Caging Small-Bodied Fish as an Alternative Method for Environmental Effects Monitoring (EEM)

Vince P. Palace,1,2* Cecilia Doebel,2 Chris L. Baron,1 Robert E. Evans,1 Kerry G. Wautier,1 Jack F. Klaverkamp,1 Jeffrey Werner1 and Suzanne Kollar1

1Department of Fisheries and Oceans, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba R3T 2N6
2Queen’s University, Department of Biology, Kingston, Ontario K7L 3N6

The utility of using fish held in cages for investigating the potential effects of industrial and municipal effluents has been demonstrated. These types of exposures are able to control the influence of some confounding factors and also provide a measure of certainty with regard to exposure. However, Canada’s current Environmental Effects Monitoring (EEM) Program for metal mines does not accept caged fish as a viable alternative for use in place of wild fish surveys. Concerns appear to be focused on the confounding influences of confinement stress on typical EEM endpoints. Confinement stress may be reduced in smaller-bodied fish relative to larger fish, increasing the relevance of their use for EEM. A standardized technique for deploying small-bodied fish in cages that may be useful for EEM programs is described.

Key words: caged fish, Environmental Effects Monitoring, small-bodied fish

Introduction

Development of the Environmental Effects Monitoring (EEM) Program for Canadian metal mines began in 1993. Administered under the Fisheries Act by Environment Canada, EEM was designed to examine the effectiveness of environmental regulations for protecting fish, fish habitat, and the usability of fisheries resources from metal mining discharges (Dumaresq et al. 2002). The methodological framework employed under EEM provides regulators with consistent scientific data regarding potential effects (Ribey et al. 2002). The EEM Program has altered the metal mining industry approach to environmental practices, shifting traditional focuses on contaminant release volumes to now encompass more holistic implications of such releases to aquatic environments. This comprehensive approach is more desirable because it has the capacity to assess the causes of environmental change, not just the signs. The EEM Program for metal mines incorporates flexibility and continues to evolve with ongoing input provided by national steering and science advisory committees.

The biological monitoring component of the EEM Program requires that mines conduct fish population surveys, fish tissue analyses and benthic invertebrate surveys at exposed and reference sites (Dumaresq et al. 2002). Since the initial development of the Pulp and Paper EEM Program, and now within the Metal Mining EEM Program, questions have arisen regarding appropriate methods for conducting fish surveys. Specifically, there have been challenges with regard to the selection of suitable reference areas, potential impacts of confounding influences, and fish mobility (Ribey et al. 2002). In situations where there are confounding influences, it has been difficult to design survey methods void of bias, representative of existing fish populations, and sensitive enough to determine whether or not effects are occurring. Current fish surveys in the EEM Program are limited by the fact that fish residency and exposure cannot be confirmed due to the mobility of many of the fish species present in receiving waters. It has been suggested that small-bodied fish species, including darters, minnows and sculpins could provide more interpretable data because of their limited mobility and, hence, more certain exposure (Gibbons et al. 1998; Munkittrick et al. 2000; Minns 1995; Hill and Grossman 1987; Mundahl and Ingersoll 1989; Fitzgerald et al. 1999). Some discussion regarding higher than expected mobility of small-bodied fish has also appeared in the literature (Linfield 1985; Clough and Beaumont 1998).

Since effects of confounding discharges can be evaluated and exposure can be assured, caged bivalves have become an accepted alternative for use in EEM where wild fish surveys are not practical (Ribey et al. 2002). Caging bivalves within chemical gradients can provide environmentally relevant information while ensuring exposure (Salazar and Salazar 1997). However, endpoints relevant to the reproductive capacity of exposed fish populations cannot be directly derived from caged bivalve studies. Fish held in cages may be able to provide reproductive endpoints and offer other advantages similar to those afforded by caged bivalves. In situations where inadequate numbers of resident small-bodied fish are present or where mobility of small-bodied fish is in
question, caging studies may, in fact, be more desirable than surveys of resident small-bodied fish. However, caged fish exposures are not currently considered a valid option to wild fish surveys under EEM. Much of the reluctance to employ caged fish surrounds the induction of physiological stress responses in fish held captive (Courtenay et al. 2002). It seems likely that the potentially smaller home ranges of small-bodied fish (Gibbons et al. 1998) may be linked to less overt confinement stress when caged than those invoked in larger fish species. The national EEM science advisory committee has recognized the potential for caged fish studies using small-bodied fish to contribute valuable information. However, they have advised that additional work is required to refine the caging approach and have recommended that the development of caging techniques be a research priority. Finally, the committee has urged that biological parameters, including growth, be incorporated as part of that development process.

