Community Level Physiological Profiling for Monitoring Oil Sands Impacts

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Oil Sands Research and Information Network

OSRIN is a university-based, independent organization that compiles, interprets and analyses available knowledge about returning landscapes and water impacted by oil sands mining to a natural state and gets that knowledge into the hands of those who can use it to drive breakthrough improvements in reclamation regulations and practices. OSRIN is a project of the University of Alberta's School of Energy and the Environment (SEE). OSRIN was launched with a start-up grant of $4.5 million from Alberta Environment and a $250,000 grant from the Canada School of Energy and Environment Ltd.

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- **Media, opinion leaders and the general public** with the facts about oil sands development, its environmental and social impacts, and landscape/water reclamation activities – so that public dialogue and policy is informed by solid evidence
- **Industry** with ready access to an integrated view of research that will help them make and execute reclamation plans – a view that crosses disciplines and organizational boundaries

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REPORT SUMMARY

Alberta Innovates – Technology Futures (AITF) conducted a review of microbial Community Level Physiological Profiling (CLPP) as a means of monitoring aquatic ecosystem health for the Oil Sands Research and Information Network (OSRIN). Relevant research was compiled from journal articles, the websites of government and non-governmental organizations, and in-house experimental results. The objective of the project was to better understand and describe the potential for CLPP to provide meaningful assessments of aquatic ecosystems in the oil sands region of Alberta to various stakeholder groups.

Ecological monitoring techniques are used to assess the effects of industrial development in the region, and to assess the effectiveness of reclamation efforts. Current techniques, while effective, are difficult and expensive to implement on a regional scale. As a group, microbial community profiling technologies offer the potential to screen multiple systems rapidly, inexpensively, and relatively easily, compared to traditional assessment methods.

CLPP has the potential to be the easiest and least expensive microbial profiling technology. However, some technical advancements must still be made before its full potential can be realized. Beyond this, a significant body of background information regarding the effect of a number of environmental variables on the profiles produced by CLPP must be compiled, both as a source of reference information and to better define the performance characteristics of the assay.

A number of organizations conduct ecological research and/or monitoring in the region. Some (e.g., RAMP, AENV) could see direct benefits from the incorporation of CLPP into their operations. Others (e.g., CONRAD, CEMA) may realize a lesser degree of benefit. Organizations focussing on specific aspects of aquatic ecosystems (e.g., DUC, DFO) are unlikely to see their missions advanced by the adoption of CLPP as an ecological monitoring tool.

Overall, we recommend investment of time and resources into CLPP and microbial community profiling in general. The expenditures required are likely to be quite small compared to the potential utility of the technology.

ACKNOWLEDGEMENTS

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1 INTRODUCTION

1.1 Oil Sands Context

1.1.1 *Scope of Impact*

Oil sands development in Alberta is proceeding on an enormous scale. Oil sands underlie approximately 140,200 km^2 of land in the Athabasca, Cold Lake, and Peace River regions. Just over 600 km^2 of this area has already been disturbed (Alberta Energy n.d.). The most obvious disturbance of aquatic ecosystems in the region is their complete removal during oil sands mining or related activities. However, the withdrawal of water for industrial use, chemical pollution, and the diversion of lotic systems could also adversely affect the ecosystems. It is not clear exactly how many aquatic ecosystems have been disturbed or will be reclaimed subsequent to oil sands development. However, roughly 39% of the oil sands region is occupied by wetlands (bogs and fens being the dominant types), and another 5% occupied by rivers, lakes, and streams. In total, the region could see the loss of up to 1,300 wetlands (Grant et al. 2008).

1.1.2 *Scale of Reclamation*

The oil sands industry is required to return land disturbed during mining to "an equivalent land capability" by the *Conservation and Reclamation Regulation* (AR 115/93) (Gosselin et al. 2010). While the actual requirements are still somewhat unclear (Cryderman 2010), reclamation of aquatic ecosystems of some type will be required, though a shift from peatland-dominated systems to areas with increased amounts of upland, non-peat forming wetlands, and lakes is expected (Johnson and Miyanishi 2008), and wetlands that are created during reclamation will have elevated levels of salinity (Trites and Bayley 2009). Even after their design and construction, monitoring of reclaimed aquatic systems will be required for years to come (Harris 2008). Given the scale of potential development, the prevalence of aquatic ecosystems on the landscape, and the long timelines involved, aquatic ecosystem reclamation in the oil sands region is a significant commitment.

1.2 Monitoring Aquatic Ecosystems

1.2.1 *Definition of Aquatic Ecosystems and the Need to Monitor*

An ecosystem may be defined as "...not only the organism-complex, but also the whole complex of physical factors forming what we call the environment of the biome" (Ricklefs 1984). An aquatic ecosystem is one existing within boundaries defined by a body of water. The surficial aquatic ecosystems that exist in the oil sands region of Alberta consist primarily of lakes, wetlands, rivers, streams and man-made bodies of water. These are, except in special circumstances, freshwater ecosystems.

This report will focus on the ability to monitor the ecological status of these freshwater aquatic ecosystems. That is, the ability to assess and describe the state of these ecosystems in

biologically meaningful terms. Given the complexity of the task, it is appropriate to define the goals behind such an objective.

1.2.1.1 Baseline Assessment

The natural state of aquatic ecosystems will vary from system to system. If a given ecosystem is going to be monitored, it is important to create a baseline dataset as a means of establishing evaluative criteria. This provides the most relevant means of evaluating the ecological impact of industrial development. In some cases, it may be possible to establish a baseline dataset by monitoring a given system for some time prior to disturbance. In other instances, such as the reclamation of aquatic ecosystems, such a strategy may not be possible. In those cases, appropriate target or reference systems must be selected. The selection of appropriate reference systems is central to ecological monitoring, and the subject of ongoing discourse (Gosselin et al. 2010, Harris 2008).

1.2.1.2 Impact of Industrial Processes

Monitoring of an aquatic ecosystem impacted by industrial development must continue beyond the generation of baseline data. Without such monitoring, the impact of development cannot be determined. Such monitoring can also be used in the selection/development of 'greener' technologies by providing insight into the relative performance of alternative industrial processes or reclamation techniques.

1.2.1.3 Assessment of Reclamation Projects

The recovery process of disturbed/reclaimed ecosystems can be expected to take years, if not decades, to complete. Although some systems have been found to self-organize with minimal intervention (Prach and Hobbs 2008, Tropek et al. 2010), or settle into predictable trajectories after just a few years of active reclamation (Grant and Koch 2007, Koch 2007), the ability to predict the outcome of most reclamation efforts may be limited (Cortina et al. 2006, Hobbs et al. 2009, Lake 2001, Zedler 2000, Zedler and Callaway 1999). A monitoring program can track the progression of a reclaimed ecosystem through successional stages, and identify deviations from the desired recovery trajectory early enough to allow effective intervention. Without an effective monitoring program, coupled with management approaches to guide recovery along the desired path, site reclamation may result in an ecosystem very different from the one intended.

1.2.2 *Traditional Aquatic Ecosystem Monitoring Methods*

1.2.2.1 Chemical

When monitoring aquatic ecosystems, chemical analyses are most often water based. Common parameters include measures of organic and inorganic carbon, nutrients, pH, dissolved and suspended solids, salinity, chlorophyll, and specific ions. Some of these parameters have multiple measures directed towards specific forms of the analyte (e.g., nitrate vs. nitrite vs. total nitrogen). Other, more specialized, assessments (e.g., acetylene reduction to measure nitrogen fixation, sediment chemistry) are available, but are only employed under specific circumstances.

It is important to distinguish chemical analyses for the purposes of ecological assessment from those performed to assess drinking water quality and/or the presence of specific pollutants. For example, specific chemicals that are indicators of the industrial disturbance (e.g., naphthenic acids for oil sands) are used to indicate contamination and/or track remediation and reclamation progress. While these analyses may share some common aspects with their ecological counterparts, their interpretive guidelines differ.

A large amount of background knowledge is available linking ecological status and water chemistry. For example, algal blooms are known to be promoted by the introduction of biologically available forms of nitrogen and phosphorus. In such circumstances, increases in total and soluble forms of nitrogen and phosphorus could be detected initially, followed closely by an increase in chlorophyll. Quantitative targets may already be available for various parameters (Harris 2008). Furthermore, the methods for the detection and quantification of specific analytes are well established and are available from multiple commercial, government, and academic entities.

On a scientific level, chemical analyses can provide important context for ecological status, but are not definitive in and of themselves. If not recognized, this limitation can lead to over-interpretation of chemical results, and the establishment of 'acceptable values' that may not be ecologically meaningful. For example, a sudden increase in dissolved nitrogen and phosphorus will pre-dispose a lake to an algal bloom event, but may not be sufficient to cause one. In such a circumstance, nitrogen and phosphorus levels may well be above a certain prescribed threshold, yet no algal bloom occurs.

By and large, samples for water chemistry are 'grab' samples. That is, a defined sample volume is collected once or on a scheduled basis. Such sampling is seldom sufficient to take into account the full range of variation present within an ecosystem. While automated sampling devices are available, they can be expensive, and still provide only a limited period of sampling. In terms of analysis, the equipment and personnel needed to perform many of these analyses incur considerable expense. The large numbers of samples required to form an integrated picture of water chemistry in a given ecosystem can be costly indeed.

1.2.2.2 Physical

Physical measurements with respect to aquatic ecosystems include temperature, turbidity, conductivity, soil/sediment density and water content, flow rates for lotic systems, water volumes and source, and topographical features such as slope, depth, and area. Most of these measures are relatively easy to collect through on-site measurements, laboratory analyses, or remote sensing.

However, not all of the data are easy to obtain. For example, it may be quite difficult to determine the proportion of water entering a lotic system from surficial vs. groundwater sources. Sophisticated survey and laboratory techniques are required for such a task. Many of the parameters investigated will also vary over time, leading to some of the same limitations described for the chemical analyses. Finally, while physical characteristics of an ecosystem can

be considered fundamental to its function, it is impossible to make meaningful statements regarding the status of that system from physical data alone.

1.2.2.3 Biological

Biological evaluations can be divided into floral and faunal assessments. Both assessments are typically achieved through surveys based on relative abundance and taxonomic identification. For floral surveys, these include terrestrial, emergent, and aquatic organisms. There has also been some work done looking at algal (eukaryotic and prokaryotic) communities as indicators of ecosystem health (Mize and Demcheck 2009, Paerl et al. 2003, Rodriguez et al. 2007). Benthic or aquatic macroinvertebrate community analyses are commonly employed faunal assessments. This approach requires the collection of water or sediment samples followed by the microscopic identification and enumeration of invertebrates.

A primary benefit of biological assessment is that it integrates the effect of ecological perturbations over time. That is, changes in the physical or chemical characteristics of an ecosystem will, over time, manifest as changes in the biological conditions. This represents a major advantage over the 'snapshot sampling' associated with chemical and physical assessments. Moreover, it is the biological status of an ecosystem that, more than any other aspect, is associated with a social value. While there may be many overlooked economic and social benefits associated with these ecosystems, it is often the floral and faunal characteristics (e.g., blue-green algae blooms, declines in fish populations) which elicit concerns from media, politicians, non-governmental organizations, and the general public. By assessing the biologic characteristics directly, one can address some of those concerns.

