Guidance for the collection and sampling of slimy sculpin (*Cottus cognatus*) in northern Canadian lakes for environmental effects monitoring (EEM)

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GUIDANCE FOR THE COLLECTION AND SAMPLING OF
SLIMY SCULPIN (*Cottus cognatus*) IN NORTHERN CANADIAN LAKES
FOR ENVIRONMENTAL EFFECTS MONITORING (EEM)

by

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Environmental effects monitoring (EEM) EEM is currently a requirement for regulated mills and mines under the Pulp and Paper Effluent Regulations (PPER) and the Metal Mining Effluent Regulations (MMER), both under the authority of the *Fisheries Act*. The objective of both of these regulatory EEM programs is to evaluate the effects of effluents on fish, fish habitat and the use of fisheries resources when facilities are in compliance with their discharge limits. Industrial developments in northern Canada now require monitoring in aquatic environments that pose significant challenges not seen in the southern regions of Canada. Low conductivity aquatic systems, shorter, colder growing seasons, and limited ice-free periods for sampling combine to create a significant gap between life history understanding and sampling guidance based on southern regions and how they can be applied in northern systems. This guidance document aims to bridge that information gap for using slimy sculpin in northern Canadian lakes when conducting Environmental Effects Monitoring (EEM) and general monitoring studies. This document also serves as a literature review of many of the relevant scientific studies on slimy sculpin that provide essential life history characteristics and a variety of collection techniques, both necessary for designing successful and productive monitoring studies.

Key words: slimy sculpin, environmental effects monitoring, northern lakes

RÉSUMÉ

Arciszewski, T., Gray, M.A., Munkittrick, K.R., and Baron, C. 2010. Guidance for the collection and sampling of slimy sculpin (*Cottus cognatus*) in northern Canadian lakes for environmental effects monitoring (EEM). Can. Tech. Rep. Fish. Aquat. Sci. 2909: v + 21 p.

L'Étude de suivi des effets sur l'environnement (ESEE). L'ESEE est actuellement exigée pour les usines et les mines réglementées en vertu du *Règlement sur les effluents des fabriques de pâtes et papiers* et du *Règlement sur les effluents des mines de métaux* (REMM), qui relèvent tous les deux de la *Loi sur les pêches*. Les deux programmes de réglementation de l'ESEE visent à évaluer les effets des effluents sur les poissons et leur habitat ainsi que sur l'utilisation des ressources halieutiques lorsque les installations sont en conformité avec leurs limites de rejets. Les développements industriels dans le nord du Canada nécessitent actuellement une surveillance dans les milieux aquatiques qui constituent des défis importants jamais posés dans les régions méridionales du Canada. Des systèmes aquatiques à faible conductivité, des périodes de végétation plus brèves et plus froides, et de courtes périodes sans glace pour l’échantillonnage se combinent pour créer un écart important entre la compréhension du cycle évolutif et les directives pour l’échantillonnage fondé sur les régions du sud du Canada, et sur la façon avec laquelle il peut être appliqué dans les systèmes situés au nord. Ce document d'orientation vise à combler cette insuffisance d'informations pour l’utilisation du chabot visqueux lors de l'Étude de suivi des effets sur l'environnement (ESEE) et des études générales de suivi des effets sur l'environnement. Ce document tient également lieu d’analyse documentaire portant sur plusieurs études pertinentes concernant le chabot visqueux qui fournissent des caractéristiques essentielles du cycle évolutif et une variété de techniques de collecte, qui sont tous deux des éléments essentiels pour l’élaboration d’études de suivi productives et favorables.

Mots clés: Chabot visqueux, Étude de suivi des effets sur l'environnement, lacs du nord canadien

1. INTRODUCTION

1.1 PURPOSE OF DOCUMENT

This document was prepared to provide guidance on designing and implementing monitoring programs in northern lakes using slimy sculpin (*Cottus cognatus*). This protocol was developed following the monitoring studies at the Diavik Diamond Mine on Lac de Gras in the Northwest Territories, carried out by DFO Science and the Canadian Rivers Institute (Gray et al. 2005b) as a requirement of Diavik's ss. 35(2) *Fisheries Act* authorization. This protocol gives guidance not only for future studies on fish in Lac de Gras, but also for other northern environmental monitoring studies that may be conducted utilizing slimy sculpin.

This document briefly describes the background to environmental monitoring using the performance of fish populations, the characteristics of slimy sculpin that make it an effective choice in monitoring studies, and the various capture techniques that have been used to capture sculpin species in temperate and arctic lakes.

2. BACKGROUND ON ENVIRONMENTAL MONITORING

Government regulations, both federal and provincial, often require monitoring to detect changes in ecosystems exposed to by-products of industrial activities. Information on potential effects are obtained via various types of environmentally protective activities that are initiated during a project's lifetime, including baseline reporting, environmental impact assessments, and regular monitoring. Regular monitoring is done on many media, including land and air, and much work has been done on developing techniques for determining the impacts of liquid effluents on the aquatic environment.

