# FACILITATING REGULATORY COMPLIANCE THROUGH ENVIRONMENTAL GENOMICS

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# EXECUTIVE SUMMARY

A review of the environmental regulations applied to the oil sands industry and municipal wastewater systems was undertaken to better understand how environmental genomics (EG) might facilitate compliance in these and other similarly regulated industries. The best potential applications of current EG approaches were identified, as were the associated research and development opportunities and challenges.

Across multiple fields of study, EG has shown potential to considerably reduce time and expense associated with biotic monitoring. Within specific applications, EG can sometimes offer additional benefits (e.g., increased sensitivity and taxonomic resolution) when compared to conventional methods.

However, there are limitations on EG's ability to satisfy regulatory monitoring requirements, some of which may be ameliorated with addition research and development:

- Estimates of population size can be imprecise.
- An inability to resolve DNA sources within taxa (e.g., live from dead, distant from local, current from former)
- Amplification/primer biases
- Complex eDNA distribution kinetics
- Sequence database deficiencies
- A lack of standardized methods

In addition, non-technical issues may limit the use of EG in a regulatory context. The potential cost and time savings associated with EG may be insufficient to overcome the institutional inertia associated with established conventional monitoring. Moreover, an objective evaluation of EG's adequacy as a monitoring tool is impaired by a lack of methodological guidance from the regulator.

At the time of writing, no regulatory requirements were identified which could be entirely satisfied by EG. However, with additional research and development, EG might facilitate regulatory monitoring of benthic macroinvertebrate and fish communities. Of these two applications, the availability of taxonomically curated "bulk" samples and the widespread acceptance of indices as meaningful ecological metrics makes macroinvertebrate community analysis the preferred option. While fish community analysis benefits from a relatively small set of target taxa and comparatively well-developed sequence databases, its dependence on eDNA as a sample material constrains EG's potential utility for the foreseeable future. The use of EG to assess plant communities, vertebrate communities, biodiversity, eutrophication, and the

microbial content of wastewater were evaluated, but presented greater technical challenges and/or lower potential utility than did benthic macroinvertebrate or fish community analysis.

If sufficiently developed, EG could be productively applied to biotic monitoring programs from pre-disturbance site assessments to post-development and reclamation monitoring programmes. EG may also be used in effects monitoring programmes conducted downstream of metal mines, diamond mines, and pulp/paper mills. In addition, EG might aid in industrial research projects, the development of novel site reclamation indicators, and the assessment of grassland well sites affected by salt contamination.

Further exploration of these opportunities is advisable prior to embarking on substantial research and development programmes.

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# 1.0 BACKGROUND

In a modification of Cordier's 2020 definition, environmental genomics (EG) can be defined as, "a suite of tools used to collect, process, and analyze nucleic acids from environmental samples to support an enhanced understanding of the environment from which those samples were drawn". Less formally, EG can be understood as the use of nucleic acid samples (usually DNA) to characterize and monitor the biotic components of an environment. Since this definition of EG approximates the broadest definition of environmental DNA (eDNA), the term "eDNA" is used herein only to refer to DNA present in the environment in a form disassociated from its source organism (e.g., shed cells, feces, etc.).

Since its first published "macrobiological" application in 2008 (Ficetola et al.), EG has promised the detection of a wide range of biota, including cryptic and hard-to-find species, from readily available environmental samples. By using soil, air, and water as samples, EG has the potential to monitor rare or endangered biota without direct contact, thereby protecting them from sampling-induced injury or death. Such a technology could greatly reduce the time and expense associated with sampling programmes and minimize the need for taxonomic expertise. In addition, there may be greater freedom in the timing of sampling programmes by reducing the need to sample during important biological events (e.g., spawning, flowering).

Though still an area of active research and development, EG would appear to be a boon to any who must routinely monitor the environment to satisfy regulators. Yet, despite its potential, EG has not been widely adopted as a tool for regulatory compliance. Why?

A review of the environmental regulations relevant to the oil sands industry and municipal wastewater systems was undertaken to better understand the needs of potential EG customers. A detailed understanding of these two potential customers was considered more useful than a high-level survey of environmental regulations in general. The best potential applications of EG within these contexts were identified, as were the technical challenges yet to be overcome. This information provides strategic direction for future EG research and development projects, allowing them to be better focused on the needs of industry, government agencies, and other stakeholders involved in regulatory monitoring, and related endeavors.

# 2.0 TERMS AND CONCEPTS

**Autecology**

The study of individual species in relation to their environment. The relevance of autecology to this review is in its ability to link EG results to assessments of the broader environment. For example, the absence of DNA from a salt-sensitive species may indicate high environmental salinity levels in the past, even if those levels are normal at the time of sampling. The autecological component of that interpretation is in knowing that a particular species is sensitive to salt. By detecting, or failing to detect, that species' DNA, EG could inform estimates of past salinity levels. Understanding the autecology of species routinely detected by EG, particularly those typically undetected by conventional methods, has the potential to expand EG's utility as an environmental monitoring tool.

**Benthic**

Of, or pertaining to, the sediment at the bottom of a body of water. The related term, benthos, refers to those organisms which live on or in that sediment.

**Biological vs. technical replicates**

In this review, biological replication, also called replicate sampling, refers to multiple samples taken at a specific time and place using a single method. For example, three 1 litre water samples taken simultaneously from a single wetland using identical equipment would be considered biological replication of that wetland. Technical replication refers to identical analyses conducted on the same sample. For example, four qPCR reactions run using DNA extracted from a single sample would be considered technical replication of that sample. The purpose of biological replication is to capture the variability inherent in an experimental unit (a wetland). The purpose of technical replication is to capture the variability inherent in an analytical method (qPCR) and/or the distribution of unique DNA sequences across aliquots withdrawn from a single sample.

**Bulk DNA**

In this document, this term is used to signify the DNA extracted from a collection of organisms or their tissues as distinct from that extracted from individual organisms or environmental samples. For example, bulk DNA could be obtained from a homogenate of flying insects caught in a net.

**CCME (Canadian Council of Ministers of the Environment)**

A Canadian inter-governmental forum for coordinating action on environmental issues. The body is composed of Ministers of the Environment from the federal, provincial, and territorial governments. The informational documents issued by the CCME are often referenced by regulations or other informational documents.

**CPUE (Catch per Unit Effort)**

A measure of fish abundance calculated by dividing the amount of fish caught by the amount of effort required to catch it. The numerator is typically in units of mass, whereas denominator's units vary according to the methodology used (e.g., angling vs. seine nets). Irrespective of the units involved, CPUE values are expected to correlate positively with the abundance of fish in the study area.

**Database**

Within this document,"database" refers to searchable online bodies of information which link DNA sequences to specific taxa. An ongoing process of tissue collection, sequencing, and error correction means that these databases tend to improve over time. Nevertheless, they still contain gaps and inaccuracies. Incomplete or flawed databases are an important factor limiting the utility of EG.

**eDNA (Environmental Deoxyribonucleic Acid)**

In its broadest definition, eDNA refers to both deoxyribonucleic acid extracted from environmental samples and the technologies used for its collection and analysis. In this review, however, eDNA is used only in reference to the former (i.e., the material). While it may be present in the environment as a free molecule in solution or adsorbed to particulate matter, most eDNA is still contained within cells which have been shed from an organism, making filtration a useful means of collection (Turner et al., 2014). In the field, eDNA is most frequently extracted from water samples but has also been obtained from soil, air, honey, feces, flowers, and other biotic structures. Some of the literature cited herein uses the acronym eDNA to refer to "extracellular" DNA, usually in reference to antimicrobial resistance genes found as free nucleic acid in the environment. While this definition does not necessarily conflict with the one used herein, the reader should be aware of the distinction should they choose to review the supporting literature.

**EIA (Environmental Impact Assessment)**

A process mandated under the *Environmental Protection and Enhancement Act* (Government of Alberta, 1993) which attempts to assess the environmental consequences of a capital project before construction begins. While they may be required for a range of projects, this document only references EIAs conducted in support of in-situ oil sands developments. **EIAs provided the bulk of "traditional methods" information for Section 3 of this review.**

**Indices**

For the purposes of this document, indices refer to quantitative metrics calculated to describe various, generally biotic, aspects of an ecosystem. These indices vary in their constituent values, complexity, and the aspect of an ecosystem which they purport to assess. To varying degrees, EG techniques may have the ability to "infer" a value for one or more of these indices. Index values are not calculated from EG results as might be done with data obtained from traditional methods. Rather, the repeated comparison of results derived from EG analyses to index values calculated from traditional data allow an empirical correlation to be established. If that correlation is sufficiently strong, a reasonable estimate of the index value can be made from EG data alone.

**Legislation vs. Regulation vs. Informational Documents**

The relationship between legislation and regulation is important context for this document. Legislation, created by elected officials, describes laws in terms of frameworks and principles while regulation, created by bureaucrats, describes the standards and means by which legislation will be implemented and enforced. A single act (i.e., legislation) may spawn many regulations while a regulation often derives its authority from only a single act. It is regulation rather than legislation which informs environmental compliance programmes. Orthogonal to the legislation/regulation axis are guidelines, codes of practice, guidance documents, best practices, directives, and other informational documents. These documents are typically created by operational personnel and subject matter experts within governmental or non-governmental bodies (e.g., CCME) and attempt to inform rather than constrain or enforce. While informational documents do not carry any legal authority in themselves, they may be referenced by regulation or legislation and are, therefore, relevant to environmental compliance programmes.

**Locus, Primers, and Target**

Locus (pl. loci) refers to a location in an organism's genome, often one chosen for amplification. This amplification is directed by primers, short strands of man-made DNA which attach to the template DNA on either side of the locus in a process known as annealing. Altering the DNA sequence of a primer will alter the location at which it anneals, and therefore the locus which is amplified by PCR. The amplified locus is usually referred to as the "amplification target" or "PCR target". However, the term "target" can also be used to denote the organism that researchers wish to detect (e.g., "PCR primers targeting rainbow trout"). The reader is advised to rely on context to disambiguate the use of "target" in the supporting literature.

The development of optimal primer sets and, therefore, amplification targets is a point of ongoing research, particularly for metabarcoding techniques. The broader the taxonomic range of biota to be included in a metabarcoding study, the more difficult it is to develop a single set of primers which perform well in all relevant species. Indeed, many studies incorporate multiple amplification targets to account for genomic dissimilarities across taxa.

**Metabarcoding**

One of two EG techniques referenced in this document and the one best suited to the characterization of entire communities. The technique involves the selection of a genomic target which is sufficiently conserved to allow amplification across a range of taxa yet variable enough to allow differentiation of those taxa based on sequence variations. The melange of products is expected to reflect the composition of the community. Because the amplification step produces a collection of products (i.e., a mixture of DNA fragments exhibiting small variations in size and sequence), this technique depends on high throughput sequencing to distinguish and quantify each one. Like qPCR, metabarcoding is not without its vagaries, some of which increase with the taxonomic breadth of the community being profiled (i.e., it is easier to profile a community composed of a few fish species within the same family than an invertebrate community composed of many phyla).

**Mock Communities**

A mock community is a precisely formulated admixture of genomic DNA extracted from species expected to be present within the community under investigation (e.g., a freshwater fish community). It functions as a control against the under- or over-representation of taxa in metabarcoding data. By subjecting it to the same analytical process applied to field samples, the mock community reveals methodology-induced biases as discrepancies between its known species composition and that reported by the metabarcoding results. Presumably, the utility of mock communities may be limited by the degree to which their species composition mirrors that of field samples, the availability of suitable specimens from which genomic DNA can be extracted, and the presence of biases induced by or prior to the extraction of DNA from field samples.

