ENVIRONMENTAL GENOMICS RESEARCH PROGRAMME

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Energy industry applications of environmental genomics: State of the science and regulatory acceptance

This Guidance is publicly available for readers to review and use. Any comments that improve the content or workability of the guidance are welcomed by the JIP. We will endeavor to address comments in future updates to the guidance, which are planned every 1 to 2 years to capture the rapid advances in this field. Please send comments to info@iogp-edna.org.

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Revision history

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Contents

Statement of Intent

This study aimed to evaluate the role of environmental genomics, particularly eDNA methods, in advancing biodiversity monitoring within the energy industry. By reviewing published literature and consulting with global experts, regulators, and industry practitioners, the study sought to identify both the opportunities and limitations of environmental genomics approaches for specific energy industry applications. This initial assessment, conducted in 2020, identified key opportunities, limitations, and knowledge gaps. This study also identified five common themes requiring further development, in order to improve the uptake and acceptance of the application of environmental genomics methods within the energy industry, which helped guide and prioritize the efforts of the IOGP Environmental Genomics Joint Industry Program (JIP). One of the common themes was to standardize approaches to using environmental genomics within the energy industry, and to this end, the JIP has developed guidance, standards, and protocols pertaining to sampling, laboratory processing, data analysis, and reporting, which can be found here: https://www.iogp-edna.org/publications/.

Executive Summary

Biodiversity monitoring is essential in the energy industry for several purposes, including to inform risk assessments, baselines and impact assessments, compliance reporting, decommissioning decisions, remediation, restoration, and detecting rare, threatened, endangered, or invasive species. Additionally, there is an increasing demand for corporate-level biodiversity data to meet sustainability reporting requirements and demonstrate nature-positive outcomes. Environmental genomics, including eDNA methods, promise to provide cost-effective and scalable solutions to meet growing industry biodiversity data needs. However, resistance by both energy industry practitioners and regulators hinders broader adoption. This study explores the opportunities, limitations and the state of the science for energy industry applications of environmental genomics while accessing its acceptance by regulatory authorities and government agencies.

In 2020, a review of published eDNA literature was undertaken, supplemented by consultations with global experts and thought leaders in the field. The International Association of Oil and Gas Producers Environmental Genomics Joint Industry Program identified key applications of environmental genomics within the energy industry. Regulators and government agencies across jurisdictions were also consulted, to document their familiarity with genomic methods, and factors hindering their acceptance. The goal of this initial inventory was to understand the opportunities, limitations and knowledge gaps for environmental genomics across several energy industry applications. This document serves as an important review of the state of the science and establishes the foundation for the continued development of environmental genomics resources by the Joint Industry Program. These growing resources can be found at https://www.iogp-edna.org/.

The literature review and consultation with approximately 100 individuals from research organizations, service providers and government agencies identified five common themes requiring further development, in order to improve the uptake and acceptance of the application of environmental genomics methods within the energy industry: i) standardization of workflows and processes, ii) understanding DNA persistence and dispersal, iii) integration of environmental genomics data with other data sources, iv) improvement of reference library databases, and v) molecular refinement of taxonomic indices.

At the time in 2020, all regulatory agencies consulted were aware of environmental genomics and its potential applications within the energy industry. However, some agencies were more advanced as a function of the maturity of the energy industry within their jurisdiction and the willingness of agencies to collaborate on technological advances. Multiple government agencies were either leading or active participants in initiatives to standardize environmental genomics methods and/or reporting requirements. However, very few of these initiatives were engaging with each other, leading to inconsistencies and duplication of effort.

Despite methodological limitations, the findings of this study demonstrate a considerable appetite and capacity among industry, scientific and regulatory communities to engage in collaborative research initiatives to advance the use of environmental genomics. It concludes by recommending collaboration among industry, scientists and regulators, to collectively address knowledge gaps and standardize approaches to employing environmental genomics within the energy industry.

1. Introduction and Objectives

To pursue environmental stewardship, sustainability goals, and to comply with regulatory mandates, energy companies undertake a variety of biodiversity studies, aimed at characterizing and/or monitoring potential changes within the environments in which they operate. These measurements apply to all stages of operations, from site selection to decommissioning, and to all environments from marine to terrestrial habitats.