Assessment of biological indicators is sensitive to scale. In some cases, a few transects or a few litres of sediment are enough to acquire a representative sample. In other cases, very large areas must be surveyed to obtain sufficient data. Often these large areas contain multiple aquatic ecosystems exhibiting different ecological states. From a logistical point of view, the biological dataset may be the most difficult and expensive to collect (e.g., when specialized equipment, such as electrofishing boats, is required for specimen collection) and analyze. The field and laboratory work can be time consuming, tedious, and sometimes arduous. Often, experts in relatively obscure fields (e.g., experts in phytoplankton or zooplankton identification) are required to generate and/or analyze the data. Such experts can be difficult to find and expensive to employ.

2 COMMUNITY LEVEL PHYSIOLOGICAL PROFILING

2.1 Description of Method

Community Level Physiological Profiling (CLPP) is a technique which produces a metabolic fingerprint for a given microbial community. We believe that this technique can be used to provide accurate, inexpensive, and rapid ecological assessments for aquatic ecosystems in the oil sands region. These assessments would provide sufficient information to identify those systems in need of more detailed evaluation or intervention. Given these attributes, CLPP may be most

useful as a screening assay, allowing multiple systems to be investigated over multiple time points without incurring prohibitive costs or time commitments.

The technique starts with obtaining a sample of the indigenous microbial community from an aquatic ecosystem. While previous investigators have used 'grab' samples of water or sediment, we have chosen to use microbial biofilm communities. Microbial biofilms are collections of microorganisms that exist in a multicellular community form in an exopolysaccharide extracellular matrix, adherent to each other or a surface (Morck et al. 2001). Samples of this type afford us a number of advantages over grab samples; these advantages are explored more fully in section 2.5.8. We collect biofilms by suspending clean acrylic plastic rods approximately 5 cm below the surface of the water by means of a float (Figure 1). These rods are left in place for one week. At the end of that time, they are harvested and transported back to the laboratory in sterile polypropylene tubes (Figure 2).

Once in the laboratory, the rods are transferred to a sterile buffer solution, and sonicated to release the biofilm. The resulting microbial suspension is transferred to 96-well microtitre plates known as Biolog EcoPlates (Biolog Inc. Hayward, California, USA[1]) and incubated aerobically at a set temperature for 72 to 96 hours. Within each of the 96 wells of the EcoPlate is an organic (carbon) compound, and a tetrazolium dye. When microorganisms are introduced into the well, they attempt to catabolize the carbon compound. If successful, they will also reduce the tetrazolium dye into a purple formazan compound. Following incubation, the EcoPlates are moved to a spectrophotometer where the optical density of each well is measured. The greater the degree to which the carbon compound in a given well has been catabolized, the higher the optical density (i.e., the darker the purple dye) of that well (Figure 3). By identifying which wells have an optical density above a specified threshold, we can profile which carbon compounds can or cannot be catabolized by a particular microbial community.

The final stage of the process is the interpretation of the carbon use profile as it relates to various ecological parameters. At present, these relationships are incompletely described, and are the subject of ongoing research.

[1] See http://www.biolog.com/

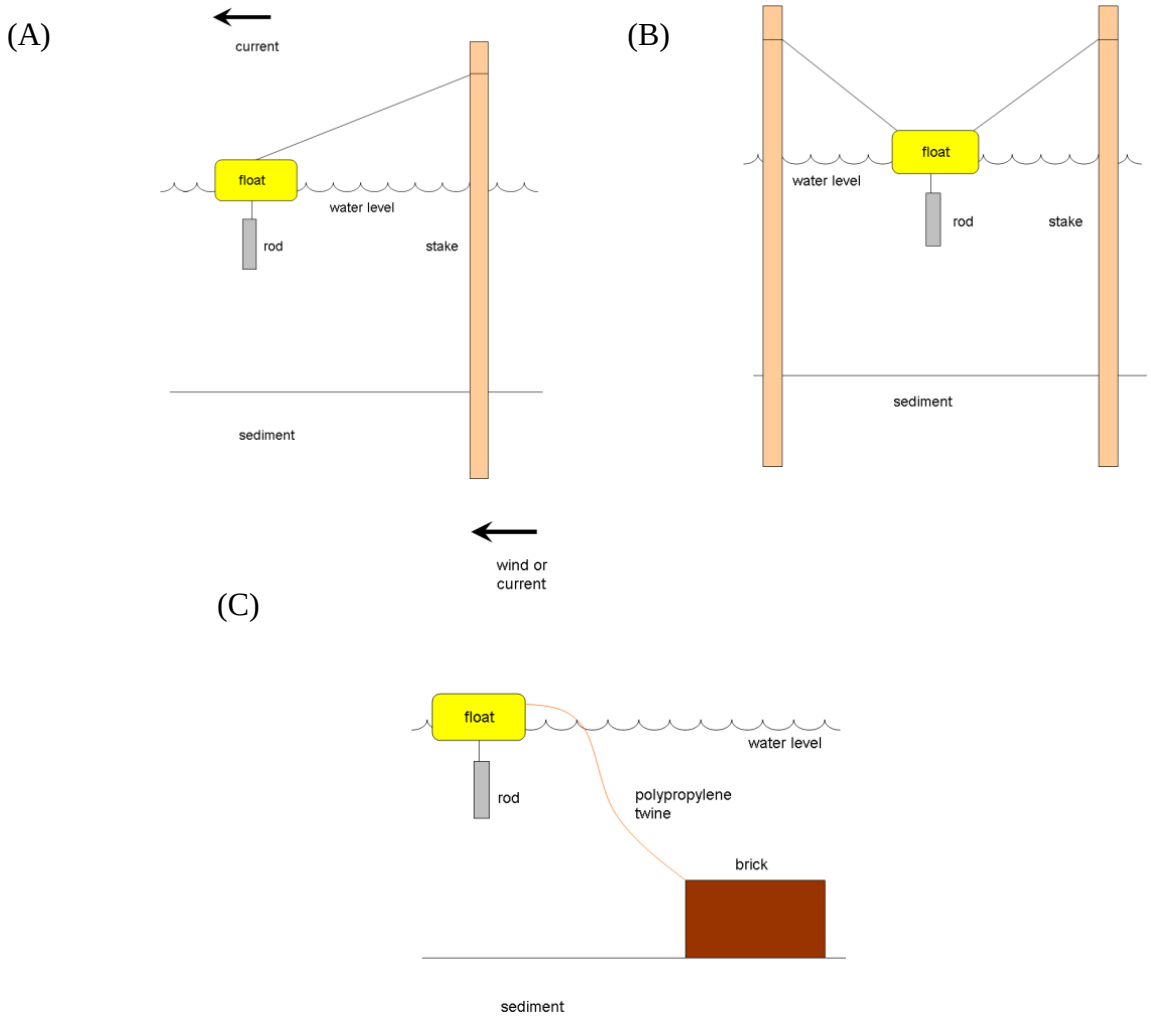

Figure 1. Sampling devices for collecting microbial biofilms from aquatic ecosystems: arrangement of sampling devices for (A) shallow water lotic, (B) shallow water lentic, and (C) deep water ecosystems.

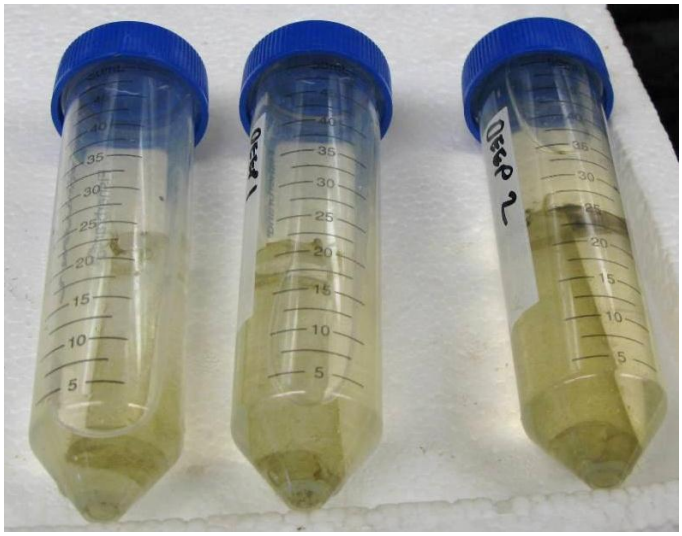

Figure 2. Acrylic rods as harvested and stored within sterile tubes.
Note the dull green colour associated with the presence of photoautotrophs in the biofilm.

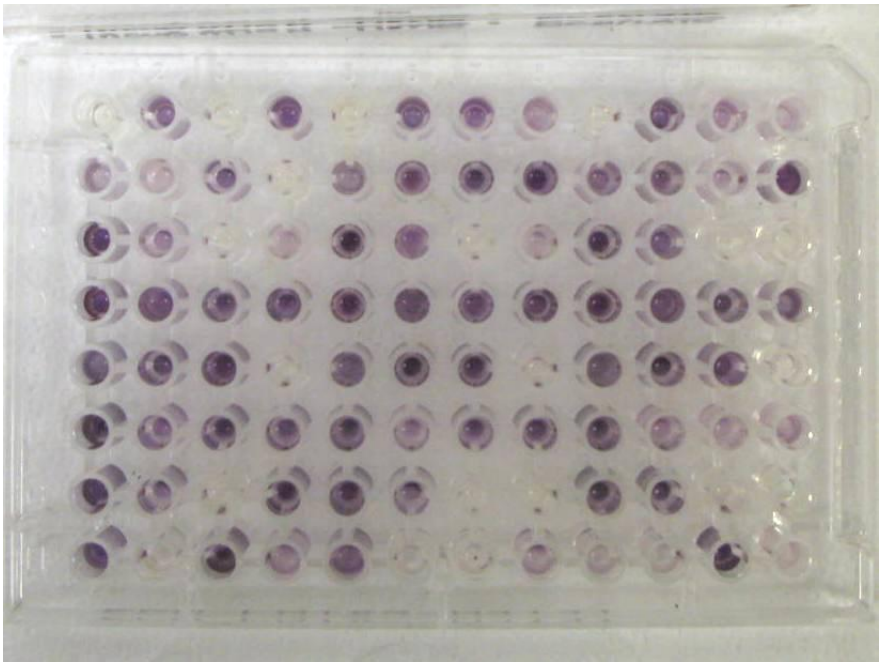

Figure 3. Photograph of a Biolog EcoPlate after incubation.
Note that some wells are purple (carbon source utilized) while others remain clear (no carbon source utilization). Note also that the intensity of colour varies, and likely reflects the degree to which the carbon source was catabolized.

2.2 Supporting Principles

Put succinctly, the character of a microbial community is primarily determined by its environment. Some researchers argue that non-environmental factors (e.g., dispersal, successional community development) are the primary determinants of microbial community structure. However, a study conducted by Van der Gucht et al. (2007) was a powerful demonstration that local environmental factors, not issues of dispersal, are the major determinants of bacterial community composition. These investigators characterized samples of bacterial communities from three separate lake systems in Spain, Denmark, and the Netherlands/Belgium. After the removal of environment-related variation in community composition, the study areas could no longer be resolved by their microbial profiles. In another study, natural microbial communities were allowed to partially develop in one river, and then transferred to another river exhibiting contrasting physico-chemical characteristics (Paule et al. 2009). The community structure changed to suit its new environment rather than continuing upon its original trajectory. Our own experiments have also suggested that for both lotic and lentic systems, environmental factors are more important determinants of the CLPP than location. In practice, however, major environmental factors such as climate, geology, and surrounding ecosystems are inextricably linked to location. Such factors must be considered when designing a monitoring program based on microbial communities.