**MOTU/OTU (Molecular Operational Taxonomic Unit/Operational Taxonomic Unit)**

Because the databases do not (perhaps cannot) include sequences covering all genotypes present in all species, metabarcoding studies inevitably produce barcode sequences for which no corresponding taxon has been identified. Nevertheless, these “unidentified” sequences exist within the dataset, sometimes in large numbers, and can be grouped into clusters based on their similarity to one another. These clusters are referred to as (Molecular) Operational Taxonomic Units (MOTUs) and are presumed to indicate the presence of a taxon which has not yet been included in a database. MOTUs are useful in inferring descriptive statistics/indices for a community but cannot be used to identify a particular taxon until the databases are appropriately updated. In this way, values for certain ecological indices (e.g., taxa richness) can be inferred without necessarily identifying every taxon represented by the metabarcoding data. There are certain risks associated with the use of MOTUs in this way. For example, a MOTU may represent taxa outside the community of interest (e.g., DNA from waterfowl might be present in benthic invertebrate samples) and thereby contribute to overestimation of community diversity.

**qPCR (Quantitative Polymerase Chain Reaction)**
The second of two EG techniques referenced in this document. While subject to various permutations, qPCR is fundamentally a technique wherein the amplification of a target DNA sequence is monitored, producing an estimate of the amount of template DNA present in the original sample. This estimate is, however, associated with variable levels of precision. Within EG, qPCR is typically employed to detect one or a few target species. As the number of target species increases, qPCR becomes less practical, leaving metabarcoding as the preferred approach.

**Read Count vs. Sequence Abundance vs. Copy Number**
These terms all speak to the prevalence of a particular nucleic acid sequence, albeit in different contexts. The definitions provided here are based on the use of these terms in the literature reviewed for this document. Outside of this relatively small body of literature, other definitions may be more appropriate.

Read count refers to the number of times, in absolute terms, a particular sequence is detected by high throughput sequencing. The number of times a particular sequence is detected (i.e., read) is that sequence's read count.

Sequence abundance refers to the prevalence of a given sequence in a, typically, mixed DNA sample prior to amplification. Usually, this value is back calculated from the results of a qPCR assay.

Copy number is a context-dependent term indicating the number of copies of a given sequence found within a larger body of DNA. In some cases, copy number is synonymous with sequence abundance (e.g., eDNA copy number would refer to the number of copies of a given sequence present within a mixed eDNA sample). In other cases, copy number refers to the number of copies of a sequence within a genome (e.g., gene copy number).

**Sensitivity and Specificity**

These terms are used to describe the performance of an analytical method, be it traditional or molecular in nature. Sensitivity refers to a method's ability to detect its analytical target, while specificity refers to a method's ability to distinguish its intended analytical target from other, possibly similar, targets. For example, the minimum number of brown trout within a body of water required for a method to detect their presence is a measure of sensitivity. Conversely, the probability that a rainbow trout will be misidentified as a brown trout is a measure of specificity.

**Traditional or Conventional Methods**

Those methods currently in use to conduct environmental research and to which EG approaches are being compared.

**Trophic**

The meaning of this term is context dependent. In some cases, it may refer to an organism's trophic level; its place in a food chain (e.g., primary producer, tertiary consumer, etc.). In other cases, it may refer to the trophic state of an aquatic ecosystem, characterized by its nutrient content and level of primary productivity (e.g., eutrophic vs. oligotrophic).

# 3.0 INDUSTRY-SPECIFIC CONTENT

Sections 3 and 4 profile the regulatory frameworks under which oil sands companies and municipal wastewater systems operate. They also provide an evaluation of EG's potential to contribute to each industry's regulatory compliance.

Each section begins with an outline of the regulatory documents reviewed and the salient points, if any, recovered. Following this outline is an evaluation of the regulatory landscape, identifying important factors influencing the potential for EG adoption. The regulatory frameworks governing these industries are somewhat "diffuse" in nature, seldom providing explicit information of obvious pertinence to prospective EG adoption. By identifying all reviewed documents, even those failing to provide explicitly relevant content, the reader is provided with an appreciation of the breadth of the survey and, perhaps, the regulatory "tone" applied to industry.

Following the regulatory review are subsections devoted to the comparative performance of EG relative to traditional methods. The relative strengths and weaknesses of EG in satisfying regulatory data requirements are articulated. Public and detailed information on the biotic monitoring methods currently employed by the oil sands industry was only available through EIAs. As a result, these documents play a central role in defining the traditional methods to which EG is compared.

These sections are not literature reviews in the traditional sense. Rather, the information is presented in point form, thereby enhancing the accessibility of the information to the reader.

# 4.0 OIL SANDS INDUSTRY

## 3.1 REGULATORY DOCUMENTS REVIEWED

- 2010 Reclamation Criteria for Wellsites and Associated Facilities for Forested Lands (AESRD, 2013 (a)).
  - Includes vegetation assessment which incorporates desirable (woody and herbaceous) and undesirable (weed) species.
  - Provides some guidance on sampling grids and plots.
  - Requires only dominant species be identified.
  - Quantification in terms of % cover, stem count, and plant count.
  - Also requires information on growth (height, branch leader growth) and health (chlorosis).

- A Framework for Reclamation Certification Criteria and Indicators for Mineable Oil Sands (Poscente, 2009).
  - Identifies a series of reclamation criteria, primarily established through approvals under the Environmental Protection and Enhancement Act, intended to evaluate the adequacy of a reclamation programme.
  - For many of these criteria, no measurable indicators (i.e., metrics) exist. In some cases, these missing indicators may represent opportunities for EG to find application in a regulatory context.

- Alberta Energy Regulator (AER) documents
  - Approvals to:
    - ConocoPhillips Canada Resources Corp. for the Surmont enhanced recovery in-situ oil sands or heavy oil processing plant and oil production site. Approval No. 48263-01-00 (AER, 2015).
    - Imperial Oil Resources Limited for the Construction, operation, and reclamation of the Cold Lake enhanced recovery in-situ oil sands or heavy oil processing plant and oil production site. Approval No. 73534-02-00 (AER, 2020 (a)).
    - Suncor Energy Inc. (amending) for the Construction, operation, and reclamation of the Suncor Energy Inc. Oil Sands Processing Plant and Mine. Approval No. 94-02-18 (AER, 2017 (a)).
    - Syncrude Canada Limited for the Construction, operation, and reclamation of the Mildred Lake Oil Sands Processing Plant and Mine,

Aurora North Oil Sands Processing Plant and Mine, and Aurora South Oil Sands Processing Plant and Mine. Approval No. 26-03-00 (AER, 2020 (b)).

- These approval documents describe the ongoing monitoring and research requirements applied to each capital project.
- Of the technical material contained within these documents, most describes the physicochemical monitoring of air, soil, and water.
- Biotic monitoring, in contrast, appears only rarely and with little detail. Criteria for acceptance of a biotic monitoring programme and its results are typically at the discretion of the regulator.
- The domains involved in the biotic monitoring programme vary from one approval to the next, but often include fish, benthic macroinvertebrates and/or terrestrial wildlife. Presumably these differences are related to differences in the character of each capital project and its environs.
- Often, regional programmes like OSM and ABMI are referenced as a means by which monitoring or research requirements might be satisfied.
- Monitoring programmes for reclaimed sites are also required, but no greater detail is provided to aid in their design or in evaluating their results.

- Canadian Natural Horizon Oil Sands Mine North Pit Extension Integrated Application and Environmental Impact Assessment (CNRL, 2019).
  - Unlike similar documents from Suncor and Devon, this document contains a conceptual framework for a fish habitat offsetting plan.
  - One of the components of this conceptual framework is an Arctic Grayling research programme, though no details are provided.

- Devon Pike 2 Environmental Impact Assessment (EIA) (Devon Canada Corporation, 2018).
  - Reviewed after the Suncor Lewis EIA and found to be very similar in character to that document.
  - Monitoring section is almost entirely concerned with Devon's participation in large multi-stakeholder monitoring organizations and projects.

- Devon Pike 2 Environmental Impact Assessment Final Terms of Reference (AER, 2018 (a)).
    - Describes the content required of the EIA for Devon's Pike 2 project.
    - Content is very similar to, if not exactly the same as, the Suncor Lewis EIA FTOR.
- Directive 001: Requirements for Site-Specific Liability Assessments (AER, 2023 (a)).
    - Methodology to estimate the costs of closing an industrial site.
- Directive 091: Rock-hosted mineral resource development (AER, 2024).
    - Concerned with hard-rock mining – not relevant to oil sands industry.
- Directive 050: Drilling Waste Management (AER, 2023 (b)).
    - Contains information regarding toxicity assessment of materials (mostly Microtox).
    - No metrics defined that are obviously amenable to EG.
- MEG May River EIA Final Terms of Reference (AER, 2016 (a)).
    - Reviewed after corresponding Suncor and Devon documents.
    - Relevant content appears to be almost identical across all three.
- Specified Enactment Direction 001 – Direction for Conservation and Reclamation Submissions (AER, 2016 (b)).
    - Describes the operational and administrative processes associated with site reclamation.
    - Mentions profiling the vegetation community prior to disturbance – describes the information required in very general terms (e.g., "Include a species list for dominant vascular and nonvascular species").
- Specified Enactment Direction 002 – Application Submission Requirements and Guidance for Reclamation Certificates for Well Sites and Associated Facilities (AER, 2019).
    - Describes the information to be submitted for reclamation certification (e.g., pre-disturbance or off-site community type), primarily referencing plant community.

- Specified Enactment Direction 003 – Direction for Conservation and Reclamation Submissions Under an Environmental Protection and Enhancement Act Approval for Mineable Oil Sands Sites (AER, 2018 (b)).
    - Describes the information required but only in very general terms – not specific enough to judge the ability of EG to provide adequate information. For example, under the biodiversity section, line 58 states, "Using Table 9, provide reclamation diversity targets to show links between target ecosites, wetland type, or aquatic feature, and target wildlife or aquatic species."
- Suncor Lewis EIA (Suncor Energy Inc., 2018).
    - Huge document.
    - This and the Devon equivalent provide the bulk of the traditional methods information against which EG equivalents are compared.
    - The monitoring portion is not particularly informative: "After the Lewis Project has been approved, Suncor will monitor wildlife resources using similar methodologies used at other Suncor facilities in accordance with the regulatory approval conditions and Suncor's internal reporting and performance evaluation requirements."
- Suncor Lewis EIA Final Terms of Reference (AER, 2017(b)).
    - Lists the sort of information required as part of the Environmental Impact Assessment.
    - Doesn't go into how data should be collected, just defines broad categories. For example, "Describe and map the fish, fish habitat (e.g., aquatic and benthic invertebrates) of the lentic and lotic ecosystems, including intermittent and ephemeral water bodies. Describe the species composition, distribution, relative abundance, movements, and general life history parameters, including their use and potential use of habitats. Provide the methods used and the rationale for the baseline data collection."
    - This is the most specific articulation of regulatory data requirements found during this review.
    - Required information is split into broad categories of Biodiversity, Aquatic Ecology, Vegetation, Wildlife, and Monitoring. These divisions are used to organize the comparisons between traditional methods and EG for the oil sands industry.

- Alberta Environment and Parks documents
  - Alberta Environmental Site Assessment Standard (AEP, 2016 (a)).
    - Provides instructions for inspecting an industrial (energy) site for potential or actual harms to the environment.
  - Contaminated Sites Policy Framework (AEPA, 2023 (b)).
    - Describes the conceptual framework used by the AER to assess the environmental risk associated with a contaminated site.
    - No explicit technical requirements are provided.
  - Reclamation Criteria for Wellsites and Associated Facilities for Peatlands (AEP, 2017).
    - Includes vegetation assessment which incorporates bryophytes, herbs, graminoids, woody species, and weeds.
    - Provides some guidance on sampling grids and plots plus the assessment of microhabitats within the study area.
    - Requires species be listed and a species richness figure be provided.
    - Quantification in terms of % cover for each structural layer.
    - Provides some guidance on aerial assessments of difficult-to-access sites.
  - Supplemental Guidance on Site-specific Risk Assessments in Alberta (AEP, 2022 (b)).
    - Another document describing risk assessment at the conceptual level.
    - No prescribed methods or metrics are provided, only generalities: "It may be necessary to develop a complete inventory of potential human and ecological receptors that may be important to a site prior to determination of the sensitive receptors, especially in the context of valued ecosystem components (VECs), endangered species, or traditional land use (TLU) considerations."
    - Frequently references CCME guidelines.
- CCME Guidance Manual for Environmental Site Characterization in Support of Environmental and Human Health Risk Assessment volume 1 (CCME, 2016 (a)).
  - Another "conceptual" document describing the circumstances under which a certain part of the community might be surveyed, and for what purpose.