To detect impacts on aquatic ecosystems, effects-based designs are replacing stressor-based programs in many areas. Stressor-based monitoring first identifies the expected effects of a project and then measures the associated endpoints, for example aluminium, and then can either compare them against existing toxicity thresholds, or to baseline levels in unexposed areas (Munkittrick et al. 2000). The stressor-based approach is common in environmental impact assessments (EIA). Stressor-based approaches, however, assume that the effects pathways are known and are predictable (Kilgour et al. 2007). They also often use toxicity thresholds developed in laboratories for one species that may not apply to others (Cairns 1986), or in multiple stressor scenarios (Munkittrick et al. 2000).

In contrast, effects-based designs use measurements of the biological components of an ecosystem to determine if meaningful changes are occurring (Gray and Munkittrick 2005). If and when changes are found, the monitoring resources can be focussed on determining the cause of the response (Gibbons and Munkittrick 1994). The specific reason(s) for those changes can, however, be difficult to determine (Hewitt et al. 2008, Gray and Munkittrick 2005).

Many programs in Canada, including Environmental Effects Monitoring (EEM), have adopted the effects-based approach. EEM programs and regulations have been designed and implemented for pulp and paper mills and metal mines (Ribey et al. 2002, Walker et al. 2002) and are pending for municipal discharges (Environment Canada 2010). EEM programs use collections of the benthic invertebrate community and fish populations to detect responses in the ecosystem to the environmental stressor(s) in question. In the basic EEM design, both fish and invertebrates are captured in reference and exposed areas and compared statistically and against critical effect sizes (Environment Canada 2005). The fish component of EEM uses (optimally) two fish species in the sentinel species approach.

2.1 SENTINEL SPECIES APPROACH

The sentinel species approach used in EEM was developed in the late 1980s and early 1990s (e.g. Munkittrick and Dixon 1989). The technique evaluates the growth, survival, and reproduction of exposed fish and compares them to fish captured at a reference site using surrogate endpoints. The overall pattern of responses of the various endpoints, such as liver size, gonad size, age, condition, and growth are compared to one or more reference sites and used to determine any limiting factors for the exposed population. For instance, response patterns of eutrophication (increased liver size, condition, gonad size, and faster growth) and metabolic disruption (increased condition and liver size, smaller gonads; Gibbons and Munkittrick 1994) are found commonly downstream of pulp mills in Canada (Lowell et al. 2003).

The sentinel species approach has several key assumptions:

- the sentinel species is representative of the receiving environment
- the sentinel species does not migrate between the reference and exposure areas
- there are minimal physical habitat differences between the reference and exposed areas and that the small differences do not strongly influence the performance of the sentinel population
- measuring the growth, survival, and reproduction of a sentinel detects meaningful (and potentially multiple) pathways of effects

2.1.1 Characteristics of ideal sentinels

The sentinels that are selected in any given study should have certain characteristics that overcome potential issues that derive from the above assumptions. There are at least three basic characteristics of suitable sentinels:

- abundant,
- sedentary,
- high site fidelity, and

- have measurable life history characteristics relevant for the assessment.

These characteristics are important because abundant fish are easier to catch and more likely to satisfy sample size requirements; a sedentary fish reflects the localized environmental state and conditions; and capturing the fish at the reference and exposed sites allows the analysis of changes to occur. These initial characteristics are necessary for a cogent analysis to proceed, while other characteristics increase the likelihood of detecting ecologically relevant changes:

- short generation times
- single-event spawning
- large reproductive effort
- benthic feeding

Early in the pulp and paper EEM program, large-bodied species were commonly used, but small-bodied species have been used more frequently in recent cycles (Munkittrick et al. 2002). Small-bodied fish are more likely to meet the criteria of useful sentinels than large-bodied fish, tend to be more abundant in the receiving environments, have a shorter generation times, and are generally more sedentary (Gibbons et al. 1998).

3. SLIMY SCULPIN AS A SENTINEL SPECIES

The slimy sculpin (*Cottus cognatus*) inhabits both streams and lakes. Slimy sculpin are almost ubiquitous across Canada, with their national range extending from the Yukon Territory in the west and north to New Brunswick in the east and Lake Ontario in the south in Canada (Scott and Crossman 1998). More recently, slimy sculpin populations have been discovered in western Prince Edward Island (Gormley et al. 2005). In streams, they occupy areas with unembedded large substrate. In lakes, they have been reported in rocky and soft substrate and in very deep to shallow water. They are considered a cool-water species with the upper thermal limit at most 25˚C, but slimy sculpin will be largely absent in waters that sustain temperatures of over 21˚C for more than a few days (Gray et al. 2005a). On a micro-habitat scale, sculpin prefer cooler areas of streams (Edwards and Cunjak 2007).