The mechanisms by which microbial communities react to their environment are complex and poorly understood. However, these mechanisms can be summarized as the interaction of the environment with genotypic and phenotypic characteristics. The environment selects for specific genotypes and induces/suppresses certain phenotypes. For example, the presence of an antibiotic agent in the environment will tend to, over time, select for bacteria with genes coding for resistance to that antibiotic. Bacteria without these genes will be killed. This is an example of the environment selecting for a certain genotype. This selection is mediated through phenotype. Prior to antibiotic exposure, even those bacteria possessing antibiotic resistance genes are unlikely to express them, as there is no need. However, within minutes of exposure, those bacteria possessing the genes for antibiotic resistance will begin to express them. This represents a phenotype that is induced by a change in the environment. Both of these principles are involved in the configuration of microbial communities to suit the environment.

Microorganisms and their community structure must be characterized by technological means. Traditionally, microorganisms have been characterized by their biochemical characteristics (Brock and Madigan 1988). Usually, these techniques are based on traits such as the ability to use specific molecules as energy/nutrient sources, or the ability to tolerate adverse environmental conditions. These techniques are usually employed to isolate/identify specific microorganisms. For example, MacConkey agar is a bacterial growth medium that uses both differential nutrient metabolism and adverse environmental conditions to select for certain microorganisms. The chemical characteristics of MacConkey agar inhibit the growth of most Gram-positive bacteria, while allowing the growth of Gram-negative bacteria. Furthermore, it can differentiate between Gram-negatives that can ferment lactose from those that cannot. These biochemical characteristics (Gram status, lactose fermentation) are very useful in differentiating certain

bacterial genera (*Escherichia*, *Klebsiella*, *Enterobacter*) from others (*Salmonella*, *Shigella*, *Proteus*, and *Pseudomonas*). While this example is based on the evaluation of pure cultures, the same approach can be used to evaluate whole microbial communities, and forms the basis of CLPP.

2.3 Detection of Changes in Natural Microbial Communities

While it stands to reason that microbial communities *should* change in response to changes in their environment, it is only prudent to look for evidence that they *do* change. Furthermore, the use of microbial communities as ecological indicators depends upon our ability to adequately characterize those communities and detect changes associated with ecologically significant shifts. The literature provides numerous examples of microbial communities changing in response to environmental variables as well as documenting our ability to characterize those changes using multiple technologies.

2.3.1 *Genotypic Assessments*

A number of genetic profiling tools have been developed to evaluate microbial community genotypes, including Terminal Restriction Fragment Length Polymorphism (T-RFLP), Fluorescent *in-situ* Hybridization (FISH), and Denaturing Gradient Gel Electrophoresis (DGGE). By far, the most commonly used technique is DGGE[2]. In this approach, DNA is extracted from the entire microbial community and a certain target sequence is amplified using polymerase chain reaction (PCR). The product of PCR is a 'soup' of different DNA sequences representing the different microorganisms present in the community. When run through an electrophoretic gel, this 'genetic soup' will resolve into discrete bands, each band representing a different group of microorganisms based on the DNA sequence of the amplified target. By observing differences in the band pattern, one can detect differences in the microbial community at a genetic level.

A number of authors have used DGGE to investigate changes in natural microbial communities (Araya et al. 2003, Boivin et al. 2006, Bouskill et al. 2010, Castle and Kirchman 2004, Docherty et al. 2006, Douterelo et al. 2010, Drenovsky et al. 2008, Duarte et al. 2009, Jin and Kelly 2007, Li et al. 2008, Montserrat Sala et al. 2008, Paule et al. 2009, Polymenakou et al. 2005, Ringbauer et al. 2006, Röling et al. 2000, Ros et al. 2008, Tian et al. 2008, Van der Gucht et al. 2007, Zeng et al. 2008). Where examined specifically, the vast majority of these studies have identified local environmental variables as the most significant factors affecting microbial community structure. Nitrogen and organic matter indices as well as pH have been identified repeatedly as important factors shaping microbial community structure (Docherty et al. 2006, Duarte et al. 2009, Polymenakou et al. 2005, Van der Gucht et al. 2007, Zeng et al. 2008). Other important environmental factors include total biomass and proportion of certain aquatic invertebrates, size of water body, abundance of autotrophic prokaryotes (Van der Gucht et al. 2007), trophic status (Duarte et al. 2009), phosphorus and redox potential (Zeng et al. 2008), the

[2] See http://en.wikipedia.org/wiki/Temperature_gradient_gel_electrophoresis

presence of livestock (Merkley et al. 2004), and chlorophyll *a* (Polymenakou et al. 2005). Given the above list of factors, it is not surprising that season, which would have an impact on virtually all of these factors, was repeatedly identified as an important variable (Anderson-Glenna et al. 2008, Bouskill et al. 2010, Ringbauer et al. 2006). Furthermore, the sudden introduction of ecologically damaging pollutants (Boivin et al. 2006, Feris et al. 2003, Li et al. 2008) had dramatic effects on microbial communities.

Running counter to the prevailing findings is the study by Bouskill et al. (2010) which concludes that microbial community diversity is primarily a function of geographic distribution rather than local environmental factors. However, even within this study, microbial community diversity demonstrates large and statistically significant Spearman Correlation Coefficients (0.618 to 0.786, $p = 0.001$ to 0.003) with respect to local concentrations of organic carbon, arsenic, copper, and zinc.

2.3.2 *Phenotypic Assessments*

Phenotypic profiling offers an alternative to the genetic methods. Phospholipid Fatty Acid Analysis (PLFA) and CLPP are the most commonly used techniques, with selected microscopic procedures being used occasionally. PLFA is a technique based on the chemistry of the phospholipids found in cell membranes. Total lipids are extracted from a sample, and the phospholipid fraction is isolated, methylated, and analyzed via gas chromatography[3] (Drenovsky et al. 2008). Since different groups of microorganisms (e.g., fungi vs. bacteria) vary in the chemical composition of their cellular membrane, changes in the phospholipid fatty acid profile indicate changes in community composition. The same technique can be used to estimate the total microbial biomass as well as the biomass of certain sub-groups. In addition, certain PLFA biomarkers, such as increases in fatty acid branching and degree of saturation, can reflect a microbial biochemical response to a number of stressors (Cordova-Kreylos et al. 2006, Jin and Kelley 2007). PLFA has been used extensively to study the microbial communities found in soil, especially with regard to chemical pollutants, biogeochemical processes, and agricultural issues (Hadwin et al. 2006, Harris 2003, Kelly et al. 2003, Langworthy et al. 1998, Moynahan et al. 2002, Röling et al. 2002, Rooney-Varga et al. 1999, Tian et al. 2008, Wünsche et al. 1995, Zelles 1999). However, the application of PLFA to aquatic ecosystem monitoring has been somewhat limited. PLFA analyses have revealed that the structure of microbial communities found in the water and/or sediment of an aquatic system are associated with levels of organic carbon, chlorophyll a (Polymenakou et al. 2005), polycyclic aromatic hydrocarbon contamination (Langworthy et al. 1998), phosphorus and water temperature (Keinanen et al. 2002), organic carbon, nitrogen, and metals (Ben-David et al. 2004, Cordova-Kreylos et al. 2006, Feris et al. 2003), and soil pH (Ben-David et al. 2004).

As described earlier in this document, CLPP is a phenotypic profiling technique based on the catabolic capabilities of a microbial community. Like PLFA, CLPP has been used primarily to study microbial communities found in the soil. A listing of more than 200 such studies appears

[3] See http://www.cnr.berkeley.edu/soilmicro/methods/BalserPLFA.pdf

on the Biolog website[4] (Biolog n.d.(a)). With respect to studies of aquatic systems, CLPP has detected shifts in the microbial community attributable to site age and source of organic material (Chazarenc et al. 2010), nutrient load (Wang et al. 2009), season (Christian and Lind 2007, Montserrat Sala et al. 2008), simulated acid mine drainage (Weber et al. 2008), nearby livestock (Merkley et al. 2004), and copper (Boivin et al. 2006).

2.3.3 *Comparison of Technologies*

A number of authors have suggested that multiple methods be used simultaneously to compensate for the limitations of any one profiling technology (Ros et al. 2008, Spiegelman et al. 2005, Widmer et al. 2001). Here we provide a brief critique of the technologies most commonly used to profile microbial communities.

DGGE is a purely genetic tool that produces a level of resolution greater than that of competing technologies. The presence of a particular band on a polyacrylamide gel represents a particular group of microorganisms. However, any phenotypic shifts present in the community will not be detected using DGGE because they are not accompanied by a shift in genotypes in the population. Quantification of particular microbial groups can only be partially accomplished by determining the *intensity* of particular bands, with the most intense bands representing a larger number of cells. However, the relationship between band intensity and abundance is not linear and is subject to biases induced by the kinetics of PCR. Furthermore, the assay is based on speed of electrophoretic migration of partially denatured DNA which in turn is determined by the guanine-cytosine (GC) content of the DNA fragments. While the technique can, in theory, separate DNA fragments that differ by as little as one base pair, in reality fragments of similar GC content and sequence will likely migrate at similar rates, and may co-exist within the same band on the gel. This means that different groups of microorganisms that possess the same or similar gene sequences cannot be resolved using DGGE. Finally, there is an issue with the 'portability' of the data. Unless individual bands are excised from the gel and readable DNA sequences obtained, the data remain as band patterns. These patterns are subject to artefactual distortions that result from small procedural differences between laboratories. The data produced by different parties would be difficult to reconcile without specialized software and/or a standardized set of protocols.

PLFA generates primarily taxonomic profiles at a level much coarser than that of DGGE, but has some ability to assess physiological status. By looking at levels of fatty acid branching and saturation, some data regarding the *level of stress* experienced by the microbial community can be generated. In addition, the technique is capable of estimating total and fractional microbial biomass within a sample. However, PLFA is subject to certain limitations in breadth of analysis, as the Archaeal community (a group of primitive microorganisms) does not routinely appear as part of the profile (Drenovsky et al. 2008). Furthermore, the technique requires a significant investment in laboratory equipment and trained personnel.

[4] See http://www.biolog.com/mID_bibliography.shtml

CLPP, in contrast to the preceding approaches, is a primarily phenotypic assessment with some reflection of the population's genotypic structure. However, without additional analysis, the phenotypic and genotypic contributions to CLPP results cannot be resolved. Despite this limitation, and others discussed below, the CLPP approach has several advantages. CLPP is less expensive and easier to perform than either of the other technologies discussed here. The expense associated with CLPP set-up is competitive with DGGE (estimated at $13,000 minimum for a thermal cycler, electrophoresis equipment, and basic gel documentation equipment and software) and significantly less expensive than PLFA (estimated $100,000 for a suitable gas chromatograph/mass spectrometer system alone). In terms of expertise, we have successfully conducted CLPP assays using personnel with little or no laboratory expertise. A brief introduction to the use of a micropipette, sonicating bath, and refrigerated incubator was all that was required. In contrast, both DGGE and PLFA require technical staff with at least a technical diploma in biotechnology or chemical technology. In our opinion, these are significant factors in favor of CLPP as a microbial profiling tool.