- CCME guidance manual for environmental site characterization in support of environmental and human health risk assessment volume 2 (CCME, 2016 (b)).
    - Little relevance to EG.
    - Primarily concerned with harvesting biota for the measurement of chemical contaminants in tissues.
- CCME guidance manual for environmental site characterization in support of environmental and human health risk assessment volume 3 - suggested operating procedures (CCME, 2016 (c)).
    - Suggested operating procedures for the collection of samples.
- Environmental Protection and Enhancement Act (GoA, 1993 (c)).
    - An administrative document that does not specify metrics or monitoring criteria.
    - Regulations under the act:
        - Activities Designation Regulation (GoA, 2003 (a)).
            - Specifies the types of industry which require authorizations under EPEA.
        - Code of Practice for Exploration Operations (GoA, 2005).
            - Specifies requirement for exploration company to revegetate disturbed land such that the plant community is consistent with the land use originally intended by owner.
        - Conservation and Reclamation Regulation (GoA, 1993 (b)).
            - Specifies, in general terms, information which must be included in an application for a reclamation certificate.
        - Environmental Assessment (Mandatory and Exempted Activities) Regulation (GoA, 1993).
            - Identifies industrial infrastructure which must have, or is exempt from, environmental assessment.
        - Oil Sands Environmental Monitoring Program Regulation (GoA, 2013 (d)).
            - Details the support (primarily financial) oil sands companies must provide to the Oil Sands Monitoring (OSM) programme.
        - Remediation Regulation (GoA, 2009 (c)).
            - Discusses the Tier 1 and Tier 2 soil and groundwater guidelines, which are uniformly physical and chemical metrics, and the administration of remediation certificates.
- Oil Sands Conservation Act (GoA, 2000 (a)).

        - An administrative document intended to account for oil sands materials as a financially valuable resource and to ensure the province receives remuneration for it.
    - Under the Act
        - Oil Sands Conservation Rules (GOA, 1988)
            - An operational document describing oil sands administrative and operational requirements. To the degree pollution is mentioned, it is limited to hydrogen sulphide gas and is dealt with in purely physicochemical terms.
- Oil Sands Monitoring Program: Annual Report for 2018-2019 (Oil Sands Monitoring Program, 2019).
    - Describes a regional programme that monitors environmental effects of oil sands development.
    - Synopsis of research projects undertaken by various parties across aquatic and terrestrial environments under the OSM programme.
    - OSM websites:
        - Deltaic Ecosystem Health – Oil Sands Region (GoC, 2022)
            - Includes invertebrates and water chemistry metrics.
        - Mainstream Benthic Invertebrates (GoC, n.d.)
            - Focuses on benthic invertebrates.
        - Monitoring Biodiversity in Alberta Oil Sands (GoC, 2024 (b)).
            - Includes migratory landbirds.
        - Assessing Ecosystem Health in Benthic Macroinvertebrate Assemblages of the Athabasca River Main Stem, Tributaries, and Peace-Athabasca Delta (1.7 Report Series) (Culp et al., 2018).
            - Recommends more investigation of causal relationships between oil sands contaminants and changes to benthic invertebrate community, and development of metrics to quantify those changes.
            - Recommends investigation of invertebrate responses to nutrients.

        - Advocates for DNA-based monitoring of benthic invertebrate community.
    - A before-after dose-response (BADR) terrestrial biological monitoring framework for the oil sands (Bayne et al., 2021)
        - Examines invasive species, plant species, migratory landbirds, mammals, amphibians.
        - Focuses on broad ecological measures (species richness, abundance, distribution, etc.).
- Oil Sands Monitoring Program: Annual Report for 2019-20 (Oil Sands Monitoring Program, 2024).[1]
    - Mentions a number of projects associated with EG at various levels of completion including a comparison of metabarcoding to traditional methods of benthic macroinvertebrate community analysis (Bush et al., 2020), a caribou census done through "DNA Estimates", and the development of a statistical tool for routine analysis of DNA metabarcoding data.
- Water Act (GoA, 2000 (e)).
    - Focuses on the means by which water resources will be allocated to various parties (e.g., agriculture vs. industry)
    - Identifies something called the Aquatic Environment Protection Strategy
        - "The strategy referred to in subsection (2) may include….matters relating to the protection of biological diversity and guidelines and mechanisms for implementing the strategy."
    - Under this act:
        - Code of Practice for Wetland Replacement Works (GoA, 2020).
            - Prohibits the transfer of non-native species into the replacement wetland.
            - Prohibits wetland replacement in a water body "frequented" by fish.

[1] While OSM annual reports from 2018/19 through 2023/24 were reviewed, only those from 2018/19 and 2019/20 contained information especially pertinent to this review

#### 4.1.1REGULATORY DOCUMENTS OUTSIDE THE OIL SANDS

- Native prairie protocol for reclamation certification of salt-affected wellsites (AEP, 2019 (c)).
  - If:
    - The affected wellsite is in a native grassland ecosystem.
    - And soil salinity does not appear to be causing a problem for the vegetation already on site.
    - And the vegetation already on site is at least 30% native grassland species.
  - Then:

    There may be no requirement to dig up the wellsite and revegetate assuming subsequent criteria can be satisfied.

#### 3.1.2 EVALUATION:

- There **are few explicit articulations** of the technical/scientific methodologies employed to generate oil sands environmental regulatory submissions.
  - Instead, there are broad guidelines articulated in nebulous terms, suggesting an onus placed on the Applicant to persuade the Regulator of a submission's adequacy rather than an onus upon the Regulator to define criteria for adequacy to the Applicant.
- Based on the EIAs reviewed here (Devon Canada Corporation (2018), Suncor Energy Inc. (2018)) industry responds to nebulous requirements by producing extensive documents containing technical information produced by traditional methods which have been validated under other circumstances (e.g., peer reviewed articles, previously accepted regulatory documents, etc.)
    - As a result, EG may represent a risk to the applicant, in that the regulator may be more likely to reject EG data than traditional data.
  - This situation may result in reluctance to adopt novel methodologies until they are so common in the literature (and preferably in previously approved regulatory submissions) that their rejection would seem unreasonable to the broader scientific community.

- Some of the data included in these EIAs comes from previously published documents, be they academic or regulatory in nature. Presumably, these data are available at little or no additional cost once they have been generated and submitted/published.
    - As a result, populating these EIAs with data is less a question of deciding what sort of data ought to be generated than it is an exercise in choosing the best of the data already available.
    - Where newly created data are included, they are typically produced using traditional methods which allow for "backwards compatibility" with previously published data.
    - This means EG data are more likely to be included if they can be translated into a form that allows direct comparison with data generated by traditional methods.
- The generation of new data, when undertaken, is not done on a project-by-project basis.
    - Instead, data generation is undertaken as a project unto itself, often focusing on a single community (e.g., vegetation, fish, landbirds, etc.) over a large geographic area encompassing multiple planned oil sands developments.
    - This practice adds to the risk and expense associated with the incorporation of EG data into EIA submissions.
        - If EG data are rejected by the regulator, it may impair multiple future developments.
        - Collecting EG data in conjunction with traditional data hedges against the risk of regulatory rejection and allows the direct comparison of EG to conventional measures, but the magnitude of the collection effort makes this an expensive strategy.
    - Mitigating the risk of regulatory rejection (by establishing adequate precedent in the scientific and regulatory literature) and the expense of its collection (increased adoption of the method and demand for molecular analysis services are expected to decrease per-sample costs) will enhance the probability of EG data appearing in EIAs.
- Unlike the bulk of an EIA, the post-development monitoring programmes appear to be both variable and poorly defined. In some cases, little information is provided other than the programme's existence (Suncor Energy Inc., 2018). In other cases, the monitoring programme may collect only physicochemical information or may be comprised only of participation in the OSM, ABMI, or other multi-stakeholder initiatives (Devon Canada Corporation, 2018). The reader may wish to interview those individuals

who manage these post-development monitoring programmes to gather more information on their design.

  - The variability of post-development monitoring programmes suggests some level of flexibility in their composition and, potentially, less resistance to the incorporation of EG data.

- Monitoring programmes undertaken during the operations of oil sands facilities appear to be, predominantly, physiochemical in nature.
  - Where a biotic component exists, the regulator provides little prescriptive information to aid in the programme's design. Moreover, it is only the discretion of the regulator which determines the acceptability of each programme. Until the criteria for adequacy are defined more explicitly, the applicant may be best served by submitting conventional programme designs and data consistent with precedent.
    - This preference for conventionality reduces the appeal of innovative monitoring methods unless the novel data are as good as or better than those produced by traditional methods.
  - Requirements to monitor reclaimed areas, typically incorporating assessments of plant and wildlife communities, are also articulated
    - Like the operational monitoring programmes, no explicit direction for the design of these programmes nor the evaluation of their results is offered. Consequently, these programmes may suffer from the same preference for conventionality.
- Requirements for various forms of environmental research are also imposed by the regulator. However, the fields of investigation are defined very broadly (e.g., wetland research).
  - In some instances, it is suggested that collaboration with regional organizations (e.g., OSM, ABMI) may satisfy at least some of the research requirements.
- The academic community, as an important participant in at least some post-development monitoring programmes, may be more accepting of EG data (Culp et al., 2018) than would be mangers in the direct employ of oil sands companies or regulatory agencies. To the degree EG becomes commonplace in the regional ecological literature, barriers to its inclusion in regulatory submissions will be diminished.

## 4.2 BENTHIC MACROINVERTEBRATES

### 4.2.1 TRADITIONAL METHODS[2]

- Samples for traditional macroinvertebrate analysis consist of the organisms themselves, collected by a variety of methods including, but not necessarily limited to, dredges and kick nets.
- Following collection, the samples were treated with a preservative (e.g., formalin, ethanol, etc.).
- The samples were transported to the analytical laboratory, where the invertebrates were manually separated from any residual sediment and sorted morphologically into a collection of classes, orders, and families.
- While collecting benthic macroinvertebrate samples, field personnel may also take physicochemical measurements of the aquatic environment.
- The invertebrate community was characterized using measures of absolute abundance (number of individuals per square meter) and relative abundance (percentage of the overall population attributable to each taxon).
- Additional descriptors of the community include taxonomic (species) richness, diversity indices (often Shannon-Weaver), equitability index, dominance index, Ephemeroptera-Plecoptera-Trichoptera (EPT) index, and EPT:C (Chironomidae) index.
- Where appropriate, summary statistics describing invertebrate communities across samples or sites were provided.
- Certain biota or developmental stages (e.g., nematodes, terrestrial invertebrates, eggs) were excluded from the results.

### 4.2.2 MOLECULAR METHODS:

- EG benthic invertebrate studies were predominantly metabarcoding studies utilizing bulk DNA extracted from invertebrates collected using traditional methods (Lejzerowicz et al. 2015, Aylagas et al. 2018, Cahill et al. 2018, Serrana et al. 2019, Beentjes et al. 2019, Bush et al. 2020, Antich et al. 2021, Meyer et al. 2021). Occasionally, water-borne eDNA was used as a sample (Marshall and Stepien 2020, Antich et al. 2021, Brantschen et al. 2021, Lanzén et al. 2021, Ji et al. 2022).