In an effort to further develop the use of caging small-bodied fish as an alternative fish survey method for EEM, we have performed a series of experiments using standardized caging methodologies (Doebel et al. 2004; Klaverkamp et al. Submitted for publication; Palace et al. 2004). The techniques used in a series of exposures in which pearl dace (Margariscus margarita), finescale dace (Semotilus semotilus) or fathead minnows (Pimephales promelas) have been caged are described for experiments conducted at Balmer Lake, northwestern Ontario, a site which has received gold mining effluents for more than 40 years. Results from these studies support the utility of the caging approach for examining typical EEM endpoints (growth, condition, liver and gonad size) as well as accumulations of elements relevant to local mining activity.

### Caging Methodology

Typically, pearl dace, finescale dace or fathead minnows are captured in 4-foot hoop nets set overnight at 1-m depths at established reference sites. Minnows are then sorted by sex and/or to obtain a uniform size class for caging purposes. Every effort is made to ensure that fish destined for each cage are handled and confined under the same conditions. To evaluate growth, juvenile fish have been deployed into the cages. Because of the mesh size of the cages, juveniles must be a minimum of 45 mm in length to be contained within the cage mesh size. Fish of this size range were reproductively immature from our study site and for the species noted. In situations where fish are reproductively mature at <45 mm and where growth in juveniles is the desired endpoint, smaller mesh size may need to be employed for the cages. Larger fish are used where gonadosomatic index (GSI) and liver somatic index (LSI) need to be quantified. Twenty juvenile or adult minnows (10 males:10 females) are deployed into sinking cages (81 × 81 × 46 cm) made from PVC pipe frames and covered with 1/8” mesh nylon netting bags with Velcro closures at one end (Fig. 1). Cages are deployed at reference sites as well as at various effluent exposure sites, and at any site suspected of contributing confounding influences. A confounding influence in this context is any chemical or physical stressor that could alter the results obtained for typical EEM endpoints separate from those that might be imposed by the effluent being monitored. Before deployment, adequate oxygen saturation and flow are confirmed and basic water chemistry parameters are measured and recorded at both the reference and exposure locations. Cages are routinely placed at a depth of 1.5 m for 14 to 28 days. After the exposure period, fish are removed from the cages, weighed and measured, and frozen individually in sterile plastic bags between slabs of dry ice. Upon return to the laboratory, fish are stored at -90°C until dissection and processing of tissues for analysis.

To prepare for analysis, fish are removed from -90°C storage and thawed on ice. Tissues are dissected from the carcass and weighed, and homogenates of tissues or whole fish can then be prepared for contaminant analysis. Condition factor (K) can be calculated as

\[ K = \frac{\text{weight}}{\text{length}^3} \times 100 \]

and potential differences in this parameter can be analyzed using ANOVA. However the preferred method for EEM is to compare fish masses between sites with body length as a covariate using ANCOVA. GSI and LSI are calculated for each fish using their respective equations: GSI = gonad weight/(body weight) × 100, LSI = liver weight/(body weight) × 100, where body weight is the total weight minus the weight of liver and gonad. Potential differences in each of these parameters are more appropriately analyzed using ANCOVA to compare the separate regressions between weight and length. Following ANOVA or ANCOVA, differences between sites can then be further evaluated using a suitable multiple comparison procedure with significance set at the p = 0.05 level.