2.4 Value of CLPP as an Ecological Monitoring Tool

We have demonstrated that, compared to other technologies, CLPP is a rapid, easy, and inexpensive means of profiling microbial communities. Similarly, CLPP's chief virtues as an ecological monitoring tool are ease, rapidity, and low cost. The technique can be used to produce screening level ecological assessments of aquatic ecosystems that would be far more difficult, expensive, and time-consuming to generate using more traditional ecological techniques. It is unlikely that microbial profiling, in any form, will ever completely replace traditional ecological assessments as a means of acquiring detailed information on individual systems. However, CLPP could produce effective screening or *triage* level assessments across multiple systems and multiple time points while minimizing cost, technical difficulty, and time commitment.

2.4.1 *Assessments Based on Genotype and Phenotype*

Both the genotypic and phenotypic characteristics of a microbial community will affect the results of a CLPP assay. Rather than a shortcoming, this duality provides CLPP with a greater sensitivity to shifting environmental conditions. While one cannot extract the presence or absence of a specific microorganism from the CLPP profile, it has been demonstrated that a shift in the genotypic composition of a microbial community can be detected (Boivin et al. 2006, Ros et al. 2008). Perhaps more importantly, CLPP can detect shifts in the phenotypic status of a microbial community even in the absence of major genotypic changes. Again, the experiments conducted by Boivin et al. (2006) demonstrate the value of this feature with respect to ecological monitoring. In these experiments, the investigators established natural microbial communities on glass slides; these slides were then exposed to high levels of aqueous copper. Upon exposure, both DGGE and CLPP assays showed dramatic changes from their pre-exposure profiles as certain groups of microorganisms succumbed to the metal. However, when the copper was removed from the system, the CLPP profile returned to its pre-exposure state, whereas the DGGE profile did not. This demonstrates the metabolic plasticity exhibited by microorganisms,

and how a phenotypic response can be used as an indicator of environmental status, even in the absence of a corresponding genotypic change. This is information that cannot be obtained from DGGE, PLFA, or any other current microbial community profiling technique.

2.4.2 *Speed*

In comparison to standard ecological assessment techniques, raw data can be generated from CLPP relatively quickly. After receiving a sample, EcoPlates can be inoculated within 20 to 30 minutes, incubations run for 72 to 96 hours, and data acquisition and entry completed within another 45 minutes. Procedures for the analysis of data vary in terms of complexity, but if we assume they are relatively simple, then 3 to 4 hours per sample is a generous allotment of time. Altogether, it is clear that even if only a single sample is processed, results and interpretation could be had within a week. Furthermore, the per-site time requirement drops if samples can be processed in batches. In comparison, vegetation and macroinvertebrate surveys may take weeks to months to produce useful data.

2.4.3 *Cost*

To outline the costs associated with a basic evaluation of a single marsh using traditional techniques, we have compiled information from past project budgets and collaborator information (Table 1). This can be contrasted with estimated costs for doing a CLPP profile for the same marsh, based on expenses from previous experimental work (Table 2).

Table 1. Costs associated with the assessment of an aquatic ecosystem using traditional methods.

Item	Description	Cost
Field personnel – time on site	One junior and one senior technician on site for 8 hours	$835
Water Chemistry	Nitrogen (NO_2/NO_3 , Kjeldahl, total), Dissolved Organic Carbon, pH, Alkalinity (partial and total), Carbonate, Bicarbonate, Solids (total, total dissolved, total suspended) Conductivity, Total Dissolved Phosphorus, Chlorophyll *a*	$110
GIS Analysis	Size, slope, density of surrounding vegetation, surrounding land use	$170
Macroinvertebrate Community Analysis	Includes ‘picking’ of samples followed by taxonomic ID	$600
Vegetation analysis	Includes taxonomic ID of specimens not immediately identifiable in field	$100
Total		$1,815

Table 2. Costs associated with the assessment of an aquatic ecosystem using CLPP.

Item	Description	Cost
Field personnel – time on site	One junior and one senior technician on site for 20 minutes	$35
Materials for sampler construction and laboratory consumables	Foam floats, acrylic rods, line, stakes, weights, buffer solutions, sterile plastic tubes, EcoPlates	$23
Laboratory Technician	One junior technician for 45 minutes	$26
Total		$84

It is clear that CLPP is a much less expensive option than traditional monitoring techniques. Admittedly, the CLPP procedure will not produce the detailed information associated with traditional assessment; but, by monitoring a microbial community, the effects of chemical, physical, and biological conditions are integrated. If the goal of monitoring is to assess many sites (as in a regional monitoring program) or to continuously monitor a single site (as in a reclamation tracking program), then a single integrated dataset is likely sufficient.

While the costs associated with travel and data analysis are expected to be similar for both the traditional and CLPP approaches, capital equipment expenses are worthy of special attention. Depending on the level of precision desired, the cost of analytical instruments necessary for just the water chemistry analysis is between $50,000 and $100,000. In comparison, the equipment necessary for CLPP includes a sonicating bath ($500), micropipettors ($2,500), a refrigerated incubator ($5,000), and a spectrophotometer ($8,000), for a total cost of approximately $16,000.

2.4.4 *Expertise*

The expertise required to generate valid CLPP data is significantly less than that required for traditional ecological assessments. For example, experts in the identification of invertebrates and vegetation are often necessary to generate relevant data for floral surveys and macroinvertebrate community analyses. Individuals such as these are relatively rare. The chemical analyses would need to be performed by someone with a considerable amount of technical training and experience. In contrast, we have successfully used undergraduate university and technical college students as well as non-laboratory technicians to conduct our field and laboratory work. Currently, all the data interpretation for microbial profiling requires experts possessing a similar level of training to those needed for traditional assessments. Consequently, part of our work has been an attempt to simplify CLPP data analysis such that most routine tasks can be done using an Excel-based template.

2.5 Scientific/Technological Limitations and Their Mitigation

2.5.1 *Bias Towards Certain Groups of Microorganisms*

The Biolog system does not treat all microorganisms equally (Douterelo et al. 2010, Garland 1997, Heuer and Smalla 1997, Konopka et al. 1998, Ros et al. 2008, Smalla et al. 1998, Widmer et al. 2001). The procedure is based on the consumption of carbon compounds as energy sources, thus excluding the autotrophs (e.g., green algae). Furthermore, the plates are incubated in ambient atmosphere, effectively preventing anaerobic metabolism. While Biolog plates have been used to profile anaerobic microbial communities (Röling et al. 2000), the procedures necessary to profile this community are demanding, and would detract significantly from the assay's virtues of speed, ease, and economy. Finally, the microorganisms most responsible for carbon compound catabolism tend to be those adapted to rapid growth under high nutrient conditions. Some of these criticisms were levelled against the Biolog GN plate which was designed to identify pure cultures of Gram-negative organisms rather than the community analysis for which the EcoPlate was designed. Nevertheless, it is reasonable to conclude that the EcoPlate may be subject to similar biases.

It is important to remember the application for which the CLPP approach is intended when considering the impact of bias towards specific microbial groups. CLPP can be developed into a screening assay for ecological status by indexing the catabolic characteristics of the microbial community against traditional measures of ecological integrity. It is only the strength and reliability of the correlation between these parameters that determines the validity of this approach, not whether the entire microbial community is represented. The literature and our experience suggest that even the limited portion of the microbial community that can be evaluated by CLPP contains sufficient information to allow meaningful assessment of aquatic ecosystems.

2.5.2 *Interference from Environmental Chemicals*

Some authors have complained that non-microbial components of samples may interfere with Biolog results (Christian and Lind 2006, Konopka et al. 1998, Spiegelman et al. 2005). This effect could be mediated by the introduction of additional catabolizable carbon sources, abiotic chemical reduction of the tetrazolium dye, or an increase in optical density due to environmental pigments (e.g., tannins). We have mitigated these issues through our sampling system. We introduce clean acrylic rods in the water column, and allow a microbial biofilm to form on them. By harvesting the material on the rod, and not the actual water, we dramatically reduce the effect of environmental chemicals on the assay. Furthermore, our sample processing procedure releases the biofilm microbial community into a sterile, chemically defined buffer solution. This enhances our ability to compare one ecosystem to another by allowing all samples to be incubated under identical conditions.

2.5.3 *Viability*

In contrast to DGGE and PLFA, CLPP requires live organisms to work. Samples must be collected, stored, and transported carefully to maintain as much of the natural community as possible. Long sample storage and transport times at non-environmental temperatures may have resulted in a lack of interpretable results in some experiments (Morrison et al. 2009). These issues can be mitigated, but not eliminated, through scheduling. For our own work, we collect our rods into small volumes of surrounding water. Air bubbles (5 to 10 mL) are maintained in the collection containers to allow continued aerobic respiration during storage and transport. The samples are maintained in a cooler but are not frozen, and are transported back to the laboratory the same day. It is important to realize that viability need only be maintained until the end of the incubation period (3 to 4 days). All of the laboratory equipment needed for CLPP could fit easily within a small portion of an ATCO-style trailer, or even in the back of a pickup truck, allowing on-site or near-site processing of samples. Once incubation has been completed, the EcoPlates can be frozen until a spectrophotometer is available.

2.5.4 *Inoculum Density*

It has been well established that inoculum density (i.e., the concentration of actively respiring aerobic microbes) can have a dramatic effect on the results of CLPP investigations (Christian and Lind 2006, Garland and Mills 1991, Konopka et al. 1997, Preston-Mafham et al. 2002). Strategies to moderate these effects are based on either a laboratory inoculum normalization step, or sophisticated kinetic analyses of colour development. Unfortunately, both of these approaches can add substantially to the time and effort required. To maintain the attributes of speed and ease, we have taken a more practical approach to mitigating the effects of inoculum density: we facilitate the formation of consistent biofilms (and therefore inoculum density) by using substrates (acrylic rods) of constant size, shape, material, and surface texture. However, we recognize that some degree of variability in our results is still likely attributable to variation in inoculum density between sites. We have devised modifications to our sample preparation protocol which should allow us to normalize inoculum density to a significant degree without incurring excessive penalties with regard to speed, ease of execution, or expense. The utility of these modifications will be determined if and when funds permit. It should be recognized, however, that the concentration of microbes within a given ecosystem is in part a result of its ecological state. The normalization of those concentrations may actually reduce the value of CLPP as an ecological monitoring tool.

2.5.5 *Seasonality*

Natural microbial communities vary with respect to season (Anderson-Glenna et al. 2008, Bouskill et al. 2010, Chazarenc et al. 2010, Christian and Lind 2007, Jin and Kelley 2007, Montserrat Sala et al. 2008, Ringbauer et al. 2006, Sutton and Findlay 2003). Any monitoring scheme based on microbial communities must be organized with this in mind. Many, perhaps all, currently monitored parameters also have seasonal variability. For example, benthic macroinvertebrates are best sampled in the spring or fall, when mature individuals of many species are present in the environment, as these individuals are easier to identify taxonomically.