[2] Summary of methods described in Devon Canada Corporation (2018) and Suncor Energy Inc. (2018)

- When traditional and EG methods were compared, the results were typically a matter of overlapping sets of taxa. Some taxa were detected by both methods, some only by traditional methods, and still others only by EG.
- The relative number of taxa detected by each method varied. Sometimes EG detected more taxa than traditional methods (Lejzerowicz et al. 2015, Bush et al. 2020, Keck et al. 2022, Ji et al. 2022), usually when an eDNA-based approach was employed, and other times fewer (Aylagas et al. 2018, Serrana et al. 2019, Meyer et al. 2021, Brantschen et al. 2021). All else held constant, those taxa most common/abundant in the sample were those most frequently detected by EG (Antich et al. 2021, Brantschen et al. 2021).
- When EG detected **more** taxa than traditional methods, the difference was attributed to:
    - eDNA "drifting" into the sample from distant locations or from sediment (Lejzerowicz et al. 2015, Marshall and Stepien 2020, Antich et al. 2021, Brantschen et al. 2021, Lanzén et al. 2021).
    - Detection of non-target biota which would normally be excluded from the results (e.g., nematodes, terrestrial invertebrates) (Lejzerowicz et al. 2015, Marshall and Stepien 2020).
    - Detection of taxa present only as body parts or secretions (Lejzerowicz et al. 2015, Ficetola et al. 2021).
    - Detection of biota too small to be included in the traditional data set (i.e., those normally filtered out with residual sediment, or too small to examine) (Lejzerowicz et al. 2015).
    - Greater taxonomic resolution than was possible using morphological analysis (Bush et al. 2020).
- When EG has detected **fewer** taxa than traditional methods, the difference was attributed to:
    - Primers which failed to amplify the barcoding target in some taxa (Lejzerowicz et al. 2015, Cahill et al. 2018, Beentjes et al. 2019, Marshall and Stepien 2020, Brantschen et al. 2021, Duarte et al. 2021, Ficetola et al. 2021, Keck et al. 2022).
    - The presence of taxa absent from the database (Lejzerowicz et al. 2015, Aylagas et al. 2018, Cahill et al. 2018, Keck et al. 2022).

    - The presence of taxa which exhibited high levels of sequence variability at the barcoding locus, resulting in reads which did not align with database records (Beentjes et al. 2019).

- To some degree, the failure to detect taxa by EG might be mitigated by using multiple barcoding primer sets (Marshall and Stepien 2020, Duarte et al. 2021, Ficetola et al. 2021, Keck et al. 2022) and/or preliminary sorting of bulk samples prior to DNA extraction (Beentjes et al. 2019).

- In general, EG methods tended to be poor at estimating the abundance of individuals when compared to traditional methods. While there was often a positive correlation between sequence abundance or read count and the abundance of individuals, that relationship was often quite loose (Lejzerowicz et al. 2015, Aylagas et al. 2018, Bush et al. 2020, Brantschen et al. 2021, Duarte et al. 2021, Keck et al. 2022). As stated by Lejzerowicz et al. (2015) "Sequence abundance is not completely disconnected from the abundance of specimens."

- EG has been reasonably successful at inferring (not calculating) various indices used to characterize benthic macroinvertebrate communities, although sometimes the means by which this inference was produced was quite sophisticated (e.g., machine learning) (Lejzerowicz et al. 2015, Aylagas et al. 2018, Cahill et al. 2018, Beentjes et al. 2019, Marshall and Stepien 2020, Brantschen et al. 2021, Lanzén et al. 2021).

- Even when metabarcoding data were analyzed on a presence/absence basis, environmental effects on the benthic macroinvertebrate communities could be detected. In comparison, traditional morphological analysis of the same samples, which included a quantitative component, could not detect the same effects (Bush et al. 2020)
    - This increase in relative sensitivity was thought to be a function of metabarcoding's greater taxonomic resolution.
    - The statistical power associated with a metabarcoding analysis can be greater than a morphologic analysis of the same samples, potentially allowing fewer samples to be collected while yielding the same explanatory value. This may represent a substantial reduction in cost for a monitoring program.

#### 4.2.3 EVALUATION:

- Of the potential applications described in this document, profiling benthic macroinvertebrate communities is, perhaps, the application best suited to EG.

- The ubiquity of benthic macroinvertebrate data in regulatory submissions, within the oil sands industry and elsewhere, implies a large potential market.
- The availability of bulk DNA samples collected by traditional methods simplifies the development of EG as an alternative analytical method.
    - Primarily, the use of bulk DNA allows development efforts to focus on EG's analytical challenges without contending with the uncertainties of eDNA distribution and kinetics in the environment.
    - Bulk DNA also allows analytical development to proceed using comparisons to traditional analyses conducted on identical samples.
    - The use of traditional samples means monitoring programmes could incorporate EG without any alteration to existing sampling protocols or sites.
- While the challenges associated with abundance estimation are nontrivial, EG has already demonstrated some ability to infer index values. This capacity should appeal to industrial and regulatory parties who already incorporate such indices into their assessments.
- At least one author has already advocated for the development of EG methods as an aid to, but not a replacement for, traditional methods of benthic macroinvertebrate analysis, suggesting some appetite for innovation in the field (Culp et al., 2018).
- A range of research and development opportunities exist for the advancement of EG as a tool for benthic macroinvertebrate community assessment:
    - The development of barcoding loci/primer sets targeting taxa (e.g., molluscs) poorly served by those commonly used today.
    - Further development of databases to incorporate more taxa, perhaps focusing on those which exhibit considerable sequence variability at barcoding loci.
    - Defining the autecology of taxa not historically part of the benthic invertebrate knowledge base, but which are easily detected by EG (e.g., nematodes).
    - The development, and perhaps production, of mock communities which can be used to gauge biases in EG results (Duarte et al., 2021).
    - The development of methods for using benthic invertebrate preservatives (e.g., ethanol) as a source of quasi-eDNA. Such methods would allow for early EG results to be reported while traditional analyses are still being completed (Duarte et al., 2021).

- Unlike other putative applications, it is difficult to imagine near- to mid-term alternatives to EG as plausible innovations in benthic macroinvertebrate community analysis.
- If pursuing this application, the current providers of taxonomic analyses (e.g., Biological Environmental Services, Cordillera Consulting) may be useful sources of information and collaboration.

## 4.3 FISH

### 4.3.1 TRADITIONAL METHODS[3]:

- The methods selected to sample a fish community were determined by the habitat and species of interest.
  - These methods included angling, electrofishing, gill netting, minnow trapping, and seine netting.
- The focus of sampling was, primarily, a relatively small set of fish species regionally valued because they were "at risk", traditionally used by Indigenous people, or valuable for subsistence/sport fishing. This species list included:
  - Walleye, Pike, Arctic Grayling, Mountain Whitefish, Longnose Sucker, White Sucker, Lake Whitefish, Spoonhead Sculpin, and Northern Redbelly Dace.
  - "Small forage fish" were quantified without speciation because they represent a food source for species important to the regulator and because their numbers can be used as an indicator of general ecosystem stress.
    - Speciation of these fish by morphology is difficult and were thought not to produce any information immediately useful to the companies or their regulators.
- While species richness, at least for those species of interest, was highlighted, much of the information focused on abundance (e.g., catch per unit effort – CPUE) and distribution metrics.
- Physiological data (fork length, condition factor, tissue analysis, fish health, and weight) were also included.
- Many of the data used in EIAs were sourced from publicly available literature and sometimes dated back several decades; this information is useful in characterizing baseline communities to track change over time.

### 4.3.2 MOLECULAR METHODS:

- EG projects have been conducted to determine fish species richness of a body of water or to detect one or a handful of target species.

[3] Summary of methods described in Devon Canada Corporation (2018) and Suncor Energy Inc. (2018)

- Community profiles produced by eDNA metabarcoding usually detected more fish taxa than were detected by conventional methods (Schmelzle et al. 2016, Shaw et al. 2016, Hansen et al. 2018, Sard et al. 2019, Czeglédi et al. 2021, Gehri et al. 2021, Hallam et al. 2021, Pont et al. 2021, Westgaard et al. 2024).
- Concordantly, the set of fish species detected by EG usually substantially overlapped, if not completely subsumed, the set of detected by traditional methods (Shaw et al. 2016, Czeglédi et al. 2021, Hallam et al. 2021, Pont et al. 2021, Westgaard et al. 2024).
- The increased species richness associated with these studies has been attributed to:
    - Traditional methods which are well suited to only a narrow range of fish sizes, life stages, taxa, and densities compared to EG methods (Shaw et al. 2016, Gehri et al. 2021, Pont et al. 2021,).
    - EG detection of dead fish or fish whose DNA has travelled to the sampling location via flowing water, predators, or human activity (Shaw et al. 2016, Gehri et al. 2021, Westgaard et al. 2024).
    - False detections of species known to be absent from the study area have occurred (D. Versnick-Brown, pers. comm., 2025).
- Studies focusing on one or a few fish species were usually conducted by qPCR of eDNA samples and usually determined that EG was a more sensitive detector of those species than were traditional methods (Schmelzle et al. 2016, Hinlo et al. 2017, Robinson et al. 2019, Piggott et al. 2021).
    - As with the metabarcoding studies, the limitations of traditional methods and the uncertainties of eDNA distribution were used to explain the comparatively high sensitivity of qPCR.
- The criteria defining valid detections were inconsistent across studies.
    - For individual studies, detection could be defined by:
        - The initial concentration of target DNA in the environment as estimated from qPCR results (Schmelzle et al., 2016).
        - The number of biological replicates which demonstrated the presence of a particular DNA sequence (Sard et al., 2019).
        - The number of technical replicates which demonstrated the presence of a particular DNA sequence (Piggott et al., 2021).

- Permissive vs. restrictive criteria for “detection” could dramatically alter the interpretation of study results (Gehri et al., 2021).

- As with other applications, EG was better suited to the detection of species than their quantification.

- The quantitative aspects of study results were often communicated as the number of sampling locations which demonstrated detection, rather than as estimates of absolute fish numbers. Presumably, this was done to avoid over-interpretation of results (Schmelzle et al. 2016, Hinlo et al. 2017, Robinson et al. 2019, Piggott et al. 2021).

- There were attempts to use initial eDNA copy number (qPCR) or read count (metabarcoding) to estimate CPUE.
  - Most, but not all, attempts showed some positive, albeit weak, correlations between these molecular metrics and CPUE (Schmelzle et al. 2016, Hinlo et al. 2017, Salter et al. 2019, Gehri et al. 2021, Ogonowski et al. 2023).
  - In some cases, however, other factors (e.g., temperature) were shown to be more important determinants of copy number and read count than was density of the target species (Ogonowski et al. 2023).
  - When a mock community was used as a metabarcoding control, the measured proportions of each species did not match the original formulation, suggesting the presence of meaningful biases in the results (Hallam et al. 2021).
  - An eDNA-based qPCR assay and imaging sonar produced nearly identical estimates of river herring numbers as they migrated up the Choptank River in Maryland, USA (Fowler et al. 2024). The statistical model used to interpret qPCR results was developed by collecting daily triplicate eDNA samples and hourly sonar recordings at a single location for five consecutive weeks. Any reduction in the frequency of sampling degraded the ability of the qPCR assay to match sonar-based enumeration. The availability of environmental information (e.g., river flow rates, water temperature) was also critical to the model. The authors advocated additional trials be conducted over multiple years at multiple locations, and with different fish species to better understand the robustness of the technique.

