Laurent, Quebec). Additionally, samples of DORM-2, TORT-2, NOAA CRM-2976, DOLT-2, and bovine liver 1577a standard reference materials (National Research Council of Canada), and two blank samples were included in each analytical run (both AAF and AAS) using the same methodologies. Analytical detection limits for As and Se was 0.002 μg/g, 0.005 μg/g for Cd, 0.05 μg/g for Pb and Ni, and 0.02 and 0.03 μg/g for Cu and Zn, respectively. The detection limit of the metallothionein assay is 2 ug/g.

Condition factor (CF) was calculated for each fish using the equation: CF = (weight (g) / length $(cm)^3$) × 100. MT concentrations in whole body homogenate were determined using the ^{203}Hg saturation assay of Klaverkamp et al. (2000). Data were analyzed using a one-way ANOVA after confirming normal distribution and equality of variance. Tukey's multiple comparison tests was then used with significance set at the $\alpha = 0.05$ level.

RESULTS AND DISCUSSION

Condition factor is a general indicator of fish health. Mean CF data in each cage ranged from 0.85 to 0.90 and were not different among fish caged at any of the sites. Cadmium and Pb were below detection limits (0.05 mg/g) at all sites. Selenium concentrations ranged from 0.84 ± 0.10 to 0.97 ± 0.09 μg/g wet weight but no significant differences were detected in mean selenium concentrations in fish from any of the caging sites.

Arsenic is the primary element of concern regarding the discharge of mining affected waters from Balmer Lake to the receiving aquatic system (Palace et al. 2003). Total As (i.e. organic As and inorganic As, as As^{3+} or As^{5+}) concentrations were significantly greater in fish caged at sites exposed to mine effluent (BCD1 and BCD4) than in fish caged at the reference sites (BCU and CHUK) (Fig 2). Dilution and chelation of As by iron or vegetation in Balmer Creek may account for lower concentrations of As at BDC4 compared with BCD1. A similar trend for As was reported for the same sites in a previous study (Klaverkamp et al. 2002a).

Two locations were used as reference sites for these experiments. Significant differences in Cu and Ni concentrations were found in fish caged at these two sites after the 14 day experimental period. Copper concentrations were greater at both BCU and BCD1 (21.97 ± 4.31 and 17.32 ± 5.62 μg/g wet weight, respectively) than at the downstream sites BCD4 (8.55 ± 2.14 μg/g) and CHUK (7.32 ± 1.08 μg/g) sites. But the only significant differences in Ni concentrations were between fish caged at BCU (7.17 ± 0.55 μg/g) and CHUK (3.01 ± 0.63 μg/g). One reason for the different Cu and Ni contents between the two reference sites and in Cu concentrations between BCU and BCD1 and the downstream BCD4 and CHUK sites may be the subtle differences in habitat types between the sites. BCU and BCD1 are characterized by flowing water, while BCD4 and CHUK are increasingly lentic in nature. Habitat

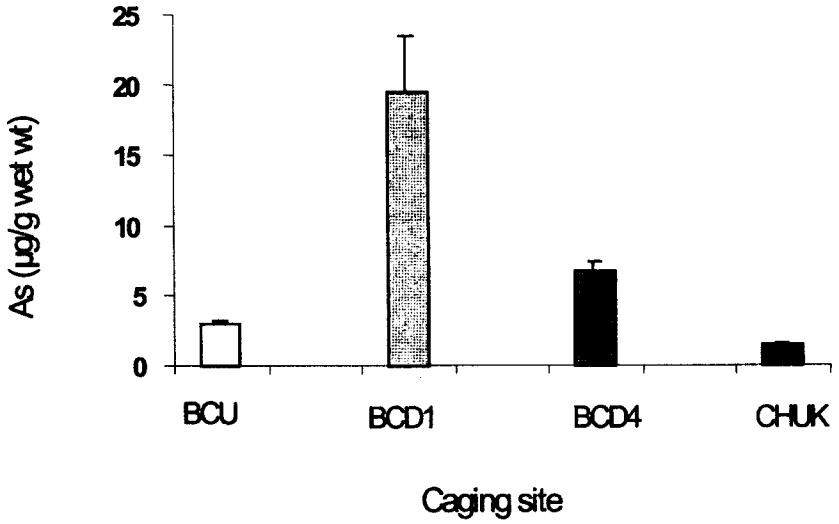

Figure 2. Mean + SE concentration of As (µg/g wet wt) observed in whole body homogenates of 14 pearl dace caged in each site. BCU and CHUK represent the reference sites for the study.

variations caused by this difference may have resulted in unique dietary constituents for fish at different caging locations. Diet shifts can greatly influence whole body Cu concentrations. In fact, diet is the most important route of Cu accumulation in aquatic animals (Eisler 1997). Further study is required to determine whether dietary shifts could be responsible for the different Cu and Ni contents at the study sites.