Conventional monitoring of biodiversity usually includes direct collection, visual observations, or acoustic recordings of species. These conventional approaches can be inaccurate and/or imprecise, as many species are difficult to visually record or detect, particularly rare, elusive or cryptic species (Boussarie et al. 2018), or difficult to identify using morphological traits. Further, surveys performed using conventional methods are often costly due to reliance on trained taxonomists, specialized equipment and significant field time requirements, or may pose safety risks due to field conditions, such as offshore environments.

Environmental genomics is an emerging field, using various molecular techniques that could offer several advantages over conventional biodiversity monitoring approaches, specifically in the areas of detection of rare or cryptic species, taxa coverage (particularly when using metabarcoding), survey and processing time, safety, and overall project cost (Lyon et al., 2017). Environmental genomics methods do not require direct specimen collection but instead rely on detecting fragments of DNA in the environment that the target organisms inhabit (e.g., water, soil, sediment, air) (Lyon et al., 2017).

Environmental genomics methods have been employed for a broad range of environmental management objectives, including conservation biology, detection of cryptic or rare species, detection of invasive species, understanding population dynamics, indicating health in aquaculture operations, wildlife forensics, trophic interactions, dietary studies, species historical patterns, ecosystem health and general community assessments (Díaz-Ferguson and Moyer, 2014; Garlapati et al., 2019; Çevik & Çevik, 2025; Chang et al., 2025).

Within the energy industry, the application of environmental genomics has, to date, been largely ad-hoc and focused on very specific or academic questions. In particular, the application of environmental genomics within legal or regulatory frameworks (such as compliance monitoring and reporting or Environmental Impact Assessment; EIA), has been limited (Hinz et al. 2022). In addition, for many industry applications, the robust science needed to validate environmental genomics applications has been either lacking or lagging. Lastly, until recently there has been little, if any, effort to standardize environmental genomics workflows and applications within the energy industry. As a result, the uptake and acceptance of environmental genomics by the energy industry, and by regulators associated with this industry, has been relatively limited.

Therefore, the objectives of this study were to help understand the key knowledge gaps and hindrances that are currently limiting the uptake and acceptance of environmental genomics within industry. The specific study objectives were to:

1) Provide a literature review the current state of science in applying environmental genomics within the energy industry
2) Evaluate and prioritize knowledge gaps, considering input from academic, regulatory and industrial perspectives
3) Provide an understanding of regulatory uptake and acceptance of environmental genomics across a range of jurisdictions, and any factors that may hinder this uptake
4) Provide recommendations for research and development opportunities that address knowledge gaps and improve uptake and acceptance of environmental genomics within the energy industry

1.1 Approach

This study focused on key environmental genomics applications for the energy industry, as identified by the International Oil and Gas Producers Association Environmental Genomics Joint Industry Program (JIP). The matrix developed by the JIP highlighted the following industry-specific applications:

- Baseline assessments - establishing pre-existing conditions
- Habitat delineation - identifying potential habitats based on biological characteristics
- Detection of key species – rare/threatened/endangered species, commercial species
- Rapid assessment of invasive species – marine and terrestrial
- Population status and dynamics – for single species
- Monitoring environmental effects of industry activities
- Remediation and restoration - requirements and effectiveness

Several focus areas were also identified that were cross-cutting among many energy industry applications of environmental genomics:

- Sampling design, including requirements for spatial and temporal replication as well as accounting for DNA longevity and drift
- Transportation and preservation of samples
- Data interpretation and comparability (indices, communication) – this includes the interpretation and integration with other physical-chemical data
- Community representation (what is there?)
- Species abundance (how much is there?)
- Distribution (where is it?)
- Accuracy of results (false positives, negatives, poor quality data, persistence of DNA) – this includes considerations for potential interferences from environmental components or other added chemicals
- Species viability (live versus dead, function, etc.)
- Measuring and interpreting change (ecologically important effect size)
- Real-time on-site measurement and analysis, including field-portability of equipment and analyses