### 4.3.3 EVALUATION:

- EG is reasonably well suited to the detection of prescribed fish species and the characterization of fish communities. Only benthic macroinvertebrates represent a better potential application for EG.
    - Other than EG, there appear to be relatively few obvious and plausible opportunities for substantial technical innovation in the field.
    - Fish species of commercial, subsistence, or sport value are likely to be of regulatory significance to a range of industries, suggesting a relatively large potential market.
    - Fish databases were the subject of fewer and less fervent complaints than seemed typical of other taxa.
        - Presumably, this is the result of research focused on a comparatively small and more closely related set of taxa.
    - The limitations of traditional survey methods are substantial and highlight EG's potential for broad application and standardization:
        - Traditional methods may be constrained to certain species and environments.
        - Field personnel may vary in their ability to use traditional methods effectively.
        - Some traditional methods may involve substantial operational costs, time in the field, and the potential for injury to field personnel.
- Chief among the challenges facing EG in this application is a dependence on eDNA.
    - Bulk DNA, were it even available, could only be generated from fish caught using traditional methods, thereby eliminating the benefits of EG.
    - No matter how well developed the analytical tools become, they will always be subject to the vicissitudes of eDNA generation, persistence, distribution, and collection.
- To a degree, the variability associated with eDNA can be mitigated through a sampling programme which is both repeated and spatially extensive.
    - Based on the studies reviewed, increasing the number of sampling locations seems to be a more effective mitigation strategy than concordant increases in

sample volume or replication (i.e., taking fewer smaller samples at many locations is more effective at mitigating eDNA variability than is taking many larger samples at fewer locations) (Shaw et al. 2016, Hinlo et al. 2017, Piggott et al. 2021).

  - In one case, triplicate samples had to be collected daily and sonar images collected hourly for 5 weeks to develop a model which would allow an eDNA-qPCR assay to match imaging sonar estimates of fish abundance (Fowler et al. 2024)

- The inability to reliably estimate the abundance or physiologic state of target species limits the applications of EG.

- Biotic indices, barring simple ones like species richness, appear to be less common in the characterization of fish communities than they are for benthic macroinvertebrate communities. This limits the benefit of index inference from EG fish data.

- None of the authors advocated the replacement of traditional methods with EG. Instead, they suggest EG provides information which is different in character to that produced by traditional methods and is best employed as a complementary rather than substitutive practice. Given this assessment, it is difficult to see how EG information could be reconciled with decades-old traditional data.

- Perhaps EG fish studies are best suited to ongoing monitoring studies of large (regional) areas, where the variations in eDNA persistence and distribution may even out over many locations and sampling periods. Such large studies are likely to involve large numbers of field personnel of variable skill, a potentially greater liability for traditional methods than for EG.

- Studies emphasizing the presence of a species rather than its number or health are applications best suited to EG.

- The limitations of traditional methods may create opportunities for EG.

  - Given the degree to which traditional methods are optimized for particular species, EG might be useful in determining what species are present at a site so that the selection of traditional methods to estimate population size is better informed.

## 4.4 VEGETATION

### 4.4.1 TRADITIONAL METHODS[4]:

- Traditional surveys characterized plant communities within well defined study areas.
  - Species of interest (rare plants, plants of traditional Indigenous value, weeds/invasive species) were identified, enumerated, and their precise locations defined.
- The range of data collected by field personnel included species identification, number of individuals, GPS location, and phenological characteristics.
  - When necessary, expert taxonomic support was provided by means of photographs and/or samples.
- Data, field and otherwise, were summarized in a series of tables and maps organized around a collection of continuous or categorical metrics:
  - Species richness.
  - Habitat.
  - Ecological land classification.
  - Cover class.
  - Wetland classification.
- Quantification was usually expressed as the geographic area (hectares, % cover) attributable to one or more categories. For species of interest, the number of individuals was provided.
- The prominence of maps in the EIA documents, some of which even specified the location of individual plants, underscored the importance of spatial information.
- There may be great potential for the automated analysis of images collected by satellite or aerial drone to produce information consistent with that produced by traditional methods (B. Eaton, pers. comm. 2025).
- Reclamation criteria provide some accommodation for aerial assessments of sites.

[4] Summary of methods described in AESRD 2013(a), AEP 2017, Devon Canada Corporation 2018, and Suncor Energy Inc. 2018

### 4.4.2 MOLECULAR METHODS:

- EG for the analysis of plant communities appears less well developed than for benthic macroinvertebrates or fish. All reviewed studies were eDNA-based studies.
- A range of eDNA samples are possible, but each presented specific challenges.
  - Soil (Carrasco-Puga et al. 2021, Duley et al. 2022, Ariza et al. 2023)
    - Soil samples produced inconsistent EG results.
    - Because of its ability to preserve eDNA, soil generated EG results which reflected both current and former plant communities (Ariza et al. 2023).
    - EG results from soil samples often, but not always, reflected the regional plant community more than the one local to the sampling location, though the demarcation between regional and local communities was indistinct (Duley et al. 2022, Ariza et al. 2023).
  - Air
    - Like soil, air samples demonstrated a lack of consistency in EG results (Johnson et al. 2019).
    - Terrestrial vertebrate eDNA collected from air samples varied with weather conditions (Section 3.5). Plant eDNA may be similarly affected.
    - Though never stated, air samples might yield eDNA mixtures that overrepresented those species which release airborne pollen or seeds, at least during certain times of year.
  - Bulk
    - While not described in the reviewed literature, the collection of bulk plant tissue for DNA extraction may be practical in some circumstances.
    - The utility of such a bulk DNA approach is untested.
- As with other taxa, the most common plants were the ones most likely to be detected by EG (Ariza et al. 2023).
- Comparisons of plant eDNA metabarcoding results to traditional survey results tended to produce two incompletely overlapping sets of taxa – some taxa were detected only by eDNA, some only by traditional methods, and some by both (Carrasco-Puga et al. 2021, Duley et al. 2022, Ariza et al. 2023).

- Taxa detected by EG but not traditional surveys were attributed to:
    - Detection of biota too small to be noticed in the survey – many of which may not even be plants (i.e., unidentified OTUs).
    - Detection of genetic diversity within taxa.
    - Detection of dead, inactive, or partial biota (e.g., leaf fragments or pollen).
    - Detection of extracellular DNA.
- Taxa detected by traditional surveys but not EG have been attributed to:
    - Primers poorly suited to the barcoding loci of some taxa.
    - Inadequate database coverage.
    - Non-homogenous distribution of eDNA.
- The inability to reliably estimate abundance impaired EG's ability to profile communities (Cordier et al. 2021, Ariza et al. 2023).
- As with other taxa, the strategy of "inferring" index values has been proposed to mitigate EG's shortcomings in taxa detection and abundance estimation (Cordier et al. 2021).

### 4.4.3 EVALUATION:

- It is difficult to see how EG could be incorporated into vegetation surveys.
    - EG produces taxa sets which only partially overlap those produced by traditional surveys, making compatibility with pre-existing data a challenge.
    - Without an obvious means of obtaining bulk DNA, EG is dependent on eDNA collected from soil or air, both of which are associated with substantial limitations.
    - EG has only limited ability to estimate abundance and no ability to determine the location of individuals nor their phenological state.
    - Except for species richness, few indices are used in the interpretation of vegetation data. This limits the opportunities to offset some of EG's known shortcomings by inferring index values.

    - EG competitor technologies are already in use. DNA-based plant identification services are already available, as is plant identification from digital imagery. Further development of these technologies seems likely.

- One potential niche application for vegetation EG, albeit one outside of the oil sands and the EIA process, is profiling plant communities already present at grassland sites exposed to salt (Alberta Environment and Parks 2019(c)). If EG can reliably quantify the native species as a proportion of the community, thereby exempting the site from further reclamation, a commercially valuable service might be possible.

- Though outside the ambit of EG, there may also be value in attempting to identify genetic markers signifying plant adaptability to reclaimed landscapes (C. Powter, pers. comm. 2025).

## 4.5 TERRESTRIAL VERTEBRATES

### 4.5.1 TRADITIONAL METHODS[5]:

- There was a wide range of traditional methods employed to survey non-fish vertebrate biota
  - Acoustics – automated recorders or playback surveys – bats, amphibians, yellow rail, common nighthawk, raptors, woodpeckers, owls.
  - Aerial and ground surveys – raptors, beaver and other semi-aquatic furbearers.
  - Winter track survey – ungulates, large carnivores, terrestrial furbearers, small canids, marten and fisher, weasels, wolverines, beaver and muskrats, mink and river otters, snowshoe hares, red squirrels, small rodents, grouse.
  - Point counts – songbirds and their allies.
  - Pellet group survey – ungulates.
  - Mist net survey – bats.
- The surveys incorporated community and species-level components, the former focused on species richness, the latter on abundance and distribution.
- In some cases, an inability to resolve individual species was acknowledged (e.g., white-tailed and mule deer could not be distinguished based on tracks or fecal pellets).
  - Such distinctions may not be important to the overall assessment.

### 4.5.2 MOLECULAR METHODS:

- Both qPCR and metabarcoding studies were conducted on eDNA samples
  - Usually water (Ushio et al. 2018, Day et al. 2019, Rose et al. 2019, Plante et al. 2021, Dooley et al. 2022, Saenz-Agudelo et al. 2022, Randall et al. 2023, Wikston et al. 2023), but sometimes air (Lynggaard et al. 2022, Johnson et al. 2023), was used as eDNA source material.
- In comparison to traditional methods, EG was found to suffer from:
  - Inadequately “universal” barcoding primers leading to biased results (Saenz-Agudelo et al. 2022).

[5] Summary of methods described in Devon Canada Corporation 2018 and Suncor Energy Inc. 2018

    - The inability to reliably estimate abundance.
        - “Taken together, our basic comparisons suggest that eDNA abundance can be, to some extent, a rough proxy of relative species abundance.” (Saenz-Agudelo et al. 2022).
    - The erratic behaviour of eDNA in a natural environment (Day et al. 2019, Rose et al. 2019, Lynggaard et al. 2022, Saenz-Agudelo et al. 2022, Johnson et al. 2023, Randall et al. 2023, Wikston et al. 2023).
    - A bias towards detection of active as opposed to passive or dormant animals (Lynggaard et al. 2022, Saenz-Agudelo et al. 2022, Johnson et al. 2023, Randall et al. 2023).
- The repeated collection of multiple samples from many sites was advocated as a strategy to maximize EG utility (Rose et al. 2019, Randall et al. 2023, Wikston et al. 2023).
- There was little discussion of inferring index values from EG data.
    - Presumably, there are few indices of sufficient breadth to incorporate all terrestrial vertebrates within a study area of any meaningful size.
    - It is unclear how an eDNA sample adequately representative of all terrestrial vertebrates would be collected.

### 4.5.3 EVALUATION:

- The breadth of target taxa and the means by which they are surveyed, makes meaningful comparison of EG to traditional methods difficult. In some cases, EG appeared to be a more effective method of detection. In other cases, the reverse was demonstrated. Indeed, variability in relative methodological performance was evident even within individual studies (Day et al. 2019, Rose et al. 2019, Plante et al. 2021, Dooley et al. 2022, Lynggaard et al. 2022, Saenz-Agudelo et al. 2022, Randall et al. 2023, Wikston et al. 2023).
- The operational attributes of EG varied across studies.
    - In one case, eDNA sampling required less field time than did traditional methods (Wikston et al. 2023), whereas another study questioned whether the EG approach was truly cheaper after the cost of laboratory labour had been included (Rose et al. 2019).

- If technically feasible, the identification of individual animals from fecal pellets or snow tracks might be valuable as an alternative to capture-mark-recapture methodologies. (Slater 2019, EcoGenomics Canada).

- While non-EG innovations were not part of this review, it is relatively easy to conceive of alternatives to EG that could produce data like that produced by traditional methods but at reduced cost. These or other competitor technologies should be considered before embarking on EG development.
  - Some combination of digital imagery captured by aerial drone and analyzed by machine learning algorithms could (perhaps already have) obviate the need for helicopter surveys.
  - Automated analysis of acoustic data is already available (https://wildtrax.ca).

