### **Holistic Monitoring Using Aquatic Bioindicators**

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## Introduction

Statoil Canada Ltd. and Matrix Solutions Inc. investigated the feasibility of using biological indicators to monitor the aquatic ecological health in the Christina River basin, northeastern Alberta. The indicator concept has a substantial history that has evolved over the past century. Originally indicator species were used to identify and characterize geographical units or type of regional habitat (e.g., central mixedwood forest). In recent decades, indicator species have been used to monitor watershed health and environmental change associated with human influences. Environmental regulations, such as water quality standards, are often based on the protection of relevant species (e.g., *Surface Water Quality Guidelines for Use In Alberta* [AENV 1999]) and; therefore, indicator species have become important measures of regulatory compliance (Carignan and Villard 2002).

Indicators of ecological health include components that reflect environmental change in a similar manner as do indicator species. These indicators of ecological health can be found at many organizational levels and represent "an element, process or property of the ecosystem" (Carignan and Villard 2002). These may include components of water quality (e.g., pH, turbidity, metals concentration), habitat area, species richness, biodiversity, algal biomass, metabolic rate, among others.

The selection of appropriate indicator species must be well thought out as bioindicators must be responsive to changes in their environment in a predictable manner. Effective bioindicators are linked to elements in the environment (i.e., the habitat and other associated species in the biological community) in a manner where environmental changes are reflected in a response by the indicator (Karr and Chu 1999). Several criteria can be used to evaluate potential indicator species. Each criterion represents an interaction between the indicator species and an important ecosystem property (Caro et al. 2004; Garibaldi and Turner 2004; Lambeck et al. 1997; Mills et al. 1993; Paine 1969; Roberge and Angelstam 2004; Suter et al. 2002).

The objective for this project was to identify potential species candidates for bioindicator monitoring in the Christina River and tributaries, and to assess the feasibility of these selected indicators for long-term monitoring. Conventional monitoring programs for projects in the oil sands region are based on predictions generated in the Environmental Impact Assessment process. The intent of this project was to develop a holistic monitoring approach where the monitoring endpoints reflect overall environmental condition in a manner not captured by conventional monitoring. The bioindicator species were chosen for being responsive to changes in their environment in a predictable manner; these species are linked to elements in the environment (i.e., the habitat and other associated species in the biological community) where changes in the elements are reflected in a response by the indicator.

Three locally important aquatic species were selected for study, these included Arctic grayling (*Thymallus arcticus*), slimy sculpin (*Cottus cognatus*), and a freshwater mussel, the fat mucket (*Lampsilis siliquoidea*) to represent key monitoring components of the aquatic community. In addition, information on low level water chemistry was collected using passive samplers (semi-permeable membrane devices [SPMDs] and diffusive gradient in thin films [DGTs]).

## Methods

Study Area – The Upper Christina River Watershed is situated in the southern oil sands region of northeastern Alberta with a drainage area of approximately 13, 200 km<sup>2</sup>. Oil extraction in this area is largely being developed with Steam Assisted Gravity Drainage (SAGD) technology. Development in the watershed is currently low; however, a number of projects are planned in the area.

Headwaters for the Christina River originate in both upland and lowland areas where wetlands such as fens, bogs and shallow lakes, are common. The mid-section of the Christina River is connected to a number of tributaries, some of which have large lakes providing source water. The river eventually confluences with the Clearwater and Athabasca Rivers near Fort McMurray. The climate is generally moist and cool with short summers and long, cold winters. Discontinuous permafrost may occur in the basin where bogs exist.

Population genetics – Fin clips were collected from grayling and sculpin for genetic statistical analysis to evaluate population dynamics. Analysis included investigating allele (gene) variability (expected and observed heterozygosity), allele diversity, and effective population size. Arctic grayling population analysis was intended to identify population structure of an indicator that represents a large spatial scale. Results were compared with those reported in literature (Stamford and Taylor 4004, 2005) to ascertain how robust the population may be. Slimy sculpin analysis was intended to establish the level of population structure of a species representing small spatial scale population dynamics. Sculpin were collected in two sections of the river (upstream and downstream); results from each were compared to assess genetic variability within the watershed.



Environmental DNA surveillance - Water samples were collected and filtered from various locations to collect Arctic grayling DNA in river water. Real time polymerase chain reaction (PCR) was used to detect the presence of DNA in filter residue from the samples. Fish slough off cells through the production of slime coating, during excretion of fecal matter (cells from the gut lining), and by scraping on surfaces; these can be detected and amplified using PCR technology.

Tissue Analysis – Slimy sculpin and fat mucket tissue was collected, frozen and for laboratory analysis of metals and polycyclic aromatic hydrocarbons (PAHs). Passive Samplers were deployed at six locations along the Christina River for 23 to 27 days duration to determine low level concentrations for metals and PAHs to compare with tissue results. The results of this comparison demonstrated the efficacy of using passive samplers to represent biologically relevant tissue concentrations.

# Results

Genetic variability in the Christina River Arctic grayling was similar to expected indicating minimal pressure from inbreeding or assortive mating. This population was found to be as genetically diverse as populations in similar systems elsewhere in northern Canada. The effective population numbers were relatively high, the population appeared to be robust and not currently at risk of going through a bottleneck.