In this study, we conducted a review of the available published literature to identify the current state of science of the environmental genomics applications of the above applications and focus areas. We also consulted professionals from academic, regulatory and energy industry backgrounds who had expertise in environmental genomics. We adopted a two-phased approach for consultation. During Phase 1, we developed and distributed a digital questionnaire to target individuals and regulatory organizations provided by JIP members with the goal to identify their perspectives on knowledge, understanding and methodology gaps. Based upon the survey responses in Phase 1, we then conducted follow-up telephone interviews in Phase 2. These one-on-one interviews were used to obtain more detailed information and perspectives regarding the survey questions, particularly considering the industry priorities provided in the above-mentioned matrix. Furthermore, a workshop discussion facilitated at the International Workshop on Environmental Genomics (IWEG) in June 2020 was used to document discussion and feedback on the same focus areas.

2. State of Science

DNA-based taxonomic and functional profiling is extensively used to characterize organismal communities in various research areas, including microbiome roles in health and disease, biomonitoring, and species richness estimation. Environmental genomics, or ecological genomics, involves studying genetic material recovered directly from environmental samples, encompassing barcoding, metabarcoding, and metagenomics.

For eDNA-based detection and identification, short genome segments unique to the target taxon are targeted. DNA barcoding uses a short DNA segment from a specific gene to identify species, with the mitochondrial gene Cytochrome Oxidase I (COI) commonly used for animals (Stein et al., 2014). Other barcode regions include ribosomal DNA (16S, 18S, 28S, 12S) and mitochondrial regions (cytochrome B) (Haarsma et al., 2016).

DNA barcoding involves extracting DNA from tissue samples, amplifying the target sequence using polymerase chain reaction (PCR), and visualizing the amplicons. Environmental DNA (eDNA) refers to genetic material shed by organisms into the environment, typically shorter fragments (~100-150 bp) than those used in barcoding (Taberlet et al. 2012a; Díaz-Ferguson and Moyer, 2014; Pilliod et al., 2013a; Thomsen and Willerslev, 2015). eDNA can be detected using species-specific primers and quantitative Real-Time PCR (qPCR) (Díaz-Ferguson and Moyer, 2014).

Next-generation sequencing (NGS) enables the simultaneous sequencing of millions to billions of DNA molecules, allowing for the detection of multiple species from complex samples. Two principal NGS approaches are DNA metabarcoding and metagenomics. Metabarcoding combines high-throughput sequencing (HTS) of targeted genes with clustering into operational taxonomic units (OTUs) or amplicon sequence variants (ASVs) to describe species assemblages (Miya et al., 2015; Flynn et al., 2015). Metagenomics, or shotgun sequencing, characterizes genomic DNA from whole communities without PCR amplification, providing both taxonomic identification and functional characterization of the environment (Gilbert and Dupont, 2011; Zepeda Mendoza et al., 2015; Ruppert et al., 2019).

eDNA analysis offers rapid species detection, quantification, and biodiversity assessment. It provides a non-invasive means of conducting large-scale ecological surveys without physically capturing or harming organisms, crucial for species-at-risk and other species of interest (Tréguier et al., 2014). eDNA methods are safer and more cost-effective compared to conventional sampling methods, allowing for fewer samples with increased confidence (Evans et al., 2017). Additionally, eDNA can detect species regardless of size or life stage, beneficial for early detection of invasive species.