## 4.6 BIODIVERSITY AND EUTROPHICATION

### 4.6.1 TRADITIONAL METHODS[6]:

- Biodiversity estimates were compiled from measures collected in other parts of the EIA.
    - Vascular plants
    - Nonvascular plants
    - Birds
    - Mammals
    - Amphibians and reptiles
- The information provided was a combination of community characteristics (largely focused on species richness) and the abundance/distribution of species of special interest.
- The OSM programme expressed interest in measurements of productivity and eutrophication in aquatic systems (Culp et al. 2018).

### 4.6.2 MOLECULAR METHODS:

- Attempts to make direct measurements of biodiversity via EG have not advanced substantially beyond the evaluation of possible eDNA sources (e.g., soil vs. feces) (Drummond et al. 2015, Cristescu and Hebert 2018, Clark et al. 2020, Van Der Heyde et al. 2020).
    - The challenges identified for benthic macroinvertebrates, fish, vegetation, and terrestrial vertebrates also apply to biodiversity estimates, but may be magnified by the considerable breadth of taxa under consideration (Craine et al. 2017, Cristescu and Hebert 2018, Wang et al. 2019, Van Der Heyde et al. 2020).
- Metabarcoding evaluations of microbial community eDNA collected from water and sediment samples have been partially successful in assessing the trophic state of aquatic systems (Craine et al. 2017, Clark et al. 2020, Gregersen et al. 2023).
    - Both prokaryotic and eukaryotic components of the microbial community have shown promise as indicators of trophic state. In some cases, the microbial indicators showed greater sensitivity to nutrient concentrations. In other cases, microbial communities collected from sediment represented a time-integrated assessment of trophic state.

[6] Summary of methods described in Devon Canada Corporation 2018 and Suncor Energy Inc. 2018

    - However, specific microbial indicators were often site-specific, reflecting physicochemical characteristics of each site unrelated to trophic state (e.g., size of river, sediment type). Their value as ecological metrics was also impaired by deficiencies in taxonomic databases, an inability to reliably estimate organism numbers, primer biases, and the limitations inherent to the bioindicators they reflect.

### 4.6.3 EVALUATION:

- A "unitary" biodiversity estimate derived from a single EG analysis seems less plausible than an after-the-fact compilation of multiple EG studies designed to assess discrete constituent communities (e.g., invertebrates, plants, fish, etc.).
    - Nevertheless, proponents of the unitary approach might first focus on the inference of the ABMI intactness index scores from EG data.
- Assessing the trophic state of an aquatic system is a more plausible application for EG.
    - By using sediment as a source of eDNA, it may be possible to "even out" the year-to-year or season-to-season variability in water chemistry/productivity.
    - However, alternative methods of accomplishing this goal have not been evaluated as part of this project.

# 5.0 MUNICIPAL WASTEWATER SYSTEMS

## 5.1 REGULATORY DOCUMENTS REVIEWED

- Approvals to Operate
  - These documents are, effectively, licenses issued by Alberta Environment and Protected Areas (AEPA), or its predecessor ministries, to municipalities (or other legal entities) allowing them to operate their wastewater systems.
  - The monitoring requirements contained within these approvals vary substantially from one authorization to the next. In general, more is required of larger municipalities than smaller ones.
  - Approvals to operate were obtained for:
    - A community wastewater system for the Meridian Beach/Raymond Shores RV park in the County of Ponoka (AEP 2018 (b)).
    - A residential development in Cold Lake (AESRD 2014).
    - City of Camrose (AEPA 2023 (a)).
    - City of Edmonton (AEP 2015 (a)).
    - City of Lethbridge (AEP 2019 (b)).
    - County of Rocky View (AEP 2021 (a)).
    - Evan Thomas Recreational Area (Kannanaskis) (AEP 2018 (a)).
    - Municipal District of Greenview (AEPA 2022).
    - Town of Banff (AESRD 2013 (b)).
    - Town of Drumheller (AESRD 2012 (a))
    - Town of Edson (AEP 2019 (a))
    - Town of Okotoks (AEP 2016 (b)).
    - Town of Taber (AEP 2022 (a)).

- Canada-Wide Strategy for the Management of Municipal Wastewater Effluent (CCME 2009).
    - A 2009 CCME document defining a set of municipal wastewater goals endorsed by the federal, territorial, and provincial ministers of the environment.
        - Not signed by ministers from Newfoundland and Labrador, Nunavut, and Quebec.
    - Goals included:
        - Establishing a minimum level of municipal effluent monitoring.
            - According to the CCME website, this goal is close to being achieved.
            - Stipulates a small battery of parameters that should be monitored.
                - Many, but not all, of these parameters can be found in the Approvals to Operate issued by AEPA.
        - Monitoring the receiving environment at a watershed level.
            - Little progress achieved.
- Code of Practice for Outfall Structures on Water Bodies (GoA 2013).
    - Describes, in general terms, the environmental studies required to enable the construction, maintenance, relocation, or removal of a wastewater outfall structure.
- Environmental Protection and Enhancement Act (GoA 2000 (c)).
    - See Section 3.1.
- Environmental Quality Guidelines for Alberta Surface Waters (AEP 2018 (c)).
    - An extensive list of water quality standards obtained from various sources (Health Canada, CCME, U.S. EPA, etc.).
- Guidance for Deriving Site-Specific Water Quality Objectives for Alberta Rivers (AEW 2012).
    - Conceptual framework only.

- Guidelines for Municipal Wastewater Irrigation (AE 2000 (a)).
  - Describes the analyses to be done on wastewater used for irrigation (mostly chemical, some microbiological) and how the suitability of receiving sites is to be evaluated.
- Guidelines for the Application of Municipal Wastewater Sludges to Agricultural Lands (AE 2009).
  - Describes characterization of wastewater sludges and the receiving site.
- Guidelines for the Approval and Design of Natural and Constructed Treatment Wetlands for Water Quality Improvement (AE 2000 (b)).
  - Mostly a multipage checklist describing the information required to use or construct a wetland as part of a municipal wastewater system.
- Municipal Policies and Procedures Manual (AE 2001).
  - References the Alberta Ambient Surface Water Quality Guidelines.
  - Describes the basis of effluent standards as the more stringent of either 1) the best practicable technology approach or 2) the water quality approach.
    - Briefly describes the biological (i.e., receiving environment) approach as a third way of defining water quality standards, but then abandons that methodology.
  - Discusses the difficulty in regulating stormwater discharges and mentions the requirement for 85% removal of particles exceeding 75 µm in diameter.
  - Identifies the City of Edmonton as the only municipality in Alberta to operate a combined sewer system (i.e. a sewer system in which both storm water and sewage flow through the same infrastructure), presumably as a portion of a larger municipal wastewater system.
- Standards and Guidelines for Municipal Waterworks, Wastewater and Storm Drainage Systems.
  - Divided into five parts:
    - Part 1: Standards for Municipal Waterworks (AEP 2021 (b)).
      - About production and distribution of potable water – not relevant to this review.

        - Part 2: Guidelines for Municipal Waterworks (AESRD 2012 (b)).
            - About production and distribution of potable water – not relevant to this review.
        - Part 3: Wastewater Systems Standards for Performance and Design (AESRD 2013 (c)).
            - Stipulates the best practicable technology standards for municipalities of different sizes.
            - Provides standards for wastewater used for irrigation.
            - References treatment wetland guidelines.
        - Part 4: Wastewater Systems Guidelines for Design, Operating and Monitoring (AESRD 2013 (d)).
            - Day to day monitoring requirements for different wastewater systems based on size of municipality, treatment technologies, and methods of discharge.
        - Part 5: Stormwater Management Guidelines (AESRD 2013 (e)).
            - Specifies that stormwater outflows (as opposed to sewer, or combined sewer outflows) must achieve 85% removal of suspended solids greater than 75 µm in diameter.
- U.S. EPA National Program Guidance for Surface Waters – Biological Criteria (U.S. EPA 1990)
    - Conceptual framework only.
- Wastewater and Storm Drainage Regulations (GoA 2012).
    - Administrative document referencing various regulatory and informational documents.
    - Relevant documents referenced the regulations:
        - Code of Practice for Wastewater Systems Using a Wastewater Lagoon (GoA 2003 (b)).
            - Lays out monitoring requirements for wastewater systems whose sole mode of treatment is a lagoon.

            - Groundwater monitoring.
            - Monitoring of water to be used for agricultural irrigation.
            - Monitoring of water to be used for irrigating parks and golf courses.
        - References the standards to which analyses must comply (e.g., Standard Methods for the Examination of Water and Wastewater).
    - Code of Practice for Wastewater Systems Consisting Solely of a Wastewater Collection System (GoA 2003 (a)).
        - Lays out analytical requirements for municipal systems which do not incorporate any treatment capacity.
        - Mandated monitoring parameters are only defined for "unauthorized releases".
        - References the standards to which analyses must comply (e.g., Standard Methods for the Examination of Water and Wastewater).

- Wastewater and Storm Drainage (Ministerial) Regulation (GoA 2003 (c)).
    - Not useful – just describes who is responsible for what.
- Wastewater Systems Effluent Regulations (GoC 2012 (d)).
    - Federal regulation for wastewater systems excluding very small systems, private systems, or industrial wastes.
- Water Law in Alberta: A Comprehensive Guide (Boutillier 2022).
    - Describes the major legislation which governs wastewater, sewage, and storm drainage in the province.
    - Doesn't go into any detail regarding environmental standards or monitoring.
- Water Management Framework for the Industrial Heartland and Capital Region Effluent Characterization Program (ECP) (AEP 2015 (b)).
    - A project report.
    - Determined the relative contributions of effluent to the North Saskatchewan River from various municipal and industrial entities.
    - Led to a follow-up project (ECP2) but have not found a report for that project.

- Water Quality Based Effluent Limits Procedures Manual (Alberta Environmental Protection 1995).
    - Contains an in-depth discussion of the methods by which effluent limits are set (i.e., best practicable technology vs. water quality-based limits).
    - Omits any substantive discussion of an approach based on the receiving environment (i.e., biologically based limits)

### 5.1.1 REGULATORY DOCUMENTS OUTSIDE MUNICIPAL WASTEWATER TREATMENT

- Metal and Diamond Mining Effluent Regulations (GoC 2024 (a)).
    - Federal legislation under the *Fisheries Act* containing environmental monitoring requirements for diamond and metal mines.
- Pulp and Paper Effluent Regulations (GoC 2024 (c)).
    - Federal legislation under the Fisheries Act containing environmental monitoring requirements for pulp and paper mills.
- Summary of Alberta Industrial Wastewater Limits and Monitoring Requirements (AE 1999).
    - Describes the effluent monitoring required of various industries in Alberta.
    - In some cases, the requirements vary substantially between individual plants/factories.
    - Monitoring focuses on physicochemical and toxicological evaluations of effluent.
    - Monitoring of receiving waters is seldom required. Even when it is, the data to be collected are not defined. The exception is the Wabamun power plant which must monitor lake temperature.
    - This document is quite old (1999) but is still, apparently, the most current version.
    - The oil sands industry is not included in this document.