So, while neither CLPP nor any of the other microbe-based technologies offer any advantage in this regard, they offer no particular liability either.

2.5.6 *Complexity of Analysis*

One challenge of CLPP, or any microbial community profiling technology, is the analysis and interpretation of data. Certainly, there has been no shortage of articles written on the subject (Garland 1996, 1997, Glimm et al. 1997, Insam and Goberna 2004, O'Connell et al. 2000, Preston-Mafham et al. 2002, Weber et al. 2007, Weber and Legge 2009). The techniques advocated by these authors offer the most detailed analysis possible. However, if the goal is to generate a fast-and-easy screening assay, these techniques are too cumbersome to be practical. In contrast to most previous investigators, we have taken a much more simplistic approach to the analysis of CLPP results. We have divided all of the carbon compounds found on the EcoPlate into categories (e.g., contains nitrogen vs. does not contain nitrogen). We then produce a category score by summing all of the carbon compounds within that category that were successfully catabolized by the microbial community. We then use these scores, combined with calculations of metabolic richness and diversity (Weber et al. 2008, Zak et al. 1994), to compare sites to each other. We hold plate incubation times and temperatures constant but do not collect kinetic data. No doubt, this comparatively rudimentary means of data analysis may result in a shallower interpretation than has been produced by others. Nevertheless, we have been able to detect statistically significant relationships between CLPP results and multiple biological and chemical parameters, the effect of ecoregion on lotic systems, and even effects associated with surrounding land use. We are pleased with the results to date, and believe that similar data analysis techniques could be used for many ecological monitoring applications.

2.5.7 *CLPP Results May Not Reflect in-situ Metabolism*

The results of CLPP analysis may not be representative of microbial metabolism *in-situ* (Garland 1997, Smalla et al. 1998), presumably because the conditions under which the EcoPlates are incubated do not resemble those present in natural ecosystems. Temperature, light, diversity and concentration of nutrients, oxygen content and any number of additional parameters are held constant in the laboratory to facilitate comparison between samples, yet can be expected to vary significantly from the conditions found at the sampling sites. As such, it must be understood that CLPP can be used as an ecological monitoring tool only after strong, reliable correlations with conventional ecological parameters have been established through empirical study.

This being said, it may be possible to alter the physical format of a microplate such that it can be incubated within the aquatic ecosystem itself. This approach removes the requirement to accurately recreate environmental conditions within the laboratory. While CLPP results obtained in this fashion would be a more faithful representation of *in-situ* metabolism, they are still unlikely to be completely accurate. Moreover, there is no model which can accurately link microbial metabolism to broader ecosystem function at a mechanistic level. As such, even a perfect assessment of *in-situ* microbial metabolism is unlikely to result in effective monitoring without a great deal of empirical study.

2.5.8 *Representative Samples*

It has been well established that microbial communities can vary over time and space (Adamus et al. 2001, Chandra et al. 2006, Garland and Mills 1991). When microbial communities are profiled based on grab samples, the profile produced reflects the microbial community present in that particular sample, which may or may not accurately reflect the broader community. Typically, this issue is ameliorated by means of multiple samples collected over multiple time points. This approach results in increased analytical costs through the processing of multiple samples per site. In contrast, our biofilm-based sampling integrates over time and, to a lesser degree, space. Almost any object immersed in a natural body of water will develop a microbial biofilm on its surface. The development of these films in terms of their microbial community is largely prescribed by the environment in which they form (Anderson-Glenna et al. 2008, Paule et al. 2009, Stoodley and Stoodley 2002). By maintaining our acrylic rods in the water for a set period of time, we achieve two goals. First, we obtain a sample of the local microbial community that reflects the environmental conditions present over that period. Second, we obtain age-matched biofilm samples, grown on identical substrates, and subjected to the same climatic conditions – providing a similar basis for comparison. We also deploy multiple samplers at a given site. Largely, this is done to provide sufficient active microbial numbers in the event that some of the sampling devices are lost due to weather or wildlife. A secondary benefit, however, is the ability to collect microbial community samples from multiple locations at a given site.

2.5.9 *Comparative Assay*

The data produced by CLPP cannot provide any absolute assessment of ecological status. Such an assessment is only possible when comparisons are made to reference sites or systems. In this respect, CLPP is really no different from other microbial community profiling technologies, or traditional ecological assessment methods. Any application of CLPP to ecological monitoring will require that some reference dataset be incorporated into the process. Reference datasets can be obtained either from specified ecosystems that are sampled contemporaneously, or from historical sampling of the test sites prior to disturbance. The selection of appropriate reference systems is central to ecological monitoring, and the subject of ongoing discourse (Gosselin et al. 2010, Harris 2008). With respect to CLPP, environmental factors affecting microbial communities, as discussed earlier in this document, should be considered when selecting the source of reference data. In the case of reclaimed aquatic ecosystems, the age of a system has a significant effect on its microbial community (Chazarenc et al. 2010, Jin and Kelley 2007, Rusznyak et al. 2008). While we would expect the microbial community in a reclaimed system to progress towards that of a natural system over time, it is not clear what that progression/succession would look like. Rather than speculate, a number of reclamation projects should be profiled from beginning to end. Time-indexed CLPP profiles from successful projects could then be used as standards by which the trajectory of ongoing reclamation efforts could be evaluated.

2.6 Results to Date

To date, we have undertaken three formal projects involving CLPP as an ecological monitoring tool. These projects are described below.

2.6.1 *Wetland Health*

This was a two-year project undertaken in collaboration with the Suzanne Bayley laboratory at the University of Alberta. This project investigated the validity of CLPP as an ecological assessment tool in a variety of marshes and man-made wetlands in the Beaver Hills Moraine. Study sites were separated into one of four categories: natural, agricultural, wet ponds, and constructed. Natural sites were located in Elk Island National Park, Cooking Lake/Blackfoot Provincial Recreation Area, and Miquelon Lake Provincial Park. These sites were best described as hypereutrophic. Agricultural sites were natural marshes which were expected to receive water from surrounding crop fields or pasture. Wet ponds were simply man-made depressions which had been allowed to fill with water over time; roadside borrow pits are a good example of wet ponds. Constructed sites were wet ponds where an effort had been made to mimic natural systems when the pond was constructed.

Our CLPP results for 2008 clearly distinguished wet ponds from agricultural sites. Wet ponds tended to be characterized by high microbial metabolic richness and diversity whereas agricultural sites demonstrated low values for each of these parameters. For microbial metabolic richness and diversity, we found statistically significant correlations with water nitrogen (nitrate, nitrite, total dissolved nitrogen), water and soil organic carbon, and soil density. When we examined specific carbon compound categories, statistically significant correlations were found with dissolved organic carbon, rake score (aquatic plant abundance), and invertebrate abundance and taxa richness. In general, decreasing microbial metabolic values indicated higher values in the environmental variables described above. This is in agreement with the findings of other investigators where lower nutrient systems exhibited higher microbial metabolic richness and diversity (Montserrat Sala et al. 2008, Wang et al. 2009).

Data from our collaborators for the 2009 study sites are still being processed. However, upon inspection of the microbial data alone, it would seem that while the relationships between site classes are similar to 2008 (i.e., agricultural sites show low microbial metabolic richness and diversity while wet ponds show high values), the profiles of individual sites vary from year to year.

2.6.2 *River Health*

This was a 1-year project conducted in collaboration with the Long Term River Network (LTRN) at Alberta Environment and Cathy Ryan at the University of Calgary. Our sampling devices were deployed at 13 sites across a number of Alberta rivers and streams, including the North Saskatchewan, Battle, Red Deer, and South Saskatchewan Rivers as well as Nose Creek in and around Calgary. Site selection was based on sampling sites used by LTRN, the Prairie Provinces Water Board, and the University of Calgary. Again, we are waiting on collaborator data to become available before analyses can be completed. However, even with incomplete

data it is clear that there is a geographic variable associated with our results. Figure 4 is a dendrogram of the study sites (1 to 13) and their associated river or stream arranged on the basis of average Euclidean distance between carbon use profiles. Those clustering under branch 'A' are west of Highway 2 (i.e., the more upstream reaches), those clustering under branch 'B' are east of Highway 2 (i.e., the more downstream reaches), and the single site under branch 'C' is found in the southeast of the province. It is interesting to note that the CLPP profiles of study sites in the same region but different river systems are more closely related than those in different regions but the same river system. This effect might be mediated by ecoregion, geography, or anthropogenic inputs (upstream or downstream, as noted above). Until further data become available, it will be difficult to attribute our results to a specific set of factors. However, based on our wetlands work, and the reports of other investigators (Duarte et al. 2009, Zeng et al. 2008, Wang et al. 2009), we believe trophic status/nutrient levels may have a great influence on the microbial communities. In general, the further downstream we sampled, the lower the microbial metabolic richness and diversity. Again, previous authors have identified environmental conditions as major factors affecting microbial community structure, and have identified variability in microbial community associated with river gradients (Anderson-Glenna et al. 2008, Paule et al. 2009).

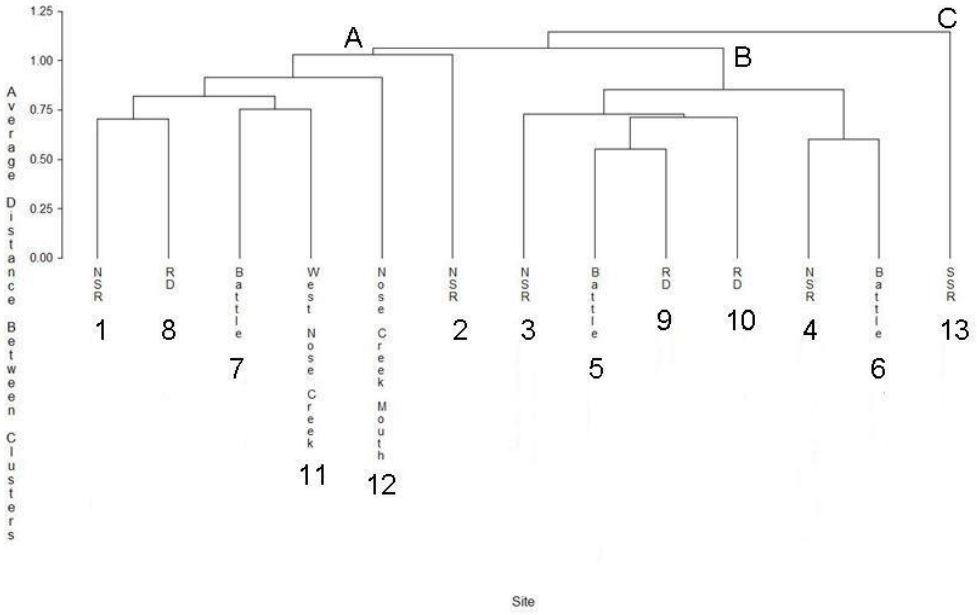

Figure 4. Dendrogram of River Health project based on average Euclidean distance between microbial profiles.
Note that individual sites are identified by the numbers underneath the river system. NSR = North Saskatchewan River, RD = Red Deer River, Battle = Battle River, SSR = South Saskatchewan River.