Figure 1. Slimy sculpin photographed on a modified measuring board for small fishes. *Photo credit: M. Gray, Canadian Rivers Institute.*

The broad distribution and possession of the above life history characteristics means that the fish reflect recent and local environmental conditions and can be used to identify changes in response to stressors in many regions, including northern areas. Slimy sculpin was selected as the sentinel species in many studies because it has a relatively small home range (Gray 2003, Keeler and Cunjak 2007), has high reproductive effort (Gray et al. 2005a, Brasfield 2007), reflects the local environment (Gray et al. 2004, Arciszewski 2007), and has a short life span (van Vliet 1964).

Slimy sculpin has been used in many studies to examine environmental impacts. In New Brunswick, the species has been used in the assessment of agricultural areas (Gray et al. 2002, *ibid* 2005a, Brasfield 2007) and in rivers with industrial and municipal discharges (Galloway et al. 2003, Arciszewski 2007). Slimy sculpin have shown increased condition and young-of-the-year (YOY) survival downstream of sewage outfalls (Galloway et al. 2003, Arciszewski 2007) and reduced growth and YOY year-class strength in agricultural areas (Gray et al. 2005a, Brasfield 2007), and reduced gonad size, fecundity, and nest size in agriculturally influenced streams (Gray and Munkittrick 2005). The impacts of environmental stressors have also been detected in both lethal (Galloway et al. 2003) and non-lethal studies (Gray et al. 2002, Arciszewski 2007) of slimy sculpin. In other regions, the slimy sculpin has been used in studies of the impacts of metal mine discharge (Dubé et al. 2005). Other sculpin species have also been used to evaluate the effects of mine drainage (Allert et al. 2009), and show high sensitivities to some metals (Brinkman and Woodling 2005).

3.1 CAPTURE METHODS

Many capture techniques have been used to collect slimy sculpin. Bottom trawls, Gee (minnow) traps, gill nets, and electrofishing have all been used to capture sculpin. Which technique to use in a particular sampling scenario will depend on the user's experience, the physical characteristics of the system being studied, and the ongoing success in the study being undertaken. For instance, backpack electrofishing units are usually used with the greatest success in streams and rivers, while minnow traps have been used predominantly in lakes. Successful capture of sculpin may, however, require deploying multiple gears, especially at sites that have not been previously sampled (Portt et al. 2006).

3.1.1 Electrofishing techniques

Because sculpin are bottom-dwelling fish and live under rocks, electrofishing has been the preferred method for capturing slimy sculpin in streams, and shorelines of larger rivers and lakes where the gradient is not too steep. Backpack electrofishing is the conventional method, but boat electrofishing can also be used to capture sculpins. Backpack electrofishing was used successfully in the Diavik Mine study by Gray et al. (2005b) along the wadable margins of Lac de Gras.

The use of electrofishing in a northern lake was initially rejected as a sampling technique because of the low conductivity water (Gray et al. 2005b). The conductivity in Lac de Gras was, however, within the operational limits of the backpack electroshocking unit. The voltage was set at the mid-range for the machine (500 volts) and a wider than normal 18" anode ring was used (11" diameter is the standard ring commonly used), allowing for more power to enter the water. Dipnetting in the rocky littoral area was a challenge, however, because the sculpin could become immobilized and sink into crevices between large boulders from which they were irretrievable.

The distribution of sculpin was patchy in the Lac de Gras study (Gray et al. 2005b). There were areas where no sculpin were observed for stretches of over 20 m, though this was generally associated with much larger substrate (boulders). The majority of sculpin were found in shallow (<40 cm) areas with smaller cobble substrate. In the Lac de Gras study, the substrate around the mining dike had areas of natural substrate and blasted rock deposits (Gray et al. 2005b). In areas of larger substrate (boulders), fish often fell or swam into crevices where it was not possible to retrieve them with a regular sized dipnet. Despite these challenges, it was possible to collect 105 fish around the mining dike in approximately 3 h (or 19 sculpin/1000 s of electrofishing). Capture was better when two nets were used – a smaller minnow net to help get fish out from crevices, and a larger net to capture fish attempting to swim away from the electric current. Two other reference sites were also sampled and yielded 6 and 10 sculpin/1000 s electrofishing time. The CPUE was reduced but was still very high compared to all prior attempts to collect fish using other methods in that lake.

Experience in other regions suggest that sculpin can best be captured with short intermittent bursts of current; this technique often means the fish will not be alerted via low-power peripheral shocking that causes the fish to execute an escape behaviour that directs them into the shocking zone of the anode.