### 5.1.2 EVALUATION

- The regulation of municipal wastewater in Alberta is primarily, though not exclusively, a function of the provincial government.
- EPEA (Section 3.1) empowers the Wastewater and Storm Drainage Regulations which reference a series of standards, guidelines, and codes of practice.
- There is little federal regulation. What federal regulation exists (*Wastewater Systems Effluent Regulations*) is empowered by the *Fisheries Act*.
- The net effect of regulation is to compel municipalities to collect specified samples, from specified locations within their wastewater systems, at specified times and have them analyzed using specified methodologies. The exact nature of the sampling and analytical programme varies from one municipality to the next and focuses almost entirely on wastewater (i.e., sewage) rather than storm drainage.
    - The one exception is the City of Edmonton that has a combined system for at least part of the city thereby including storm drainage by default (AEP 2015 (a)).
- The variability in sampling and analysis requirements is explained by variation in the size of municipalities and differences in their wastewater management strategies.
    - It is recognized that full primary, secondary, and tertiary treatment of wastewater is beyond small municipalities – both financially and technically. This applies to both water and sludge, though in the case of the latter, agricultural application (either directly, or via composting) seems to be the preferred approach to disposal.
    - With respect to the required sampling, the most common analyses are measurements of biochemical oxygen demand, suspended solids, nitrogenous compounds, and phosphorus – though there may be some variation in the specific assays (e.g., 5-day Biochemical Oxygen Demand ($BOD_5$) vs. Carbonaceous Biochemical Oxygen Demand (CBOD)).
    - Measurements of coliform bacteria (total and fecal), pH, and whole effluent toxicity are less common.
    - Measurements of conductivity, sodium adsorption ratio, *Escherichia coli*, residual chlorine, and specific cations are relatively rare.
- Samples are taken from different points within the wastewater system – there is no sampling of the receiving waters/environment except in very specific circumstances.

- Why is monitoring of the environmental effects not required? In the absence of such monitoring, how are wastewater standards validated?
  - Both the provincial and federal regulators appear to be aware of the potential for environmental damage, even from effluent meeting the required standards. However, the need for consistent and easily quantifiable end-of-pipe pass/fail criteria is prioritized over ongoing effects monitoring in receiving waters.
  - Presumably, the ambiguity around the causes and mitigation of downstream effects (i.e., it may be uncertain what proportion of an adverse effect can be blamed on any one emitter, nor is it immediately clear how pass/fail criteria must be modified to mitigate adverse effects), means that very little effort is made to identify such effects in the first place.
  - Wastewater standards are validated using two methods.
    - First is the “Best Practicable Technology” approach in which effluent standards are based on the performance characteristics of the best technologies which could be employed by a municipality given its financial and technical constraints (Alberta Environmental Protection 1995).
    - The second approach is to simply accept the criteria put forward by other organizations (e.g., CCME, Health Canada, U.S. EPA, etc.) and/or to measure the toxicity of whole effluent using laboratory-based toxicological techniques (e.g. 96 hr. $LC_{50}$ in rainbow trout) (Alberta Environmental Protection 1995).

- The rejection of effects monitoring limits the potential of EG as a regulatory tool for municipal wastewater. Conceivably, molecular alternatives to the toxicological assays used to assess whole effluent toxicity could be developed. However, apart from concerns regarding laboratory fish welfare, there seems little utility in pursuing this application.
  - Some work has been done looking at the use of fish embryos or immortalized cell lines as alternatives to live whole fish (Burden et al. 2020, Kolarova et al. 2021, Scott et al. 2023, Villeneuve et al. 2023, Flynn et al. 2024). While the results of these assays have shown some correlation to whole fish testing, they are still in the early stages of development. Moreover, most are based on physiological

measures of viability, rather than transcriptomics and, as such, deviate significantly from the definition of EG set out at the beginning of this document.

  - It may also be possible to develop molecular alternatives to the bacteriological assays conducted on wastewater effluents (i.e., total and fecal coliforms, *E. coli*). While this topic will be explored in Section 4.2, it is also a deviation from EG as defined at the beginning of this document.
  - Were such assays developed, they would have to be endorsed by recognized authorities in the field (e.g., Standard Methods, ISO 17025).

- Outside of routine effluent testing, there are some special circumstances where EG may prove relevant to municipal wastewater issues.
  - The placement, construction, installation, maintenance, replacement, or removal of an outfall structure may necessitate study of the surrounding aquatic ecosystem with a focus on fish populations, fish habitat, or other important aquatic species (GoA 2013). Indeed, the Okotoks Approval to Operate (AEP 2016 (b)) lays out certain requirements for the study of benthic invertebrate and algal communities in the Sheep River. The reader is referred to the fish and benthic invertebrate portions of Section 3 for more information on the potential applicability of EG to these investigations.
  - If a municipality is considering the use of a natural or constructed wetland as part of its wastewater treatment system, the province requires some assurance that plants, waterfowl, fish, and other "significant" biota will not suffer excessively as a consequence (AE 2000 (b)). There may be some opportunity for EG to provide relevant information in this regard (see Sections 3.3, 3.4, and 3.5).
    - However, substantial field-level investigations are only required if ecological concerns cannot be dismissed by literature reviews or casual inspection of the site. Therefore, the opportunities for EG application may be quite rare.
- While conducting this survey, two alternative applications for EG became apparent.
  - Federal regulation of the effluents emitted by metal or diamond mines and pulp and paper mills require that benthic invertebrate and fish communities downstream of facilities be monitored (GoC 2024 (a) (c)). These investigations may be at least partially amenable to EG techniques. Again, the reader is referred to the benthic invertebrate and fish portions of the oil sands section (Sections 3.2 and 3.5).

- In some cases, provincial regulation requires "receiving waters" to be monitored by certain industries. However, this finding is based on a 25-year-old document for which no more contemporary version is available (AE 1999). Moreover, the details of this monitoring requirement are not specified (with the exception of the Wabamun power plant). Nevertheless, the reader may wish to investigate chemical plants, coal mines, fertilizer plants, gas plants, power plants, and pulp & paper mills as potential users of EG-based environmental monitoring.

## 5.2 MICROBIOLOGY

### 5.2.1 TRADITIONAL METHODS:

- Fundamentally, the bacteriological evaluation of wastewater, and to a greater degree drinking water, focuses on Fecal Indicator Bacteria (FIB). This is a very broad and anthropocentric category encompassing a wide range of bacterial taxa that have, historically, been used to indicate the presence of fecal material (Villemur et al. 2015, U.S. EPA 2021).

- Fecal material brings with it the potential for organisms capable of causing disease in humans. The bacteria found in the lower gut of homeotherms have been used as a sensitive, though not always specific, indicator of feces and therefore the potential presence of enteric pathogens.

- The range of bacteria, viruses, protozoa, and multicellular parasites associated with the fecal-oral transmission of human disease is vast. As a result, the detection and quantification of each potentially pathogenic species, while theoretically possible, is too technically demanding and expensive to be practical at scale. In contrast, FIB are relatively easy to detect and quantify (Maheux et al. 2014, Villemur et al. 2015, Health Canada 2020 (a)).

- The value of FIB to the assurance of potable water quality is obvious, their value to wastewater operations is less so. In many instances, regulations do not require FIB to be monitored at all. It seems they must be monitored only when direct human exposure to wastewater is plausible (e.g., wastewater irrigation of parks, golf courses, or agricultural fields) or when a wastewater system incorporates a tertiary treatment capacity (i.e., systems serving large communities) (AE 2000 (a) (b), CCME 2009).

- FIB is a catch-all term referring to three nested sets of bacterial taxa: total coliforms, fecal coliforms, and *Escherichia coli* (Washington State Department of Health 2025).
    - These sets vary in application according to their relative sensitivity (conferred by cell density within feces) and specificity (conferred by their absence from environments outside a homothermic gastrointestinal tract) to fecal material.

- Total coliforms are the broadest group of FIB, comprised of roughly 20 genera and 80 species. While the name suggests a colonic origin, they are, in fact, widespread throughout the environment. This makes them low-specificity indicators of fecal material. However, their ubiquity and comparative resistance to disinfection makes them good indicators of system integrity (i.e., broken pipes) and disinfection effectiveness; a role more pertinent to potable waterworks than wastewater systems (Maheux et al. 2014, Health Canada 2020 (b), Washington State Department of Health 2025).

- Within the total coliforms is a subset of organisms called fecal (thermotolerant) coliforms. These organisms are more tolerant of high temperatures (44° to 45°C) than is typical of more environmentally adapted taxa. These organisms are more specific to feces, but less concentrated therein than the total coliforms, making them a somewhat less sensitive fecal indicator (Health Canada 2020 (b), Washington State Department of Health 2025).
- The most specific of the fecal coliforms is *E. coli*, though some environmental isolates have been documented. This species is present at lower cell densities in feces than the fecal and total coliforms. Although some isolates of *E. coli* can cause serious disease in humans (e.g., those producing shiga toxin), the majority are harmless (Jang et al. 2017, Villemur et al. 2015, Health Canada 2020 (a), U.S. EPA 2021, Washington State Department of Health 2025).
- According to Alberta's municipal wastewater regulations, where FIB must be monitored they should be quantified as Colony Forming Units (CFU – a culture-based metric) per unit volume of water. For total coliforms, wastewater systems should not release more than 1,000 CFU/100 mL. Fecal coliforms cannot be present at more than 200 CFU/mL, and *E. coli* must not exceed either 100 or 200 CFU/mL (the threshold value seems to vary from one municipality to the next).

### 5.2.2 MOLECULAR METHODS:

- Proponents of a molecular approach to the detection and quantification of FIB might suggest that culture-based approaches be replaced with ones based on DNA. They would point to the various advantages of molecular assays:
    - A molecular test is likely to produce results more quickly than one which is based on the growth of an organism (Maheux et al. 2014, Jang et al. 2017, Walker et al. 2017, Health Canada 2020 (a)).
    - For *E. coli* in particular, molecular methods can be used to provide some level of microbial source tracking and/or virulence assessment. That is, the genetic characteristics of an isolate may reveal an environmental or fecal origin. If the latter, the most likely host species and the isolate's potential to cause disease in

humans might also be outlined (Higgins et al. 2007, Villemur et al. 2015, Jang et al. 2017, Ragot et al. 2023).

- Because molecular techniques do not require cellular replication, they can detect Viable but Non-Culturable (VBNC) cells. The VBNC state is a common bacterial response to environmental stress wherein the cells remain alive but do not replicate. When conditions improve, those cells may eventually revert to their replicative state. Common disinfection techniques (e.g., chlorine, UV irradiation) can induce the VBNC condition, suggesting culture-based methods may underestimate FIB when applied to disinfected waters (Health Canada 2020 (a)).
- A laboratory already processing water samples for the molecular detection and quantification of FIB could expand its repertoire to include known but difficult to culture pathogens (e.g., *norovirus*, *Cryptosporidium parvum*). This would enable pathogens to be detected directly rather than relying on indicator organisms (Maheux et al. 2014).
- It may be possible to detect and quantify the presence of antibiotic resistance genes which, even outside of host cells, may have some public health significance (Haberecht et al. 2019, Yuan et al. 2019, Ikuma and Rehmann 2020).

- There are at least as many drawbacks to molecular detection and quantification as there are benefits.
  - While molecular detections can be completed more quickly than cultures, it is not clear that this relatively modest increase in speed (several hours vs. a day) is meaningful to wastewater system operators, nor that it would justify the increased cost or technical demands.
  - It is not clear that finding the source of a particular isolate or evaluating its pathogenic potential would be important to wastewater system operators on a routine basis. Indeed, the human origin of wastewater is not in question. In special circumstances, such as an outbreak of waterborne disease, source tracking may be more useful. However, it is likely that under such circumstances, parties other than the wastewater operators (e.g., public health officials) will bring their specialized expertise and tools to bear.
  - There may be little benefit to the direct detection and quantification of potential pathogens over conventional FIB assays. Barring the dissociation of pathogen load from FIB numbers, the response to excessive levels of either is likely to be identical intensified disinfection or dilution.