2.6.3 *Elk Island National Park (EINP) Lake Monitoring*

This project was undertaken in September 2010, at the behest of EINP management, to describe the chemical status of small lakes within the park, and to investigate the possibility of airborne deposition of organic pollutants from nearby industry. Sampling devices were deployed in 18 different sites throughout the park, and samples of water and sediment taken for chemical analysis. While no site demonstrated obvious ecological impairment, Spearman rank correlations demonstrated statistically significant correlations between a few water chemistry (conductivity, alkalinity, pH, and total suspended solids) and microbial parameters (polymer use, carboxylic acid use, amine use, and metabolic evenness). When compared to natural wetlands studied in the region as part of the 2008/09 wetland health project, no significant differences were detected in terms of microbial metabolic richness or diversity, although individual sites did appear to vary significantly from year to year. Ordination procedures based on the microbial data have separated the study sites into two reasonably distinct clusters. Differences in soil and/or vegetation are still under investigation as variables which may explain this clustering.

2.7 Future Development

2.7.1 *Refinement*

Our work to date has focussed on demonstrating that CLPP can be used to make meaningful statements regarding the ecological status of a given aquatic ecosystem. If we are to further develop the technology, the operational details of the technique must be addressed. For the most part, this involves refinement of our existing techniques and the accumulation of background information. This refinement could occur through a series of experiments conducted over the next 3 to 5 years should funding become available.

Variability in inoculum density is a primary concern. We suspect the precision of the assay could be greatly improved if a quick and inexpensive method for standardizing the inoculum density could be developed. We suspect a procedure involving filtration, followed by ATP measurement, and then centrifugation/dilution could achieve this goal. In addition, the use of dispersal agents/buffers to aid in the break-up of sampling biofilms without compromising viability or altering optical density could further improve assay precision.

Sampling techniques should be optimized. We have good evidence to suggest that our assay is not particularly sensitive to sampling location. However, other variables such as optimum residence time, the suitability of epilithic biofilms (biofilms that grow on the surface of objects, such as rocks, *in-situ*) as samples, the quantitative effects of season and weather events, and maximum sample storage times, remain to be determined.

Data acquisition procedures could be simplified to allow the use of non-proprietary technology and to expand the potential monitoring capability of CLPP. To date, we have used a Biolog microstation, running in ‘automatic’ mode. In this mode, a desktop computer applies a proprietary algorithm to optical density data and sets threshold values to evaluate carbon-compound catabolism in each well. These threshold values are important to the interpretation of results, to the point where data from other Biolog microplates (Biolog n.d.(b)) are very difficult

to reconcile with EcoPlate results. To use non-proprietary technology and to expand our array of carbon compounds through the use of non-EcoPlate microplates, a new algorithm must be developed.

Data analysis routines must be simplified, standardized, and 'packaged' before CLPP could be fielded as a frontline screening tool. We continue to modify our own analytical methods to evaluate CLPP as an ecological monitoring tool. We have made some significant progress, but more could be done to make this technology more appealing to industry and government.

We must be able to demonstrate the broad applicability of CLPP as an ecological monitoring tool before it would be accepted by many users. Long term monitoring of a number of different systems in different regions with different levels of ecological impairment would greatly aid in the interpretation of results and setting of historical values for end-users. The best way to accomplish both goals is to undertake a series of studies which demonstrate the ability of the technology to detect important differences in the ecological status of various aquatic ecosystems. The results of these studies could be compiled, packaged, and made available to end-users of the technology.

Finally, performance specifications must be defined. We suspect CLPP analysis can provide a rapid, broad, but fairly shallow status assessment of any given aquatic ecosystem. However, any industry or government user of the system would expect detailed information on the capabilities and limitations of the technology prior to adoption. Such benchmarks have yet to be defined.

2.7.2 *New Applications*

This document has focussed on the value of CLPP as an ecological monitoring tool. However, the technology could be useful for other applications.

A great deal of work has been done studying microbial communities in polluted environments, resulting in a body of work far too large to review comprehensively. A number of studies have used microbial profiling techniques either as a means of identifying microorganisms important to the degradation of a pollutant, or as a means of measuring the success of site remediation (Hadwin et al. 2006, Harris 2003, Kelly et al. 2003, Langworthy et al. 1998, Moynahan et al. 2002, Röling et al. 2002, Rooney-Varga et al. 1999, Tian et al. 2008, Wünsche et al. 1995). As noted in the Hadwin study (2006), the current format of CLPP may not be a good choice for this application simply because the conditions under which the Biolog plates incubate do not resemble those on site. The solution to this issue may be to modify the physical and chemical characteristics of the microplate such that it can be incubated *in-situ*. This may allow us to assess carbon utilization patterns far more accurately than can be done in the laboratory. We have had some initial success with filling the microplate wells with agar. This allows the carbon compounds to serve as an energy source, and the tetrazolium dye to undergo its colour change, without allowing those chemicals to diffuse into the surrounding water. Further development might involve a filter top and modification of the carbon compound array to obtain the most relevant information and protect the agar plugs. Such modifications would also make CLPP more useful for studying biogeochemical processes.

Of particular interest to the oil sands industry are issues surrounding the presence, source, degradation, and impact of naphthenic acids. At least one attempt has been made to use CLPP as a means of identifying wetlands affected by naphthenic acids (Hadwin et al. 2006). During that study, sediment samples were collected from various wetlands in the Athabasca region, and used as inocula for the Biolog EcoPlate. Sediment samples were centrifuged, resuspended in ice-cold PBS buffer, vortexed, and then incubated with agitation at 20°C under (presumably) aerobic conditions for 10 days. As we have described in the preceding paragraph, the conditions associated with sample processing and incubation probably do not reflect those found *in-situ*. In addition, naphthenic acids are not present in the EcoPlate as a carbon source. Unsurprisingly, this particular study found no evidence to suggest the presence of naphthenic acids could be detected using CLPP.

However, we suggest that modifications to the EcoPlate system to allow *in-situ* incubation and the inclusion of naphthenic acids of varying molecular weights as carbon sources may well produce better results. In fact, there is evidence to suggest that while small molecules can often be degraded by one or a few species, the breakdown of high molecular weight hydrocarbons requires the combined metabolic capabilities of a diverse microbial community (Tian et al. 2008). As such, the value of investigations into the microbial biodegradation of naphthenic acids depends upon the integrity of the microbial community. This integrity, in turn, is determined by the degree to which the natural environment of the microbial community can be replicated. In our view, this is best accomplished through *in-situ* assessment.

3 EXISTING PROGRAMS IN THE OIL SANDS REGION THAT MIGHT BENEFIT FROM THE CLPP APPROACH

There are a number of organizations or agencies in the mineable oil sands region that monitor some aspect of the aquatic ecosystems in the region[5]. Here we provide an overview of these organizations, including a summary of their objectives and monitoring programs relevant to aquatic ecosystems, and discuss the utility of the CLPP approach to achieving their objectives.

The adoption of the microbial CLPP approach by individual organizations would depend on demonstration that it produces reliable results that index the ecological health of aquatic ecosystems. The only way to achieve this is through continued research on the method. Given that this approach is so cost-effective, it is possible that individual companies may want to fund methods research, as the potential benefit of the CLPP technique outweighs the slight risk involved in investing in this type of research.

[5] See also Lott, E.O. and R.K. Jones, 2010. *Review of Four Major Environmental Effects Monitoring Programs in the Oil Sands Region*. OSRIN Report No. TR-6. 114 pp.

3.1 Cumulative Environmental Management Association (CEMA)

The Cumulative Environment Management Association (CEMA) is a society composed of a number of stakeholders[6] interested in managing the cumulative effects of development in the mineable oil sands region of Alberta (Cumulative Environmental Management Association n.d.). The goals of CEMA include developing and promoting management frameworks, best practices, and implementation strategies for the region that protect human health, and which sustain and restore the environment, including air, water, land and biodiversity. CEMA's scope includes balancing oil sands development with managing the impacts of this development on land, water, and air. This includes developing best management approaches to protect landscapes, vegetation, soil, watersheds, aquatic ecosystems, and surface water quality and quantity, and to minimize potentially harmful air emissions and their effects. In addition, CEMA addresses the need to reclaim landscapes in the post-mining environment, including both terrestrial and aquatic ecosystems.

Although CEMA funds technical work, such as reviews of existing information or data (e.g., Westworth Associates Ltd. 2002), or the design of monitoring networks (e.g., Worley Parsons 2010), the organization does not fund ongoing monitoring programs. However, there are numerous organizations that do monitor the environment and ecosystems in the oil sands region, and CEMA does provide information on monitoring systems to these agencies. Therefore, CEMA is in a position to promote the use of microbial CLPP as a tool for monitoring aquatic ecosystem health in the region.

3.2 Regional Aquatics Monitoring Program (RAMP)

The Regional Aquatics Monitoring Program (RAMP) is a multi-stakeholder agency which presently includes 22 organizations (Regional Aquatics Monitoring Program n.d.) with representation from municipal, provincial, and national government agencies, industry and First Nations communities. The mandate of RAMP is to monitor, evaluate, and report on the state of the aquatic environment in the Regional Municipality of Wood Buffalo (RMWB) and any changes that may have resulted from the cumulative effects of resource development in the region.

RAMP is a science-based monitoring program that addresses the aquatic monitoring needs of all RAMP stakeholders. This includes collection of baseline data to characterize natural variability in the region, monitoring required for regulatory compliance or community agreements, comparison of data to predictions made in Environmental Impact Assessments (EIAs), and monitoring to detect cumulative effects on aquatic systems in the oil sands region. In addition, RAMP acknowledges and incorporates traditional environmental monitoring into its activities, and communicates results of its monitoring and assessment activities to RMWB communities, regulatory agencies and other interested entities. The program is constantly evolving in response

[6] 48 member organizations as of January 2011

to monitoring results, advances in technology, changing concerns of local communities, and changes in project approval conditions.

RAMP uses field sampling and surveys to annually collect data on six key aspects of the aquatic environment: (1) climate and hydrology, (2) water quality, (3) benthic invertebrate communities, (4) sediment quality, (5) fish populations, and (6) acid sensitive lakes. Sampling sites are located on the Athabasca River and its tributaries, the Athabasca River delta, and important lakes and wetlands in the region. A large number of variables are sampled within these six key components; a subset is subjected to in-depth analysis to provide an indication of the health of aquatic ecosystems in the region. Sampling such a large number of parameters is costly and time-consuming, but does have the advantage of providing a fairly complete picture of changes in the aquatic environment. However, access to a relatively rapid, repeatable, and technically tractable method such as microbial CLPP may be attractive to RAMP in the future, once the method has been thoroughly tested. Specifically, we can envision RAMP using CLPP as a means of expanding the number of sites they monitor, identifying sites that warrant further evaluation, or reducing monitoring costs for existing sites.

3.3 Canadian Oil Sands Network for Research and Development (CONRAD)

The Canadian Oil Sands Network for Research and Development (CONRAD) is a non-profit organization which promotes and facilitates collaborative research in science and technology related to the oil sands of Alberta. CONRAD is presently comprised of 15 industry members, two universities, one industry association and five government agencies.