A main drawback of backpack electrofishing in lake systems is that it is limited to shallow water (<1 m) and the conductivity of the water must be adequate (>10 µS/cm). The depth limit can become a concern where spatial segregation by size of fish is occurring. Additionally, netting fish in areas with many interstitial spaces can be difficult if the fish sink into crevices; this could be especially problematic in areas with low water velocity or irregular flow patterns.

If backpack electrofishing units are used, standardized operating procedures should be used with the exception that a larger, 18" anode ring should be utilized as it permits additional power to be sent out in as needed. Collections should be made by at least two experienced electrofishing personnel. For low conductivity water, the initial voltage setting should be 300-500 V and then settings should be modified based on the response of fish in the response zone.

An alternative method for capturing small biota in rocky substrates and lake bottoms was developed at the Great Lakes Water Institute, at the University of Wisconsin. They have coupled a submersible remote-operated vehicle (ROV) with an eletroshocking unit and a suction sampler. This allows them to collect fish eggs and YOY fish (e.g. Marsden and Janssen 1997; http://www.glwi.uwm.edu/people/jjanssen/index.php). Suction sampling devices have also been used successfully in the collection of juvenile lobsters with many references to this technique in popular and scientific publications.

3.1.2. Trapping

Toolik Lake

Trapping techniques have been used successfully by personnel at the long-term ecological research (LTER) station located at Toolik Lake in the State of Alaska, USA (O'Brien et al. 2004). The investigators have commonly deployed custom traps (jars with lucite funnels) to capture sculpin at various depths (see Hanson et al. 1992, McDonald and Hershey 1992, O'Brien et al. 2004). These investigators have also used foldable mesh minnow traps (A. Hershey, pers. comm.). The minnow traps have finer mesh than regular Gee traps and are more proficient at capturing the small YOY sculpin, but YOYs have also been captured in the jar traps (McDonald et al. 1982). The researchers from Toolike Lake found that the interface between the cobble of the littoral area with the softer substrate in the profundal zone of the lake had the highest abundance of slimy sculpin, corresponding to the density of their preferred chironomid prey (McDonald et al. 1982). In the same research region, researchers also found that in lakes with piscivorous predators (e.g. Lake trout, *Salvelinus namaycush*) that slimy sculpin were found more often on the coarse substrate than on the muddy bottoms of the study lakes (Hanson et al. 1992). There is clearly a trade-off with location of prey items and exposure to predators.

In terms of catch-per-unit-effort (CPUE), passive traps are useful but do produce relatively low catch rates. For Hanson et al. (1992), 24 traps were deployed within 25 m of the shore in each study lake for five weeks from June to August, and checked every three days. CPUE ranged between 0.087 and 0.39 sculpin per trap, meaning that approximately 2-9 sculpin were collected each day while deploying 24 traps in one given study site. For McDonald et al. (1982), CPUE would equate to about 8 sculpin per individual trap over a period of more than 25 days.

Riding Mountain National Park

Slimy sculpin is also being used by Parks Canada to monitor ecological health of lakes within the Riding Mountain National Park in western Manitoba (B. Reside, pers. comm.). This group of investigators is deploying Gee-type minnow traps for periods of 48 hours with multiple traps (8-10) deployed as an array in Clear Lake. Originally, the traps were baited with dog food, or glow sticks, but the researchers found that unbaited traps or traps baited with bread were more efficient at capturing sculpin in the lake (approximately 10 m deep; B. Reside, pers. comm.).

Deepwater Sculpin (Myoxocephalus thompsonii)

Custom traps were built to capture deepwater sculpin in many lakes across Canada (Sheldon et al. 2008). The traps were designed to lie flat on the bottom and offer the maximum possible catchment area at 0 -15 cm above the lakebed. It was important that the benthic fish traps be collapsible to reduce volume during transport. The traps were made out of 6 mm wire mesh (90 cm long x 45 cm wide x 15 cm high; Sheldon 2006). The reduced height of the trap, combined with the length of the funnel, caused the slope angle of the funnels to be less than 12^{o}, minimizing the vertical travel distance for fish entering the trap. The researchers predicted that this, along with the large trap catchment area, would significantly increase catch per unit effort of deepwater sculpin relative to traditional basket minnow traps. Of 46 deepwater sculpin captured in the traps, 40 were captured in the benthic fish traps. Subsequent to this trial period, only benthic fish traps were used (Sheldon 2006). On a cautionary note, CPUE information for deepwater sculpin is not directly applicable here since the deepwater sculpin is naturally less abundant and lives in a very different habitat than the slimy sculpin. Given that, 155 sculpin were captured in 20 lakes using more than 60 h of deployment for each of 15-30 traps per lake (elucidated from Sheldon 2008).