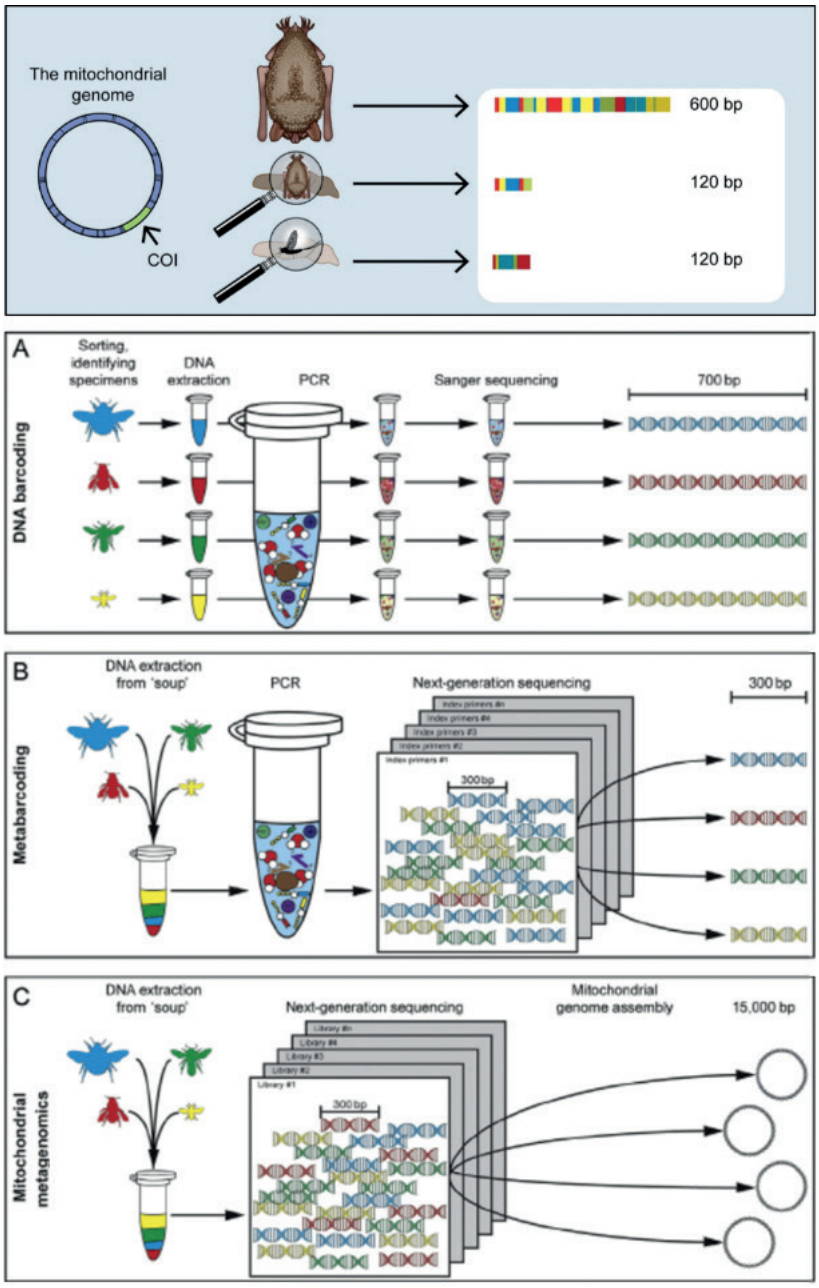

Figure 1 – Barcoding, metabarcoding, and metagenomics pipeline (modified from Haarsma et al., 2016; Gill et al., 2016)

2.1 Industry Focus Areas

2.1.1 Potential Industry-Specific Applications

Exploration in remote areas, such as deep-water ecotypes, requires baseline assessments for environmental permitting and monitoring. These assessments measure potential environmental changes and assess impacts on biodiversity and ecosystem services (BES). eDNA techniques offer rapid species detection, quantification, and biodiversity assessment, providing a safer and more cost-effective sampling method compared to conventional approaches (Evans et al., 2017). However, eDNA detection depends on various factors, including species life history, population density, and environmental conditions (Fediajevaite et al., 2021).

Baseline Assessments

Baseline assessments establish initial conditions to monitor environmental effects of energy industry activities. eDNA techniques can enhance spatial and temporal coverage due to reduced labor intensity and faster data acquisition. Using eDNA in baseline assessments ensures consistency and comparability across a project's timeline.

Studies have shown eDNA's potential for broad-scale biodiversity surveys and detecting species in various environments. For example, Lacoursiere-Roussel et al. (2018) detected 181 species in Canadian Arctic ports, and Closek et al. (2019) assessed marine vertebrate biodiversity in the California Current ecosystem. These studies highlight eDNA's ability to complement traditional baseline assessments.

Methodological considerations for accurate baseline studies include size trimming, sample replicates, multiple DNA markers, and pilot studies (Cowart et al., 2015). eDNA techniques can also estimate species abundance and distribution, providing valuable information for future monitoring.