- Challenges exist around the sensitivity, breadth, and quantitative capacity of the molecular assays.
    - To date, the sensitivity of molecular tests has proven inadequate to match drinking water standards, though they may be adequate for wastewater applications (Health Canada 2020 (a) (b)).
    - Any molecular test must detect a wide range of *E. coli* isolates and/or total and fecal coliforms while minimizing false positives. There has been some progress in this regard by incorporating multiple sequencing targets (Maheux et al. 2014, Walker et al. 2017, Health Canada 2020 (a)).
    - While a molecular technique need not enumerate every cell, it must produce results that align predictably with CFU measurements. Moreover, it must be able to distinguish live cells from those killed by disinfection but whose DNA remains detectable (Walker et al. 2017, Acharya et al. 2020).
- There may be a relatively small potential market for molecular detection and quantification techniques. Based on the Approval to Operate documents, only a minority of municipalities are required to do any bacteriological monitoring at all. So, even if the technical challenges were overcome, there may be insufficient demand to justify the development effort.

### 5.2.3 EVALUATION:

- While it may be possible to replace culture-based FIB assays with molecular alternatives, it is not clear that there is a compelling practical or financial reason to do so. Nor would such an application fit the rubric of "environmental monitoring".
- Any newly developed molecular methods would likely have to be validated by outside authorities (e.g., Standard Methods for the Examination of Water and Wastewater, ISO 17025, etc.) before they would be accepted by the regulator.
- Under special circumstances, there may be a demand to track primary pathogens or antibiotic resistance genes in wastewater. However, it is not clear if or when such circumstances would occur, nor which target(s) would be of interest.

# 6.0 DISCUSSION AND CONCLUSIONS

## 6.1 WHAT IS NOT

Before the opportunities for EG to support industrial compliance with environmental regulation can be summarized, it is first necessary to summarize the constraints and limitations to which EG will be subject.

1) **Though difficult to quantify, the demand for biotic information to satisfy environmental regulations may be low and inconsistent.** By its nature, EG produces information on the biota present within an environment. If government requires industry to produce relatively little of this type of information, or to produce it inconsistently over a project's life, then the commercial opportunities for EG will be similarly limited.

2) **Regulators do not define the criteria by which the acceptability of an environmental monitoring technique would be judged**. This is important because it becomes difficult, if not impossible, to pre-define metrics that EG would have to achieve in order to be "good enough" to be accepted by regulators.

3) **By and large, authors in the field do not endorse EG as a total replacement for traditional monitoring methods**. This is important because it defines an upper bound to reasonable expectations of EG as an environmental monitoring technology. Furthermore, it suggests that EG is most likely to find real-world application in situations where traditional methods are considered somehow inadequate or undesirable.

### 6.1.1 THOUGH DIFFICULT TO QUANTIFY, THE DEMAND FOR BIOTIC INFORMATION TO SATISFY ENVIRONMENTAL REGULATIONS MAY BE LOW AND INCONSISTENT.

When compared to physicochemical monitoring, government appears to mandate relatively little ongoing industrial monitoring of biota inhabiting the "receiving environment". Where such data are required, they most commonly support pre-disturbance site assessments or evaluations of reclamation programmes. This limits the potential regulatory applications of EG. While the current project cannot estimate industrial expenditures on biotic monitoring, it seems reasonable to assume that the perceived value of EG is driven largely by its comparative rapidity and low cost. Therefore, the perceived value of EG is expected to vary directly with total industrial expenditures on biotic monitoring. Where overall expenditures of time and money on biotic monitoring are high, the perceived value of EG's cost and/or time savings will be substantial, potentially warranting the "risk" of adopting a novel monitoring technology. Where expenditures on biotic monitoring are low, EG cost savings may be inadequate to justify the risk of regulatory rejection.

The perceived value of EG cost savings may be further reduced by an emphasis on biotic monitoring during the early (pre-disturbance) and late (reclamation) stages of a capital project. Without an ongoing financial burden, the cost-savings associated with EG may be underappreciated.

By conducting ongoing biotic monitoring on a regional scale, the Oil Sands Monitoring (OSM) programme, the Alberta Biodiversity Monitoring Institute (ABMI) and Ducks Unlimited Canada (DUC) may represent stabilizing influences on EG demand. However, these programmes are, for the most part, more focused on answering research questions than fulfilling regulatory requirements.

### 6.1.2 REGULATORS DO NOT DEFINE THE CRITERIA BY WHICH THE ACCEPTABILITY OF AN ENVIRONMENTAL MONITORING TECHNIQUE WOULD BE JUDGED.

While there is significant direction on the physical, chemical, and toxicological characterization of effluents, the regulators offer almost no direction on the environmental monitoring techniques used to profile resident biota. To the degree the regulators offer any guidance on such matters, it is typically in the form of conceptual frameworks rather than explicit performance characteristics. At best, the regulators refer applicants to methods described by authoritative sources in the field (e.g., CCME Guidelines, Standard Methods for the Examination of Water and Wastewater), though the degree to which endorsement by these sources is a prerequisite for acceptability remains unclear. The resulting situation is one in which the applicant is best served by data previously accepted by the regulator or data generated by the same means, thereby favouring the use of conventional rather than innovative methods. Therefore, any new method must be widely considered as conventional before its data are likely to be incorporated into regulatory submissions.

The lack of methodological direction from the regulators also suggests that they will not compel applicants to adopt EG, even if it is widely regarded as a conventional technique. Instead, EG will have to be adopted through a bottom-up process beginning with the applicants and their contractors.

### 6.1.3 BY AND LARGE, AUTHORS IN THE FIELD DO NOT ENDORSE EG AS A TOTAL REPLACEMENT FOR TRADITIONAL MONITORING METHODS.

Of the documents reviewed, none advocated the replacement of traditional methods by EG, though a complementarity to the former is sometimes identified. The reasons for this are threefold. Firstly, and perhaps most importantly, the estimates of target organism abundance produced by EG are often inconsistent and correlate poorly with those obtained from traditional methods. Second, the set of taxa detected by EG (generally through metabarcoding) often differs substantially from the set detected by traditional methods. Finally, methods relying on eDNA cannot distinguish between the current and past inhabitants of a site nor between local and distant biota. Indeed, many of the same challenges were identified by Marnane et al. in their 2022 white paper.

## 6.2 WHAT IS

Despite the constraints described above, there are opportunities for EG to facilitate compliance with environmental regulations, albeit often outside the oil sands industry or municipal wastewater systems. Federal regulations require pulp and paper mills, metal mines, and diamond mines to regularly monitor the benthic macroinvertebrate and fish communities downstream of their operations (Section 4.1.1). While the development challenges for EG may be substantial, the repeating nature of these analyses may confer significant value to the comparative cost savings often attributed to a molecular approach. The environmental surveys required for major capital projects (Section 3.1), wetland construction (Section 3.1 and 4.1), and the reclamation of salt-contaminated grassland wellsites (Section 3.1.1) are other possible industrial applications for EG but are likely to be episodic and potentially procyclical in nature. There are also opportunities to enhance the utility of EG as a means of profiling benthic macroinvertebrate communities through the development of innovative techniques and products (Section 3.2).

## 6.3 RECOMMENDATIONS

### 6.3.1 PICK A DIRECTION

It will be necessary to focus Alberta's EG development efforts if this technology is going to facilitate industrial compliance with environmental regulations in the near to medium term. What follows is a collection of options from which a proactive strategy could be derived.

### 6.3.2 ENGAGE WITH ENVIRONMENTAL CONSULTANTS AND ACADEMICS

Consultants and academics leading or participating in environmental surveys for industry, particularly those incorporating aquatic biota, may be useful contacts in determining the potential for EG as a supporting technology. Indeed, the knowledge possessed by these experts is likely to include surveys conducted for a range of customers and industries, possibly expanding EG's opportunities beyond the scope of this review.

### 6.3.3 ENGAGE WITH THOSE LEADING POST-DEVELOPMENT MONITORING PROGRAMMES

The oil sands industry is unlikely to fully accept EG as a means of fulfilling its regulatory obligations until the technology is widely understood to be conventional if not commonplace. Consequently, the efforts of InnoTech and other entities focused on the development, validation, and application of emerging technologies should focus on promoting familiarity with EG across the industry. Given EIAs which, at times, seemed to be near duplicates of each other, the post-development monitoring programmes described in these documents were conspicuous for their inconsistency across submissions and their ambiguity. This suggests ongoing monitoring programmes may exhibit a certain degree of flexibility in their composition and, potentially, an opportunity to experiment with new monitoring techniques. The individuals responsible for these monitoring programmes may possess important information on the programmes' constraints, imperatives, and the potential for EG's incorporation.

### 6.3.4 SUPPORT THE UBIQUITY OF EG DATA IN THE LITERATURE

The Oil Sands Monitoring (OSM) programme may be another means of promoting the inclusion of EG data in regulatory submissions. Given the regionality of OSM projects and the propensity of EIAs to draw upon publicly available data, EG contributions to OSM or ABMI projects may find their way into regulatory submissions, thereby establishing an important precedent. However, some precautions should be observed before pursuing this strategy. While some EG data relevant to the oil sands industry have already been identified (Culp et al. 2018, Slater 2019, Bush et al. 2020), a more detailed examination of the project reports, academic papers, and EIAs originating from the oil sands region should be undertaken. If EG data are already plentiful in the literature yet absent from the most recent EIAs (those reviewed for this document were at least six years old), then the contribution of even more such data may yield little effect. Additionally, the public availability of EG data contributed to monitoring projects must be ensured. Restrictions on the availability of EG data will only discourage their inclusion in regulatory submissions.

### 6.3.5 EMBRACE NICHE APPLICATIONS WHERE THEY DO NOT CONFLICT WITH THE MAIN RESEARCH AND DEVELOPMENT PROGRAMME

So long as they do not detract from the broader R&D programme, niche applications and "one-off" projects can provide the opportunity to cultivate new proficiencies and contacts while demonstrating the potential benefits of EG approaches. At the same time, transparency regarding EG's challenges and limitations will be crucial in maintaining professional credibility.

As part of their regulatory approvals (AER 2015, 2017, 2020 (a) (b)), oil sands companies are required to conduct a variety of environmental research projects. While the content of these projects was not reviewed, the fields of investigation identified in the approvals (e.g., biodiversity, wildlife sustainability, aquatic ecology) suggest that EG might be a valuable tool. Indeed, InnoTech has already provided EG services to support CNRL's Arctic Grayling research (CNRL 2019).

In 2009, the Cumulative Environmental Management Association (CEMA) released a report on the criteria by which the reclamation of oil sands mine landscapes was to be evaluated (Poscente 2009). For many of these criteria, no reliable indicator (i.e. metric) had been developed. Perhaps EG might be useful in developing some of these "missing" indicators. In particular, EG may be well suited to profiling aquatic vegetation and soil fauna communities. The development of new EG indicators would likely require a demonstration of how these approaches can be applied at an operational scale. It should be noted, however, that it may be difficult to obtain financial support to develop new indicators which neither industry nor government currently assess.

Applications outside the oil sands or municipal wastewater systems may warrant investigation. A greater understanding of the federal regulatory requirements imposed on pulp and paper mills and metal or diamond mines would help to determine what, if any, opportunities they represent for EG. To the degree such information is available, the exact analyses required, the number of samples analyzed, the frequency of their collection, the quality control processes applied, and the criteria used to interpret results would aid in gauging EG's potential to support these industries.

Within Alberta, salt-contaminated wellsites in grassland regions may represent a potential application for EG. To make that determination, it would be important to know how many salt-contaminated wellsites are present within grassland areas, what proportion are slated for reclamation, the frequency with which reclamation exemptions are pursued based on the existing plant community, and the means by which such assessments are currently conducted.

#### 6.3.6 ENHANCE THE ABILITY OF OTHERS TO USE EG FOR BENTHIC MACROINVERTEBRATES

Finally, it may be worth reviewing the opportunities for the technical advancement of EG as a means of profiling benthic macroinvertebrate communities (Section 3.2). This work could be done in conjunction with some of the other directions described above. To the degree this work makes EG more easily usable by the broader research community, it will enhance the visibility of the technique in the literature and enhance its conventionality, making it more attractive to industry and government. Technical development projects and demonstration projects will be necessary to realize EG's potential as a means of facilitating compliance with environmental regulations.

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