Summary of Trapping

The benefits of trapping are that it is a relatively benign and passive sampling technique and many traps can be deployed at once. The main limiting factor with this technique is that the level of effort required to capture sufficient numbers of slimy sculpin for a monitoring study is high. Factors such as the size of the mesh, the orientation of the traps, the number of traps, the deployment duration, the trap shape, and the aperture height may also influence the success of trapping for sculpin. Ostensibly, many flat traps with a wide funnel, fine mesh, a low aperture, long (i.e.48 h) deployment during the late summer will have the best chance of successfully capturing sculpin. However, a pilot study in one or many lakes may be required to identify the best techniques for sampling sculpin in any particular northern lake. For instance, the Riding Mountain study group was instructed to use glow sticks in their traps, but they found that their success was higher without a light source (B. Reside, pers. comm.). Even with these modifications, capture rates were estimated at about 0.04 fish/trap day.

The capture rates of sculpin using these trapping techniques, however, is likely to be highly variable and this should be considered when designing a monitoring program; pilot trapping studies are recommended and required to determine effort and the likelihood success of a project.

3.1.3 Trawling

Trawling techniques have also been used to capture sculpin in deep lakes (Foltz 1976). A bottom survey of Lake Michigan was done by the USGS using a 12 m trawl (Madenjian et al. 2005). The trawl was pulled for 10 min along predetermined transects that were spaced 9 m apart in depths of water that ranged from 9 to 110 m. These surveys captured both slimy sculpin and deepwater sculpin. Bottom trawls targeting sculpin species have also used in other Great Lakes (Brandt 1986, Owens and Noguchi 1998, O'Brien et al. 2009), smaller lakes across Canada (Sheldon et al. 2008, Carney et al. 2009), and Bear Lake in the western United States (Ruzycki et al. 1998).

The benefit of trawling for sculpin is that it is relatively less labour intensive than other active techniques, such as backpack electrofishing. The problems with trawling are that it requires more equipment, including boat and trawl, it is more labour intensive, it is potentially highly disruptive to benthic habitat and bycatch, and can be less effective in areas with rocky substrate (Stockwell et al. 2007). Additionally, hazards such as large woody debris and other benthic obstacles in boreal lakes may limit the use of trawling depending on the size of the lake and benthic complexity.

3.1.4 Artificial substrates

Artificial substrates have been used in spawning experiments on mottled sculpin (Downhower and Brown 1979) and slimy sculpin (Majeski and Cochran 2009). In these studies, the experimenters laid out tiles (Downhower and Brown 1979) and other substrate materials (Majeski and Cochran 2009) in streams and monitored them periodically for sculpin colonization. Majeski and Cochran (2009) found that although slimy sculpin would colonize the artificial substrate, the fish did not use them as nest sites. Downhower and Brown (1979) also had success in colonization.

This has potential as a capture technique since these studies use a segregation device to envelope the artificial substrate and isolate the fish beneath the tile prior to its removal. This technique could be used to sample fish in northern areas with difficult conditions, but it has never been used for monitoring. CPUE would be expected to be low in northern lakes where population abundance is not as high as southern systems.

3.2 SUMMARY OF CAPTURE METHODS

Although many studies have used trapping to capture sculpin in lakes, their capture efficiency is low and many traps must be used. Collapsible traps are recommended (A. Hershey, pers. comm.) since they are easier to transport long distances and in the field. The benefit of backpack electroshocking is in its much higher efficiency, but this will also be dictated by the substrate, the abilities of the crew, and the conductivity of the water. In new sampling areas, multiple gears should be initially used to determine which method works best in which area. Because the sample size requirements of non-lethal analyses is relatively high and productivity of northern systems is low, using minnow traps or other passive gears may not yield enough fish in the time designated for the study.

4. SAMPLING CONSIDERATIONS

There is little information on the life history of slimy sculpin in northern lakes, but work in other areas of Canada show that habitat and behaviour of fish are critical factors to consider in the success of sampling programs. Sculpin show relatively low mobility and have more restricted movement patterns in their spring spawning period. During the spawning period, males construct nests that they guard carefully during the spawning seasons, limiting their movements. In southern Canada, spawning starts around 8˚C, generally occurring in early to mid May. Hanson et al. (1992) trapped YOY sculpin in Toolik Lake, Alaska during the first week of July. Assuming a similar incubation period as that observed in Northern Saskatchewan of 28-29 d (van Vliet 1964), that puts spawning in the northern Toolik Lake at about the beginning of June. This suggests that slimy sculpin may be spawning at lower water temperatures coinciding with ice-out in that system (Hanson et al. 1992).

Male sculpin guard nests for a considerable period of time, and incubation times in the North may be longer due to slowly warming water and consequent slower developmental rate. At warmer temperatures, eggs take up to four weeks to incubate and hatch, so it is possible that males are guarding nests during early July. Using traps to collect fish during the spawning and egg incubation period will be less successful because active foraging is not a predominant activity.