Currently, eDNA-based approaches are not used alone for baseline assessments but are compared to traditional methods. Ongoing studies, such as those in the Labrador Sea and Eastern Coast of Canada, aim to develop and evaluate eDNA techniques for industrial applications. The need for substantial reference library development highlights the early stages of this approach.

Detection of Key Species

Detecting key species, such as rare, sensitive, or economically valuable species, is crucial in areas considered for industrial development or being monitored for environmental effects of energy industry activities. eDNA sampling techniques have been shown to increase the detection of targeted key species compared to conventional methods (Pilliod et al., 2013b; Schmelzle and Kinziger, 2016; Smith and Goldberg, 2019).

eDNA techniques can avoid misidentification or false negatives common in conventional surveys, improving management and conservation planning. For example, Franklin et al. (2019) used qPCR methods to detect rare forest carnivores in snow samples, achieving higher or equal identification rates compared to standard PCR techniques. Similarly, Spear et al. (2015) detected a rare aquatic salamander in 71% of sites where they were present or had been historically.

Rodgers and Mock (2015) demonstrated the potential to detect terrestrial species of conservation concern from eDNA in watering holes. However, qPCR techniques are recommended for targeting key and/or rare species due to their higher sensitivity (Wilcox et al., 2013). Primer specificity is also crucial to avoid false negatives and positives, which could impact management and conservation planning.

In summary, eDNA sampling techniques improve detection rates of key species, providing better understanding of their presence, distribution, and abundance. This method is becoming more common due to its improved sensitivity compared to conventional methods.

Early Detection of Invasive or Competitive Species

eDNA methods enhance the detection of invasive or competitive species, allowing for early detection and effective monitoring of their persistence (Dejean et al., 2012). Early detection enables control measures to prevent the spread of invasive species, reducing environmental impacts caused by human activities.

Metabarcoding provides a method for early detection of invasive species in bilge and ballast water, which are common vectors for aquatic invasive species (Zaiko et al., 2015b; Fletcher et al., 2017). For example, Zaiko et al. (2015b) identified many non-indigenous species in ballast waters using metabarcoding that were not detected by conventional methods.

Ports are commonly monitored for invasive species. Brown et al. (2016) used metabarcoding to survey commercial ports, identifying 24 non-indigenous species, 11 of which had not been previously reported in the given region. Similarly, Lacoursiere-Roussel et al. (2018) detected spatio-temporal variation of metazoan eDNA distribution in Canadian Arctic ports, highlighting the importance of establishing eDNA baseline data for continued monitoring.

Early detection examples include identifying invasive Japanese oyster drill egg capsules in imported oysters, preventing their spread (Darling and Mahon, 2011). Mauvisseau et al. (2019) developed an eDNA assay for early detection of an invasive crayfish species, successfully detecting it in known locations. Dufresnes et al. (2019) mapped the invasion of an Italian crested newt using eDNA surveys and population genetics, providing evidence for eradication programs.

eDNA methods for early detection require careful assay development with low detection limits. For example, Pochon et al. (2013) reported successful detection of marine invasive species at relative abundances greater than 0.64%. Pie et al. (2017) developed a qPCR assay for detecting an invasive mussel, with detection limits as low as 0.225 pg of target DNA.

Targeted eDNA sampling for early detection of invasive species has fewer limitations than metabarcoding complex environmental samples. However, management decisions based on false negatives and positives could have significant economic and ecological consequences. Therefore, eDNA techniques should be validated by conventional sampling as regulatory confidence builds.

Of all industry-specific applications of eDNA, detecting invasive species is the most widely used and accepted. Numerous studies have evaluated eDNA's utility for detecting and controlling invasive species, highlighting its extensive benefits for early detection and management.

Population Status & Dynamics (Single Species)

Understanding population status and dynamics is crucial for areas considered for industrial development or environmental monitoring. Conventional methods, such as visual observation and tissue sampling, can be challenging for elusive or rare species, leading to increased use of eDNA sampling in population genetics studies.

Population genetics examines genetic differences within and between populations, essential for effective stock management and understanding region-specific sensitivities to environmental stress. eDNA techniques have successfully assessed population structure and genetic diversity (Elbrecht et al., 2018; Parsons et al., 2018; Sigsgaard et al., 2016), showing promise for tracking and managing commercial and threatened species (Bernatchez et al., 2017).