The goals of CONRAD are to (1) improve performance in the oil sands industry through superior new technologies, (2) improve effective and quality of research pertaining to the oil sands, and (3) develop technologies that will improve the environmental performance of the oil sands industry (Canadian Oil Sands Network for Research and Development n.d.). The overall objectives of the organization are to bring together the research needs and ideas of industry with agencies that sponsor and/or do research, to encourage and facilitate collaborative research. Projects of potential interest to CONRAD are judged based on scientific merit and their commercial applicability to the oil sands industry. The main areas of interest to CONRAD include environment research, *in-situ* recovery, surface mining of oil sands, extraction of bitumen, and upgrading of bitumen and heavy oil.

CONRAD is not a monitoring organization, but one which directs research questions and funding, and helps in the development of collaborative research teams. As such, the microbial CLPP approach has potential for inclusion in research projects related to the environment in the oil sands region. These could include such topics as development and testing of cost-effective aquatic monitoring methods, response of microbial communities to specific types of effluent from oil sands processing (e.g., tailings), or tracking changes in microbial communities following construction of aquatic habitats during reclamation.

3.4 Alberta Biodiversity Monitoring Institute (ABMI)

The mandate of the Alberta Biodiversity Monitoring Institute (ABMI) is to provide reliable, science-based data on the state of biodiversity within the province of Alberta to people and agencies that manage resources within the province (Alberta Biodiversity Monitoring Institute n.d.). ABMI samples a subset of biota (approximately 2000 species), habitat, physical characteristics, and human disturbance levels at points distributed throughout the entire province.

ABMI uses standardized and scientifically-reviewed sampling protocols to ensure that data are comparable across sites and years. The program collects field data on a wide range of organisms, including plants, invertebrates, vertebrates, and the habitats where they occur. Aquatic ecosystems which are sampled include wetlands, lakes, and rivers. Variables that are sampled at wetlands include physical characteristics (bathymetry, wetland zones), site capability (ecosite characterization), riparian characteristics (vegetation, snags, and percent area covered by water, rock, bare soil, bare soil, lichens and non-vascular plants, forbs, grasses, shrubs and trees), human-caused site disturbance, and water physicochemistry (Alberta Biodiversity Monitoring Institute 2008). Parameters sampled at rivers include the physical characteristics of the river, water physicochemistry, benthic invertebrates, benthic algae, fish, and incidental vertebrate observations (Alberta Biodiversity Monitoring Institute 2007a). Parameters sampled at lakes include physical characteristics of lake sampling area (e.g., depth transects), water physicochemistry, phytoplankton, zooplankton, fish, and incidental vertebrate observations (Alberta Biodiversity Monitoring Institute 2007b).

Currently the mandate of the ABMI is to sample biodiversity in Alberta. The CLPP approach does not provide information on biodiversity directly; rather, the method provides an index of the health of a system (e.g., wetland) as a whole. In addition, the ABMI is presently focused on using sampling of a subset of species that occur in Alberta to examine changes in biodiversity at regional scales. The program does not sample microorganisms. However, ABMI is developing new approaches to monitoring rare and elusive species that are not generally detected using their standard protocols, and ways to examine biodiversity at more local scales. Therefore, there is a small possibility that the microbial CLPP approach may be of interest to the ABMI at some future time.

3.5 Alberta Environment (AENV)

Alberta Environment (AENV) currently runs long-term monitoring programs for both large lakes and rivers in the province. The lake monitoring program includes the Alberta Lake Management Society's (ALMS) Lake Watch program (Alberta Lake Management Society n.d.), Alberta Environment's Parks Program and Long Term Lake Monitoring Network, and lakes sampled under RAMP (see above; Alberta Environment n.d.(a)). These agencies are cooperating to use standardized methods so lakes can be compared across the province. Most lakes in the program are sampled once every few years. Standard protocols for Lake Watch and Alberta Environment usually include collection of water, zooplankton, and phytoplankton samples. Although biological samples are collected, they are not generally processed, but are archived for potential analysis in the future. RAMP lakes are generally sampled for water chemistry, including

conventional parameters, nutrients, major ions, contaminants, toxicity, and metals; a subset of lakes is sampled for other parameters such as benthic macroinvertebrates.

Alberta Environment also runs the Long Term River Network. Twenty-eight sites located in major rivers across the province are sampled on a monthly basis for water physicochemical parameters (Alberta Environment n.d.(b)). Sampling stations are often established upstream and downstream of point sources of pollution, such as cities. There is no routine biological sampling associated with the long-term river monitoring network, except for sampling of bacteria such as fecal coliforms. However, the river sampling work we did in collaboration with the Long Term River Network originated from a conversation with AENV personnel (Anne-Marie Anderson, Richard Casey, and John Willis), so there are opportunities to use data collected during routine monitoring in research projects.

Recently, a panel of 12 experts from the fields of health/epidemiology, science, public administration/risk/economics/systems thinking, and regulatory/government/industry were chosen to help create a new environmental monitoring system for the oil sands region of Alberta (Government of Alberta 2011). The goal is to develop a monitoring system that addresses air, land, water, and biodiversity in northeastern Alberta, with an expectation that such a system could eventually be expanded across the entire province. Initially the panel will focus on building a system to monitor the condition of the lower Athabasca River and the effects of human activity on the river; this will then be expanded to the entire northeastern region and then the province (Alberta Environment n.d.(c)).

Given current monitoring activities, and the forthcoming regional environmental monitoring system, development of microbial CLPP as a screening tool for the status of aquatic systems should be of interest to Alberta Environment. Given the scale of the oil sands region, the numerous and varied types of aquatic habitats in the area, and the need to monitor reclaimed aquatic systems to ensure regulatory compliance, development of a rapid, technically-tractable, and cost-effective ecosystem health monitoring tool would be of tremendous benefit in the future, as it would allow sampling of many more sites than could be monitored using current technologies. CLPP could be used to flag sites that are compromised in some way, thereby directing additional sampling and analysis to determine the cause of impairment at relevant sites. At present, it appears that samples are collected, but not necessarily analyzed, from numerous sites, which is inefficient.

3.6 Ducks Unlimited Canada (DUC)

Ducks Unlimited Canada's Western Boreal Program[7] has been collecting waterfowl abundance data in Alberta since 1999 using aircraft. Surveys have been done in a number of areas in the boreal forest, including the Peace Athabasca Delta, and the area around Utikuma Lake. These surveys target a number of different life stages of waterbirds, including breeding, moulting, and fall staging, waterbird pairs and broods. DUC is presently investigating the effects of landscape

[7] See http://www.ducks.ca/conserve/programs/boreal/index.html

change on duck abundance and distribution at regional and local scales in the boreal ecoregion of Alberta.

In addition to ongoing monitoring of waterbirds, DUC is involved with wetland restoration, presently being the only certified wetland restoration organization in Alberta. DUC also participates in a variety of research projects with other agencies, including universities and industry. Much of the monitoring currently done directly by DUC is related to waterbird production, distribution and habitat, with additional remote-sensing work on the size, characteristics, and landuse around numerous wetlands and lakes. Little routine work is presently done related to other wetland/lake parameters, such as water chemistry, except as part of individual research projects. Adoption of microbial CLPP as a tool for monitoring aquatic health by DUC might occur in the future once the method is proven in the field and the relationships between the microbial community and the status of the system are understood. However, adoption of this method would depend on the method providing data needed for DUC to understand patterns of waterbird abundance in relation to the CLPP signal, and this will require several years of empirical research.

3.7 Department of Fisheries and Oceans (DFO)

The role of Fisheries and Oceans Canada in Alberta is primarily enforcement. Therefore, this federal department samples fish populations in reaction to specific events (e.g., chemical spills or leaks into a lake or river) or when building an enforcement case. Fisheries and Oceans personnel will sometimes assist other agencies (e.g., Alberta Conservation Association, Alberta Sustainable Resource Development) in their fish sampling programs, but do not run any large-scale sampling programs of their own.

DFO is tasked with enforcing fisheries policies related to fish habitat loss during industrial, and other, activities. In cases where projects result in harmful alteration, disruption and destruction (HADD) of fish habitat, compensation is required to ensure that no net loss (NNL) of the productive capacity of a system is achieved (Department of Fisheries and Oceans n.d.).

Because the mandate of DFO is very specific, and because the agency uses a "user pays" policy (e.g., the party creating the habitat disturbance must pay for habitat compensation activities, including monitoring), there would likely be little interest within the agency for the microbial CLPP approach to monitoring aquatic systems. In the case of DFO, the disturbance is known, often localized, and plans to address the impact are in place before the impact occurs. Compliance monitoring is often related to fish populations themselves (e.g., richness, abundance, distribution) and the quantity and quality of habitat elements (e.g., coarse woody debris in the stream, presence of pools, dissolved oxygen levels). Therefore, the CLPP approach is probably not useful for DFO.

3.8 Monitoring by Other Organizations

3.8.1 *Alberta Conservation Association (ACA)*

The Alberta Conservation Association (ACA) takes an active role in monitoring fish populations within Alberta, with ACA personnel sampling fish in numerous stream reaches and lakes. There are few repeat visits in the stream sampling, but lakes are often sampled once every five to seven years; these lakes are principally large angling lakes. Streams are sampled with electroshocking equipment, while lakes are sampled with gillnets.

The ACA also sponsors, and participates in, research projects. The ACA funds research by university students on biodiversity, conservation biology, and by the general research community on fish and wildlife species and their habitat. ACA personnel participate in a variety of projects; those that pertain to aquatic systems usually examine fish populations or their habitats (e.g., Jokinen 2005, Stevens and Council 2008), though parameters such as water chemistry are not often measured.

3.8.2 *Alberta Sustainable Resource Development (ASRD)*

Alberta Sustainable Resource Development (ASRD) samples fish for two reasons: enforcement and scientific research. For enforcement, fish are sampled to determine population status; this information is used when setting catch limits and fishing seasons at specific water bodies. At a provincial level, the ASRD enforcement group samples priority water bodies, including lakes and rivers, approximately once every five years. The sites that are sampled depend partly on public pressure and harvesting pressure. At the regional level, managers may choose to sample additional water bodies. The principal focus of this sampling is estimating the population size of game fish and related parameters such as fish health and contaminant studies. Lake sampling is done using gillnets, while river sampling is done with electrofishing boats.

Research projects may focus on a particular fish species or water body, or may be more general and examine questions such as the relationship between landuse and the fish populations in an area. Habitat and water quality information is sometimes collected as part of research projects.

3.8.3 *Canadian Wildlife Service (CWS)*

The Canadian Wildlife Service (CWS) tracks changes in wetland distribution and use by ducks across the prairie regions of Manitoba, Saskatchewan, and Alberta. This involves flying transects across the prairie regions and counting wetlands and ducks, followed by ground-truthing a subsample of the flight transects to gather additional information, such as depth of waterbodies. In addition, the Prairie Habitat Joint Venture (PHJV) Habitat Monitoring Program collects data on land use change, and changes in wetland status over time at a number of sites across the prairies (Watmough et al. 2002). CWS protocols for sampling wetlands do not typically include collection of water quality data.