Non-lethal methods for assessing growth, survival, and reproduction have been developed for assessing small-bodied fish populations (Gray et al. 2002); these are included in the EEM guidance documents (Environment Canada 2005). The collection of younger age classes can provide useful information about the status of the sample populations. For instance, length-frequency distributions can be used to identify young age classes, assess growth over time, and size at age when coupled with some aging analysis. In most studies of sculpin, there are high proportions of YOY fish (Gray et al. 2002, Arciszewski et al. 2007, RAMP 2010) indicating successful reproduction. YOY sculpin were not collected in Lac de Gras (Gray et al. 2005b), since they were likely too small at this time of the year to be shocked and netted. The use of 1+ fish as a surrogate for YOY analyses, however, is not direct since over-winter survival of the previous summer's 0+ cohort is integrated into estimate of reproductive success.

The best sampling time for slimy sculpin in northern lakes would be late August or even into September, as close to ice-up as is reasonable to ensure enough time to sample. This permits an increased chance of capturing YOY sculpin, and also more meaningful data on mature fish gonad sizes. In southern Canadian regions, male sculpin are best sampled in the fall, as they start gonadal recrudescence before the winter, while female sculpin are best sampled shortly before spawning (Brasfield 2007). Since sampling mature female sculpin at the optimal time is not plausible in northern lakes (i.e. would have to occur prior to ice out), information on gonad size in the later summer or early fall in northern lakes may suffice for comparison between test and reference sites.

The sex of mature slimy sculpin can be assessed non-lethally by visual inspection of the presence of the urogenital papilla in males and its absence in females (Figure 1).

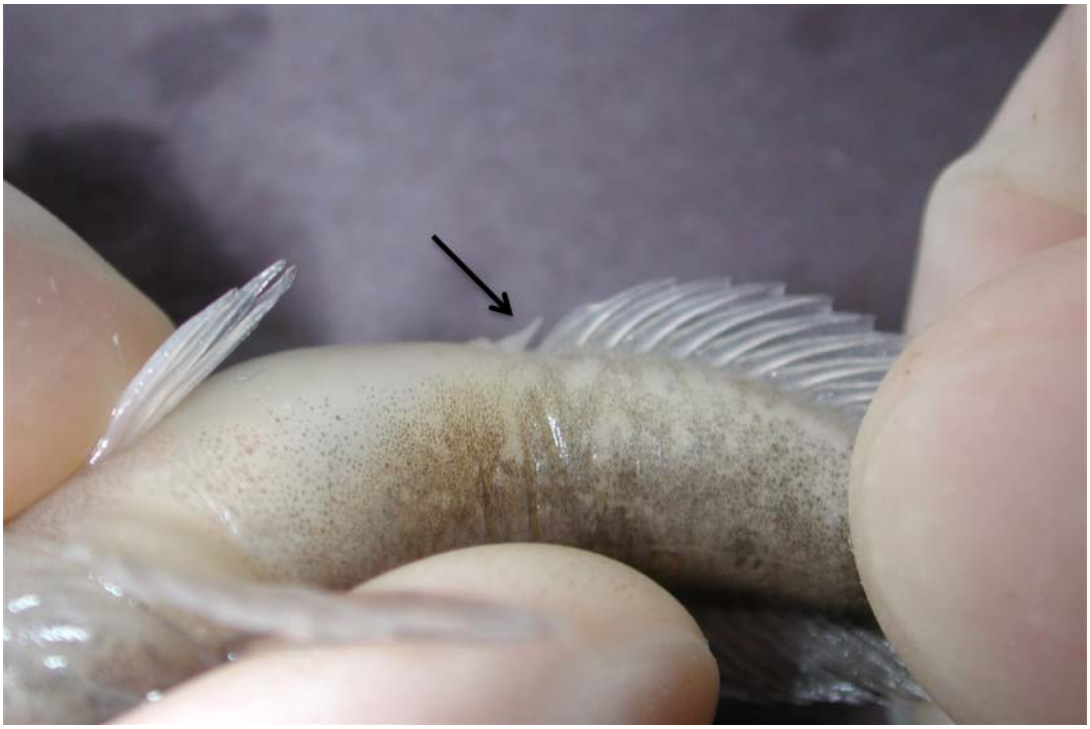

Figure 2. External sexing of mature slimy sculpin is possible by observing the presence (male) or absence (female) of the genital papilla located anterior to the caudal fin on the ventral side of the sculpin. *Photo credit: C. Porrt, Portt & Associates.*

4.1 STUDY DESIGN

The success of a sampling program will be strongly affected by the decisions made about the study design. Future studies should follow a similar site selection process as recommended in the Technical Guidance Documents for pulp and paper EEM (Environment Canada 2005) and in the Lac de Gras study (Gray et al. 2005b). The exposure areas are chosen for their proximity to the source of impact, such as a mine outfall, runoff entry point, waste rock leachate, or sediment plume. If possible, a minimum of three reference sites should be located in areas of natural substrate, outside of the influence of mine activities and preferably in locations that can be utilized as long-term reference sites (sites that will not be subsequently impacted by industrial activities). Three reference sites will provide valuable information on natural variability in the area due to differences in natural physical, chemical, biotic, and abiotic factors unique to each site. Ambient environmental conditions should also be recorded at these reference sites (e.g. water temperature, water quality) to help explain the natural differences that will be invariably found among reference sites. Collecting data on the ambient environmental conditions may also be useful in future development of regional monitoring studies. If possible, temperature and other probes should be deployed for as long as possible.