Haplotypes, sets of DNA variations inherited together, are valuable for understanding population dynamics. Parsons et al. (2018) used eDNA to extract population-specific mitochondrial sequences for harbour porpoises, identifying eight unique sequences. Sigsgaard et al. (2017) isolated whale shark mtDNA from seawater, recovering similar haplotype frequencies as tissue samples. Meekan et al. (2017) used a whale shark copepod to recover new whale shark haplotypes, confirming limited genetic structuring of the global population.

eDNA can also provide information on reproductive status. Spear et al. (2015) observed temporal trends in eDNA associated with reproductive activities of an aquatic salamander. Kamoroff and Goldberg (2018) suggested increased eDNA shedding during mating season for fish.

Species abundance estimation using eDNA has shown significant correlations with qPCR and ddPCR (Skinner et al., 2020; Lacoursière-Roussel et al., 2016; Doi et al., 2015; Salter et al., 2019). For example, Fukaya et al. (2018) estimated jack mackerel abundance using eDNA and qPCR, with results similar to echo sounder methods. Mizumoto et al. (2018) correlated eDNA concentration with fish density and body size in a rare salmonid species.

Buxton et al. (2017) assessed seasonal variation in great crested newt populations using eDNA, observing peaks corresponding with breeding and larval abundance. However, eDNA concentrations were influenced by environmental factors such as temperature and body condition.

eDNA methods can reliably estimate species distribution, often increasing detection compared to conventional methods. Sigsgaard et al. (2015) detected a European freshwater fish in historical locations where it had not been observed since 1995. Aylward et al. (2018) used target capture DNA sequencing to monitor an endangered lemur species, demonstrating eDNA's potential for environmental management.

Integrating quantitative eDNA measurements, hydro-geomorphological scaling, and ecological models can enhance understanding of species distributions and population status. Although the use of eDNA for population studies is emerging, it holds great promise for future applications and additions to the population genetics toolkit (Adams et al., 2019).

2.1.2 Monitoring of Environmental Effects of Energy Industry Activities

Energy industry activities can impact surrounding environments, such as through the deposition of drill cuttings affecting benthic communities via physical smothering, organic enrichment, and chemical contamination (Breuer et al., 1999). Monitoring and minimizing these effects is a regulatory requirement globally, and eDNA techniques are emerging as cost-effective and efficient alternatives to conventional methods.

Community Representation

Anthropogenic disturbances can alter community composition, detectable using metabarcoding (Chariton et al., 2015; Lanzén et al., 2016; Laroche et al., 2017; Stoeck et al., 2018a,b; Xie et al., 2018). Metabarcoding captures a wider range of taxonomic groups, including potential indicator taxa sensitive to pollutants (Lanzén et al., 2016). Studies have shown that DNA-based monitoring can supplement conventional methods by providing additional information on community composition and detecting patterns between environmental variables and community composition.

For example, Chariton et al. (2015) predicted ecological conditions of estuaries using metabarcoding of benthic eukaryote communities, while Brannock et al. (2017) assessed nematode composition following the Deepwater Horizon oil spill, detecting decreased diversity post-spill. Laroche et al. (2017) and Laroche et al. (2018a) found that bacterial eDNA was most responsive to environmental impacts, accurately reflecting anthropogenic effects when OTUs were trimmed based on shared presence in eDNA and eRNA datasets.

Cordier et al. (2019a) assessed the impact of offshore gas platforms on benthic and pelagic communities, finding significant changes up to 50 m from platforms. Xie et al. (2018) detected long-term ecological effects of residual oil pollution using eDNA metabarcoding. Studies targeting multiple taxa have identified shifts in community structure and potential indicator taxa responding to pollutants (Bik et al., 2012; Lanzén et al., 2016).

eDNA techniques can complement conventional methods by increasing taxonomic resolution and providing a deeper understanding of coral and sponge ecology (Everett et al., 2018). Cordier et al. (2019a) demonstrated that eDNA analysis of sediment samples using the AMBI index can accurately predict impacts from energy industry activities.