In general, the types of data collected by these resource-oriented agencies do not include a general assessment of the health of the aquatic environment. Monitoring activities are specific to understanding the status of the targeted group (e.g., fish, waterfowl) and would not benefit from

the general assessment of site health that is offered by microbial CLPP, barring, of course, the identification of ecological conditions incompatible with the needs of the target group. However, the CLPP approach might still be appropriate for some of the research funded by agencies such as the ACA, or for research conducted by agencies such as ASRD. In addition, once technical development of the CLPP technique has proceeded, the tool might be easily applied to routine monitoring of fish and waterfowl habitat, and used to designate sites that may be in need of additional management or research to ensure the habitat remains healthy for target species.

3.8.4 *Individual Companies*

Many individual companies in Alberta's oil sands rely on organizations such as RAMP for routine monitoring of aquatic habitats at site and regional scales. In addition, oil sands companies include various types of sampling in their Environmental Impact Assessment reports which are required for all mineable oil sands projects in Alberta, and provide some information regarding pre-disturbance conditions at a site. Some companies also do routine monitoring (water chemistry data is commonly collected) as part of their reclamation activities or research programs, and additional monitoring may be done on their leases during research by other agencies (e.g., universities). For example, the Carbon Dynamics, Food Web Structure, and Reclamation Strategies in Athabasca Oil Sand Wetlands (CFRAW) , which involves a number of academic research teams, is working on reclaimed wetlands in the mineable oil sands area, and samples a wide range of parameters at reclaimed and reference sites. Both Suncor and Syncrude provided support for a study characterizing microbial communities of wetlands in the oil sands region (Hadwin et al. 2006).

4 CONCLUSIONS/RECOMMENDATIONS

Microbial communities can be used as environmental indicators. Empirical evidence has demonstrated that these communities are shaped by the physical, chemical, and biological aspects of their environment. While the theoretical framework is currently insufficient to predict how a given microbial community will respond to a specific environmental stressor, broad empirical descriptions of cause-and-effect relationships already exist. If the technologies for profiling microbial communities continue to be developed, it is probable that microbial community profiling will be able to provide a rapid, inexpensive, and relatively easy means of monitoring the status of aquatic ecosystems.

Of the microbial community profiling technologies available, CLPP is the most cost effective. The profiles produced by CLPP reflect both the phenotypic and genotypic characteristics of a microbial community for minimum investments of time and money. The capital equipment costs are similar to, or far less than, those associated with other profiling techniques. CLPP also has the potential to be far less expensive, time consuming, and technically demanding than traditional ecological assessment techniques, while still producing a level of detail sufficient to identify 'at risk' systems requiring more intensive investigation and/or intervention.

In terms of application, CLPP may be best employed as a screening assay. Parties engaged in the broad ecological assessment of large numbers of sites, or the frequent monitoring of a few

ecosystems, would benefit most from CLPP. Alberta Environment, via some of its aquatics monitoring programs, and RAMP are among those groups whose work could be significantly advanced by the adoption of CLPP as a monitoring tool. For these groups, the ability to screen multiple sites quickly, easily, and inexpensively would be a substantial improvement over the status quo. Other groups including CEMA, ABMI, and CONRAD may have some interest in CLPP as a research or monitoring tool. Groups concerned only with specific aspects of aquatic ecosystems, such as DUC and DFO, are unlikely to derive a substantial benefit from CLPP or any other microbial profiling technology.

Another application for CLPP is the tracking of reclamation projects over time and across a variety of spatial scales (e.g., site, mine, and regional). In this capacity, CLPP may be of particular interest to individual oil sands companies and to Alberta Environment. Again, it is the potential for rapid and relatively inexpensive assessment of multiple sites over time that makes CLPP attractive as a reclamation monitoring tool. Pending the compilation of appropriate and time-indexed 'target values', the development of a given reclamation project could be tracked, and any deviations from the expected trajectory identified, allowing for timely investigation and intervention.

Advancements must be made in two fields before CLPP could be widely employed as an ecological monitoring tool. First, some technical refinements must be made to improve the precision of the assay. Standardizing inoculum density and optimization of sampling and data analysis procedures are expected to enhance the performance of the assay. Second, a broadly based body of background information must be compiled in order to better define the capabilities of the assay, and aid in the interpretation of results.

The oil sands industry can benefit from CLPP while contributing to its development. The organizations concerned with environmental research (CEMA, CONRAD) or monitoring (AENV, RAMP) in the region can promote the inclusion of CLPP in their projects. Such an arrangement promotes the iterative improvement of technical procedures and the accumulation of background data. Projects examining the characteristics of reclaimed aquatic ecosystems, and their progression towards a more natural state, are particularly useful in this regard. CLPP data collected during these projects can be used in future monitoring programs as a reference dataset. More importantly, this approach will allow the industry to test the application of the technique, with minimal risk and investment.

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6 GLOSSARY OF TERMS AND ACRONYMS USED IN THIS REPORT

6.1 Terms

Aerobic

Living or occurring only in the presence of oxygen. This in contrast to the term anaerobic, meaning living or occurring only in the absence of oxygen.

Archaea

A group of microorganisms, previously known as archaebacteria, which possess certain characteristics of prokaryotic and eukaryotic cells.

Adenosine Triphosphate (ATP)

The molecule which is the source of energy for most metabolic processes in living organisms.

Autotroph

An organism that can synthesize complex organic substances from simple inorganic components using either light (photoautotroph) or inorganic chemical reactions (chemoautotroph) as an energy source.

Biofilm

Collections of microorganisms that exist in a multicellular community form in an exopolysaccharide extracellular matrix, adherent to each other or a surface. The biofilms discussed in this document are related to, but much less developed than, the benthic microbial mats that some readers may be familiar with.

Catabolism

The set of metabolic reactions which break down molecules into smaller units, usually associated with the release of energy.

Denature

In the context of DNA, denaturation (also known as melting) is the process by which double stranded DNA unwinds and separates into single strands by breaking of hydrogen bonds.

Ecoregion (Ecological Region)

An ecologically and geographically defined area larger than an ecosystem, but smaller than an ecozone. Ecoregions contain characteristic, geographically distinct natural assemblages of communities and species. See http://www.tpr.alberta.ca/parks/heritageinfocentre/docs/NRSRcomplete%20May_06.pdf for more information on Alberta's ecoregions.

Electrophoresis

An analytical technique used to separate biological molecules, most commonly by size or mass, when the charge to mass ratio is held more or less constant. By establishing a spatially uniform electric field across a separation matrix (see polyacrylamide gel below) the biological molecules in question will migrate through the matrix at a rate inversely proportional to their mass, or in the case of DGGE, the degree of denaturation.

Epilithic

On the surface of rocks or stones.

GC Content (Guanine-cytosine content)

The percentage of nitrogenous bases found within a given DNA molecule that are either Guanine or Cytosine. Since Guanine-Cytosine pairs are bound by 3 hydrogen bonds rather than 2 for Adenine-Thymine pairs, DNA molecules with a high GC content are more thermostable, compared to those with a low GC content. With respect to DGGE, GC content is a major determinant of denaturing (melting) conditions. DNA molecules with a high GC content will tend to be more resistant to denaturation than will molecules with a low GC content.

Genotype

The genetic constitution of a cell or organism usually with respect to a specific character under consideration.

Gram-Positive/Negative

An empirical division imposed on bacteria as based on the staining procedure developed by Hans Christian Gram in 1884. Gram-positive bacteria will stain purple through the uptake of crystal violet. Gram-negative bacteria will not retain the violet stain, and will instead take up the counterstain (safranin or fuchsin) and appear red or pink. The morphological differences associated with Gram status relate to cell wall construction and the presence of an outer membrane, outside the cell wall, in Gram-negative bacteria.

Heterotroph

An organism that depends on complex organic substances for energy.

Inoculum

A substance or organism that is introduced into surroundings suited to cell growth. In this case, inoculum refers to the microbial suspension introduced into the wells of the Biolog EcoPlate.

Kinetic

In this case, the study of rates of metabolic processes.

Lentic

Of or relating to still waters. Lakes and marshes would be considered lentic ecosystems.

Limnetic (zone)

The open, deeper portion of a body of water.

Lipid

A broad group of naturally occurring hydrophobic or amphiphilic molecules that include the fats, waxes, sterols, fat soluble vitamins, monoglycerides, diglycerides, phospholipids, and others.

Littoral (zone)

That part of a body of water that is close to shore. With respect to our CLPP evaluations, we have defined the littoral zone as that area containing emergent vegetation.

Lotic

Of or relating to actively moving waters. Rivers would be considered lotic ecosystems

Metabolism

The set of chemical reactions which occur within a living organism to maintain life.

Microbe

A microorganism.

Phenotype

Any observable characteristic or trait of a cell or organism. Phenotypes result from the expression of genes as modified by various environmental factors. For example, skin colour is the result of melanin pigments. The expression of the genes controlling melanin production is modified by environmental factors (i.e., intensity and duration of sunlight).

Phospholipids

A subgroup of lipids which contain a phosphate group and form the major component of cell membranes through their ability to form lipid bilayers.

Polyacrylamide Gel

A separation matrix used in the electrophoresis of biological molecules (proteins, nucleic acids) composed of crosslinked acrylamide subunits.

Polymerase Chain Reaction (PCR)

A technique in molecular biology to amplify a single or few copies of a piece of DNA across many orders of magnitude to result thousands to millions of copies (http://en.wikipedia.org/wiki/Polymerase_chain_reaction).

Saturation

Within the context of Phospholipid Fatty Acids, this term is used to refer to the double bonds present within a fatty acid molecule. Saturated molecules have no double bonds. Unsaturated molecules have at least one double bond.

Sonicate

To disrupt with (ultra)sound waves.

Taxa

Plural of taxon. Taxonomic groups such as phyla or genera.

Tetrazolium

A class of organic compounds that, when reduced by living organisms (usually through an enzymatic reaction), form another organic compound called a formazan dye. The formazan dyes vary in colour from blue to red to orange. In the EcoPlate assay, the formazan dye is purple (http://en.wikipedia.org/wiki/Formazan).

Triage

Sorting and allocating resources on the basis of need or likely benefit.

6.2 Acronyms

ABMI	Alberta Biodiversity Monitoring Institute
ACA	Alberta Conservation Association

AENV	Alberta Environment
ALMS	Alberta Lake Management Society
ASRD	Alberta Sustainable Resource Development
ATP	Adenosine triphosphate
CEMA	Cumulative Environmental Management Association
CFRAW	Carbon Dynamics, Food Web Structure, and Reclamation Strategies in Athabasca Oil Sand Wetlands
CLPP	Community Level Physiological Profiling
CONRAD	Canadian Oil Sands Network for Research and Development
CWS	Canadian Wildlife Service
DFO	Department of Fisheries and Oceans
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic acid
DUC	Ducks Unlimited Canada
EIA	Environmental Impact Assessment
EINP	Elk Island National Park
FISH	Fluorescent *in-situ* Hybridization
GN	Gram Negative
HADD	Harmful Alteration, Disruption and Destruction (of fish habitat)
LTRN	Long Term River Network
NNL	No Net Loss (in reference to fish habitat)
OSRIN	Oil Sands Research and Information Network
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction

PHJV	Prairie Habitat Joint Venture
PLFA	Phospholipid Fatty Acid (analysis)
RAMP	Regional Aquatics Monitoring Program
RMWB	Regional Municipality of Wood Buffalo
SEE	School of Energy and the Environment
T-RFLP	Terminal Restriction Fragment Length Polymorphism