4.2 FISH ENDPOINTS AND DATA ANALYSIS

The endpoints from different types of surveys overlap considerably, but not all are available in all surveys (Table 1). For instance, non-lethal sampling integrates YOY analyses, while lethal programs focus on adult, or mature, fish endpoints.

Table 1. Endpoints for the survey types and the statistical tests used to analyze the data.

Endpoint	Survey Type		
	Lethal	Non-lethal	
		Single trip	Multiple trips
Length	✓	✓	✓
Weight	✓	✓	✓
Condition	✓	✓	✓
Liver weight	✓		
Gonad Weight	✓		
Age	✓		
Tissue metals	✓		
YOY Length		✓	✓
YOY Weight		✓	✓
YOY Condition		✓	✓
YOY Growth			✓
Size distributions		✓	✓
Sex Frequency	✓	✓	✓
Proportion of YOY		✓	✓
CPUE	✓	✓	✓

All non-lethally sampled fish will be measured for length (± 1 mm), weight (± 0.01 g) and released at the site of capture. Multiple non-lethal collections at the same location will provide useful information on size distributions that can be used to assess growth, survival, condition, and reproductive success. An electrofishing effort of a maximum of 12,000 seconds of electrofishing time (i.e. time the machine is delivering electric current in the water), should be considered as adequate effort to try and capture the objective number of slimy sculpin at each site in northern lakes. The presence of fish at the reference sites and the absence of fish at the exposure site should be interpreted as an impact (Environment Canada 2005). Catch-per-unit-effort (i.e. no. fish/second.) should be documented and will provide a measure of relative abundance between sampling areas.

The target sample size for non-lethal analyses is recommended to be 100 adult slimy sculpin from each site. It is unlikely that YOY sculpin will be collected during the summer collection due to their small size. The small size of the fish makes them less susceptible to the electric current, and also allows the fish to slip through the mesh of the net. To overcome this, a finer mesh size could be used for the landing/dip net, but a finer mesh will be more difficult to sweep through the water. Multiple collections of fish in the same year can also be used to document growth rates of fish (RAMP 2010). Of the 100 sculpin collected, 10 mature males and 10 mature females may be sacrificed and sampled for liver size, gonad size, and tissues taken for other objectives such as metal and metallothionein (MT) analysis. Normally EEM requires the collection of 20 adult males and 20 adult females from each site but removing those numbers of mature fish from these northern, underproductive lakes might not be sustainable. Using experienced personnel who can externally sex sculpin will also reduce any excessive lethal sampling in order to reach the target of 10 male and 10 females. Accumulation rates could be estimated by selecting a size range of fish.

An examination of the external and internal condition of each sacrificed fish will be conducted to determine the presence of abnormalities, lesions, tumours and parasites. Lethally sampled fish should be weighed (± 0.01 g) and measured for length (± 1 mm). Livers are then extracted and weighed (± 0.01g). The gut can be removed, weighed and contents examined and noted. Gut contents will be preserved in individual vials containing a 70% ethanol solution and returned to the laboratory for further analysis or confirmation. Otoliths can also be removed for fish aging purposes and stored separate from other tissues. Otoliths can be stored dry or in propylene glycol, but avoid exposure of the fish or otolith to formaldehyde as this can cloud the otolith, and interfere with accurate age determination.

Gray et al. (2005b) found a high incidence (up to 35%) of a cestoid parasite *(*Order: *Pseudophyllidea)* in slimy sculpin from Lac de Gras (Figure 3). The incidence and intensity of parasites should be recorded, and where possible, record the weight of the parasite(s) to provide a indication of parasite infection levels as well as providing a mechanism for adjusting the body weight when calculating somatic indices during the data analysis stage. For fish that are heavily parasitized, they will be unable to allocate sufficient energy to reproduction (e.g. significantly smaller gonads for a given age/size), and so these fish may have to be excluded from some or all of the data analysis due to the confounding factor of the parasites. Each fish liver and carcass should be placed in individually labelled sterile plastic bags and frozen immediately on dry ice. Tissues should be transported as soon as possible to an appropriate laboratory where they will be stored at -90˚C until they are analyzed for metals and other chemical parameters of interest.