In the absence of contamination, fish contain Zn concentrations ranging from 4 to 20 µg/g wet weight (Spear 1981). Fish caged in sites BCU and BCD1 had Zn concentrations near this range (22.26 ± 3.11 and 24.14 ± 2.66 µg/g wet weight, respectively), while those caged in BCD4 (38.33 ± 2.48 µg/g) and CHUK (32.88 ± 1.75 µg/g) had significantly higher concentrations. Higher Zn concentrations at BCD4 may have been influenced by the sewage effluent release point and/or the municipal waste disposal site, as these types of facilities have been shown to release Zn to the aquatic environment (Spear 1981). Zinc accumulation was also observed in fish caged at the CHUK reference site. It should be noted that a highway crosses the Chukuni River approximately 1 km upstream from this caging location. Road surface runoff is a source of Zn to aquatic environments (Spear 1981). The community of Red Lake is located 4 km upstream of the CHUK site, and represents another potential source of Zn. Klaverkamp et al. (2002a) also showed higher Zn concentrations in sediments at the CHUK site relative to BCU.

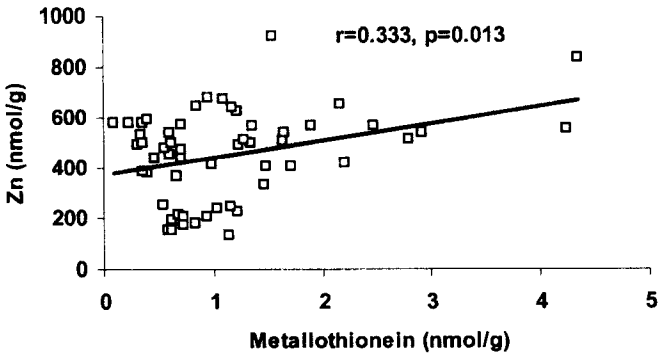

Figure 3. Scatter plot of Zn against metallothionein concentrations in whole body homogenates of pearl dace caged at each site (n=55).

Concentrations of the metal binding protein, metallothionein (MT), were significantly higher in fish caged downstream of mining activities (BCD1 = 9.21± 2.02 μg/g) and BCD4 = 11.74 ± 1.89 μg/g) compared to fish caged at the BCU (4.27 ± 0.64 μg/g) reference site. Metallothionein was also significantly elevated in fish caged in the CHUK reference site (6.88 ± 1.01 μg/g) when compared to fish caged in the BCU reference site. Linear regression analysis showed a weak but significant relationships between MT concentrations and the molar equivalent of Zn ($r = 0.333$, $p = 0.013$) levels in individual fish caged at all sites (Fig 3). Stronger relationships were not obtained when the molar equivalents of MT-inducing metals (Cu, Zn, As) were summed in any combination. In spite of the fact that Cu is known to induce MT synthesis, Cu concentrations were not positively correlated with MT induction in fish from this study. Previous work has also shown that including Cu in statistical analysis did not significantly improve relationships between tissue MT and total metal/metalloid concentrations (Klaverkamp et al. 2002b). Although Cd is known to induce MT synthesis, levels of Cd in this study were below detection limits and were, therefore, not included in regression analysis with MT. Arsenic is not traditionally associated with MT induction, but was included in the analysis because previous studies have shown increases in the production of this protein in fish exposed to the metalloid (Pedlar et al. 2002).

These studies have shown the utility of using caged small-bodied fish for the evaluation of specific endpoints in the environmental effects monitoring of metal mining effluents. Accumulation of arsenic, the primary element of concern, was demonstrated in fish caged at sites downstream from mining activity relative to reference locations. The use of MT as a monitoring tool for exposure to certain metals in the metal mining EEM program was also supported, as evidenced by a significant relationships between this parameter and Zn concentrations. Further

research is required to evaluate the potential of using small-bodied fish to assess the bioavailability and potential to accumulate As, Se, Cd, Cu, Ni, Zn, and Pb from water and sediments. Such studies could be conducted in a variety of mining communities across Canada, such that the generalized applicability of this approach may be assessed. Additionally, future research should focus on identifying optimum exposure times to allow sufficient accumulations of metals for endpoint analyses, while maintaining acceptable health of the caged fish.

Acknowledgements. This work was supported by Placer Dome North America and Goldcorp. Inc. C. Doebel received an NSERC undergraduate student research award to perform these studies.

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