### Discussion

When selecting fish species for the fish survey of EEM, the most important considerations are abundance, relevance to the study area and ease of measuring the EEM endpoints (Munkittrick and McMaster 2000). Small-bodied fish are most often more abundant than larger sport or commercial fish species, but do offer some challenges in terms of measuring EEM endpoints. Specifically, to measure liver and gonad sizes, adequately sized fish must be used for caging and care must be taken in dissecting tissues. Furthermore, multiple spawners and live bearers require special attention regarding measurement of reproductive variables. Where fractional spawners are chosen, it is recommended that they be sampled prior to initiation of the first spawning period (Munkittrick et al. 2002).
Caged exposures have been used extensively over the past decade for examining the potential effects of municipal effluents on endocrine function in fish (reviewed in Christiansen et al. 2002). Use of the technique, however, has been limited within the context of EEM, largely based on information that caged fish are subject to levels of stress that may confound results (Courtenay et al. 2002). Many of these previous studies have used large-bodied fish. Because of their limited mobility, smaller bodied, forage fish may be subject to less confinement stress when deployed in cages (Munkittrick et al. 2000; Minns 1995; Hill and Grossman 1987; Mundahl and Ingersoll 1989; Fitzgerald et al. 1999).

Recently, the national EEM science committee recommended that survival and growth in juveniles could be used as integrative measures of stress in caged fish. In a series of experiments where 20 juvenile pearl dace per cage were deployed at an upstream reference site and downstream of gold mining effluent discharges (4 cages and a total of 80 fish per site), survival was nearly 100% after 2 and 4 weeks of caging at both of the sites. Because survival is only a gross measure of stress, condition was also assessed in caged juvenile pearl dace (Fig. 2). A subsample of resident fish captured from a reference site was measured after the initial collections. Additionally, resident fish were captured and measured from the same reference site when cages were retrieved after 2 and 4 weeks. The repeated measures of size in resident fish were performed to enable an assessment of growth for comparison against the growth measured in the fish caged at either reference or effluent-exposed locations. Condition was similar in resident pearl dace during the initial collections and at the 2- and 4-week periods (Fig. 2). There were also no significant differences in condition of the fish caged at either the reference or effluent-exposed sites at either of the sample times. The results suggest that fish continued to forage while they were in the cages and that confinement stress was minimal. Furthermore, we performed gut content analyses of confined and resident pearl dace and observed similar proportions of forage items in the gut of these two groups (Palace et al. 2004).

While caging techniques can be useful for separating the potential effects of confounding influences, the exposure of native fish collected from reference areas to different conditions at exposure areas could impact parameters of interest separate from the potential impacts of the efflu-
ent being considered. For example, differences in the chemical and physical (e.g., temperature, conductivity, pH, dissolved oxygen, hardness) environment between the reference and exposure areas can alter the results for measured endpoints (Russel et al. 1996; Larsson et al. 2000; Peters 1999). For this reason, pre-caging characterizations of reference areas to ensure similar characteristics to the intended exposure areas are recommended. These parameters are also typically monitored throughout the caging period at both the exposed and reference areas.

Application of an EEM approach requires that liver and gonad size also be evaluated as measures of energy metabolism and reproductive function, respectively. Tissues from juvenile fish similar to those used for the studies described above are not large enough to provide accurate measures of liver and gonad size. However, we have previously deployed adult small-bodied fish for caging durations of 4 weeks to obtain tissues and derive LSI and GSI measures. The altered LSIs and GSIs that we have measured in past experiments have been consistent with those that would be expected based on the chemical makeup of effluents and nutrient availability (Klaverkamp et al. Submitted for publication).

Preserving the usability of fisheries resources is a primary focus of the Fisheries Act and the EEM Program. In situations where contaminants are enriched in effluents, fish tissue content of the contaminant in question must also be assessed (Ribey et al. 2002). Arsenic is the primary element enriched in mine effluent at the Balmer Lake study site. Water concentrations of this element become elevated in late summer and early fall and often exceed regulatory guidelines (Palace et al. 2003). We have previously used caged fish exposures to determine the potential for arsenic accumulation and the influence of confounding influences at several sites in the study area. Table 1 contains mean total As concentrations in whole bodies of fish caged for two weeks at two reference sites and at near-field and far-field effluent exposure sites. Arsenic concentrations were significantly greater in fish caged at both effluent exposure sites than in fish caged at the reference sites (ANOVA followed by Tukey’s, p < 0.05). Additionally, arsenic concentrations were greater in fish caged at the near-field site than in fish caged at the far-field site, suggesting that geographical extent of contamination could be at least initially investigated using caged fish exposures. Doebel et al. (2004) suggested that lower arsenic concentrations in fish caged at the far-field site could be explained either by dilution or chelation of arsenic by iron in the heavily vegetated downstream waters of Balmer Creek. Most importantly, arsenic concentrations in caged fish were reflective of enriched sediment and water concentrations of the element at the exposure sites (Klaverkamp et al. 2002), demonstrating the utility of short duration exposures for examining arsenic exposure and uptake.