Figure 3. Cestoid parasites of the Order Pseudophyllidea are tapeworms that can be found in the body cavities of fish as an intermediate host. They are characteristically flat, white in colour, and size will depend on the space in which they are able to survive. *Photo credit: J. Stefka, Natural History Museum UK.*

4.2.1 Power analysis

Power analyses are used in the EEM program during study design to determine the number of fish required to detect a given critical effect size at a given level of power. Power analysis is very important and often not well-understood and often not reported in monitoring study reports. This information is critical to the interpretation of data collected and also for designing follow up or future studies in the same region.

A priori power analyses

During the design phase of a study, *a priori* power analyses can be used to determine the sample size (number of fish) required to detect an effect (a pre-determined critical effect size) prior to sampling. This sample size can be calculated using the following:

- a critical effect size
- the probability of type I error “α”
- the probability of type II error “β”
- an estimate of reference variability

In cases where the required sample size calculated for one endpoint (e.g. condition) is greater than that calculated for another (e.g. relative gonad weight), the greater sample size should be used.

In EEM, CESs are used to help identify differences that are important and where more information and effort could be focused in order to understand the ecological significance of these differences (Lowell 2005). Although the values below are based largely on fish responses collected through the National pulp and paper mill EEM program, these responses have been determined to be biologically and ecologically significant and may at least provide a relative level of biological significance in an area like the north where responses patterns and environmental stressors are quite different and currently unknown. For fish gonad and liver sizes, relative to body weight, a CES of 25% is suggested, and for fish condition (i.e. length versus weight relationship (not condition factor)) a CES of 10% is suggested (Lowell 2005).

Reporting environmental monitoring data across different sites and regions should also include 95% confidence intervals as a practice, providing critical information on how well the estimated mean represented the expected mean and can allow practitioners to better interpret non-significant results.

4.2.2 Data Analyses

Assuming that the statistical assumptions are met for parametric statistical tests, simple comparisons of fish age, length, and weight can be made between fish collected in the exposure and reference areas using analysis of variance (ANOVA). Analysis of covariance (ANCOVA) may then be used to analyze liver weight and gonad weight against body weight by site (Environment Canada 2005). Sex ratio and size distributions can be compared statistically by Chi-square and Kolmogornov-Smirnov tests. Length-frequency distributions should be constructed for both the exposure and reference areas. Histograms of length should be prepared to visually compare the distributions between sites. Descriptive statistics should be reported for all variables, which includes arithmetic mean, minimum, maximum, quartile range, standard deviation, standard error, 95% confidence intervals, and sample size. Fulton's condition factor (k, Ricker 1975), liversomatic index (LSI) and gonadosomatic index (GSI) should be calculated and included in the summary statistics:

Condition Factor: $$k = 100{,}000\left(\frac{Body\ weight\,(g)}{Total\ length\,(mm)^3}\right)$$

Liversomatic Index: $$LSI = 100\left(\frac{Liver\ weight\,(g)}{Body\ weight\,(g)}\right)$$

Gonadosomatic Index: $$GSI = 100\left(\frac{Gonad\ weight\,(g)}{Body\ weight\,(g)}\right)$$

Comparisons of the concentrations of metals and MT in fish tissue and all supporting fish endpoints will be made using ANOVA. If the data have significant departures from normality the non-parametric Kruskall-Wallis test would be used. Data should be analyzed by sex. Relationships between MT concentrations and metals capable of inducing production of this protein (i.e. Cd, Cu, Zn, Hg, Ni, As) will be evaluated using linear regression analysis with metal concentration set as the independent variable and MT concentration as the dependent variable. Multivariate approaches are also useful to better interpret and understand the fish monitoring data. The level of alpha will be set at 0.05 for all tests.

5. REPORTING

A data report will be prepared and submitted in both electronic and printed form. The report will present the raw data (in an appendix) and the results of the statistical analyses. Catch-per-unit-effort (CPUE) and water quality parameters, including pH, temperature, dissolved oxygen and conductivity, should be presented in a table. Other measurements, such as condition, length, and weight, should be presented in a figure with the mean ± 1 standard error.

The report should provide an interpretive discussion of any differences in fish metal concentrations, MT concentrations and all other fish endpoints between the exposure, or test, and reference areas.

6. CONCLUSION

The objective of this guidance document was to provide an overview of slimy sculpin research related to environmental monitoring applications, as well as insights for future ecological and monitoring studies in northern Canada lakes. Characteristics of the slimy sculpin that make it an ideal sentinel species include its low mobility and high site fidelity, ubiquitous distribution through northern regions of North America, relatively high abundance in cool-water systems, short life-span, high reproductive output, easy-to-measure biological and physiological parameters, and benthic position of the food web. The efficiency and timing of collection needs to be considered for abundance estimates, as well as for changes in population structure and physiological parameters that occur during the year. Understanding basic biological information of a given species aids the interpretation of responses in environmental monitoring studies.

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