Sculpin and sculpin habitat were not found in upper tributaries of the Christina River, but rather in the mid to lower sections of the river where optimum habitat was present. The population appears continuous within its range. No significant differences in allele frequencies were found between the upstream and downstream sculpin populations in the river and there appeared to be little restriction of gene flow between these populations.

The environmental surveillance for DNA detected grayling DNA in samples collected at a number of locations. Positive grayling DNA, negative controls and trout control were used to confirm results. These findings demonstrate the potential for this technology in monitoring aquatic species as well as investigate aquatic biodiversity and rare species identification. Further method improvements are expected to increase resolution and certainty of environmental DNA sampling analysis. The results of the tissue analysis provided good baseline information on metals in sculpin and mussel tissues; however, no PAHs were detected in tissue from either species. Metal tissue concentrations were compared with analytical results from the passive samplers. Metal concentrations in the DGTs reflected sculpin and



mussel tissue metal analyses, thus strongly demonstrating the reliability of DGTs in assessing aquatic impacts.

The SPMD analysis indicated variability in river water PAH concentrations and demonstrated PAH concentrations can be detected at low levels and measured in a manner that is relevant to environmental monitoring.

# Discussion

The importance of this work was that it demonstrated how innovation and modern technologies were applied to develop a holistic monitoring program intended to ascertain incremental environmental changes. This approach encompassed local community and societal values as well as provides industry better assessment tools to manage their presence in the region.

The challenge of many environmental monitoring programs is relating information collected in the field with a particular development or activity. Through project planning and mitigation, most SAGD oil sands projects area able to minimize or eliminate direct impacts on aquatic systems. Detecting indirect impacts and unintended consequences of development is a challenge; however, industry is recognizing its need to understand potential small and incremental changes to limit their environmental footprint.

Changes in the aquatic environment associated with development may be reflected in the aquatic ecosystem (e.g., in hardness and alkalinity, redox potential, pH, trophic changes, algae, etc.). In turn these conditions will affect the bioavailability of metals and other constituents, and consequently the tissue concentrations observed aquatic organisms. This may result in selective pressures that affect demographics, breeding relationships and differential reproduction causing a change in population structure and genetics. Any resulting changes in fish distribution could disrupt the current rate of gene flow in the river. Upstream fish could become isolated resulting in inbreeding, thus increasing the population's vulnerability, especially at the upstream edge of its range.

Due to their role in the environment, grayling, sculpin and mussels can reflect the environmental integrity of the watershed. They are linked to many components of the aquatic system and thus respond to changes in ecological processes, increases in pollution and changes to habitat. Arctic grayling are sport fish important to local indigenous peoples and the recreational fishing community. Slimy sculpin and mussels are sensitive to change and respond quickly to environmental perturbations. All three species are sensitive to watershed activities, especially pipeline and road development, logging and



overfishing. Small incremental environmental changes that may or may not be detected through standard monitoring methods (e.g., low level water quality analysis) may be revealed through minor changes in the population as detected through population genetics, tissue analysis and other parameters.

Population genetics are not fundamentally new, they are used to manage fish stocks by government biologists; however, the methods applied in this program are unique in that they have not previously been applied to environmental monitoring by industry. Population genetics can be used to measure the strength of fish populations, detect potential bottlenecks and if a population is vulnerable to local extinctions. Long term population monitoring can validate the effectiveness of environmental mitigation programs or help direct the management of industrial activities consistent with corporate environmental objectives.

Environmental DNA surveillance is a novel approach to monitoring and demonstrates creativity and continued future technical method development. Environmental DNA monitoring has largely been confined to academia and research. It has been applied in only a few monitoring scenarios including assessing the extent of the invasive Asian carp in the United States. We collected environmental water samples and amplified fish (general bony fish sequences) and Arctic grayling DNA to determine their presence at select locations in the Christina River and a large tributary. The results demonstrated that environmental DNA surveillance has numerous applications for monitoring biodiversity in aquatic and terrestrial assessment programs.

Methods to monitor water quality using sculpin and fresh water clams have been developed by other industries (i.e., pulp and paper, mining) where distinct and defined impacts are known. However, these methods are not commonly applied where indirect environmental changes are of interest. Our studies found strong relationships between metal concentrations in sculpin and mussel tissues and results from passive samplers deployed in the Christina River thus improving our understanding of environmental pathways between water quality and aquatic organisms. This evidence can help us better use passive sampling technologies and relate the results to aquatic species.

Traditional monitoring methods may not detect environmental changes in a manner that is observed though monitoring of bioindicators. Societal values regarding environmental wellbeing have shifted requiring industry to provide a more holistic understanding of the environments being developed. The



result obtained from this program allows us to address some of these gaps and provide a means of securing our future environmental sustainability.

This study demonstrates that these aquatic species have a number of attributes that can be measured in a manner that reflects environmental conditions. Information provided from monitoring unique aspects of multiple species can reveal environmental dynamics at a number of levels not detected from traditional monitoring. Future work should investigate relatedness between the degree of environmental change and observed responses measured in aquatic organisms.

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