While the approach that we have described has proved useful for delineating the potential for As to accumulate in fish, similar previous experiments have resulted in no significant relationships between concentrations of Cu, Zn, Pb or Cd in water or sediment and those in fish caged for short-term exposures (4 weeks). Positive relationships have been observed between water and/or sediment concentrations and fish tissues for Ni and Se (Klaverkamp et al. 2002). Tuvikene et al. (1999) reported that rainbow trout (Oncorhynchus mykiss) caged for 3 weeks in a riverine environment, accumulated metal concentrations (Cd, Pb, Cu) in their tissues (muscle and liver) that were reflective of resident fish captured near the sites of caging. With reference to Metal Mining EEM Programs, it should be noted that no relationship was evident for Hg in this study. The same conclusion was reported for catfish caged for 2 weeks at a site with high concentrations of the heavy metal (Schlenk et al. 1995). It should be recognized that many contaminants in general, and some metals specifically, will require longer than 4 weeks to accumulate and to elicit potential effects. Moreover, bioavailability and accumulation of each specific metal will be determined not only by its physical chemical properties but also by biological, chemical and physical factors, including dietary conditions, redox conditions, abiotic and biotic transformations, lipid solubility, pH, complexation with organic matter, the presence of sulfur compounds, and the availability of inorganic complexes (Paquin et al. 2003). All of these factors need to be considered when evaluating the potential for a given metal to accumulate in fish and to elicit responses.

Factors affecting accumulation rates are even more relevant when considering the potential for contaminants to impact typical EEM endpoints, including liver and gonad size. Questions often arise regarding the ability of caging studies to detect impacts on liver and gonad size over the necessarily short durations of caging studies. Strategies to enable the technique to detect potential effects can include caging during periods of peak growth and gonad maturation. For example, by caging rainbow trout during the summer months when growth was more rapid, Harries et al. (1997) were able to detect signifi-

### Table 1. Total arsenic concentrations in whole bodies of pearl dace caged for 14 days at reference and effluent exposed sites

<table>
<thead>
<tr>
<th>Caging site</th>
<th>Total arsenic µg/g wet weighta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upstream reference</td>
<td>3.01 ± 0.22</td>
</tr>
<tr>
<td>Additional reference</td>
<td>1.40 ± 0.12</td>
</tr>
<tr>
<td>Effluent exposed - near-field</td>
<td>18.10 ± 4.14</td>
</tr>
<tr>
<td>Effluent exposed - far-field</td>
<td>6.68 ± 0.85</td>
</tr>
</tbody>
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aMeans labelled with different letters are significantly different from each other based on ANOVA and Tukey’s test (n = 14, p < 0.05).
cant differences in liver and testicular sizes after 3 weeks. The authors linked these changes to the contaminant profiles of the caging environments.

**Summary and Conclusions**

We have described a standard protocol for caging fish over short durations (2 to 4 weeks) that has proved useful for determining the accumulation of selected metals and their effects in small-bodied fish species. The approach allows typical EEM endpoints to be monitored, including liver and gonad size in adult small-bodied fish and growth in juveniles. However, some issues remain to be resolved before the technique can be employed in formal EEM monitoring programs. For example, optimal loading densities to minimize confinement stress and to ensure adequate food supplies need to be described. For longer-term exposures or where food items will be limiting it may also be necessary or desirable to provide external sources of feed to the caged fish. Methodologies will need to be developed for these additions.

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