Species Abundance

Estimating species abundance using eDNA is challenging due to limitations in obtaining counts or biomass. While no specific studies have estimated abundance for monitoring environmental effects of energy industry activities, eDNA has been used to investigate spatial and temporal changes in biodiversity and genetic-based biotic indices to estimate ecological change.

Species Distribution

Monitoring changes in species distribution, particularly benthic communities, is crucial for assessing anthropogenic impacts. eDNA analysis can enhance species detection across larger areas, being more cost and time-efficient (Stein et al., 2014). However, measuring species distribution in offshore environments is challenging due to eDNA dispersion in moving water masses. Further research is needed to address data gaps regarding dispersal and persistence in deep-sea environments and to develop a comprehensive reference database for diverse biological communities.

In summary, eDNA techniques offer a promising tool for monitoring the environmental effects of energy industry activities, providing valuable information on community composition, species abundance, and distribution. However, further research and methodological refinements are necessary to fully realize their potential in industrial applications.

Biotic Indicators

eDNA can characterize various ecosystem aspects and predict ecological change using biotic indices. These indices often use bioindicator species to interpret data. For example, nematodes have varying sensitivity to pollutants, and indices integrating their responses are used in environmental assessments (Bongers and Ferris, 1999). Metabarcoding eDNA monitoring produces similar conclusions to conventional methods, using meiofauna and microfauna as indicators (Lanzén et al., 2016). Buchner et al. (2019) found that presence/absence data can yield similar ecological assessments to abundance-based data when interpreted using biotic indices.

Bacterial communities are effective bioindicators, with metabarcoding data detecting spatial and temporal changes, aiding in predictions of anthropogenic impact (Dowle et al., 2015; Stoeck et al., 2018a). The microgAMBI index assesses marine sediment quality by characterizing microbial diversity via metabarcoding (Aylagas et al., 2017). However, bacteria's varied responses to stress require careful taxon assignment to avoid misinterpretation (Borja, 2018).

Macroinvertebrate taxa or soft-bottom benthos can also be bioindicators. Species tolerance profiles, such as those developed by Borja et al. (2000), detect anthropogenic changes. These profiles, applied to eDNA metabarcoding data, can assess pollutants for routine monitoring (Carew et al., 2013).

Transcriptomics

Transcriptomics, the study of RNA transcripts, provides insights into active and dormant cellular processes. It links phylogeny to function in natural communities and can analyze ecological functions without relying on reference databases (Poretsky et al., 2009). Rivers et al. (2013) used transcriptomics to measure microbial responses to the Deepwater Horizon oil spill, identifying degradation pathways and microbial diversity changes. Jenny et al. (2016) used transcriptomics to identify stress response genes in American oysters, improving understanding of hydrocarbon toxicity impacts.

Change in Ecological Function

Functional profiling assigns functional traits to closely related organisms using evolutionary models. Programs like PAPRICA use metagenomic data for this approach. Laroche et al. (2018b) assessed the impact of offshore energy activities on benthic bacterial communities, finding unique network signatures and reduced community cohesion at impacted sites. They concluded that functional community structure and co-occurrence network properties could complement taxonomic indices for impact assessments.

eDNA data and biotic indices can detect ecological change, with promising applications for monitoring energy industry activities. Developing supervised machine learning (SML) models to infer biotic index values from eDNA data without taxonomic assignments may overcome reference database limitations (Cordier et al., 2019b).

The use of eDNA for monitoring environmental effects is gaining popularity among researchers, environmental managers, and industry. Recent studies have assessed eDNA approaches for measuring ecological change and environmental impacts (Lejzerowicz et al., 2015; Pawlowski et al., 2014, 2016a,b; Stoeck et al., 2018a), including energy industry-related impacts (Cordier et al., 2019a; Lanzén et al., 2016; Laroche et al., 2018a; Mason et al., 2014; Xie et al., 2018; Yeung et al., 2011). Continued research and development may lead to regulatory acceptance of eDNA techniques for monitoring programs globally.

2.1.3 Remediation and Restoration

Microbe-mediated bioremediation is an ecologically sound and cost-effective method compared to traditional non-biological methods (Lovley, 2003). It utilizes microbial diversity to degrade, transform, or accumulate pollutants like radionuclides, metals, pharmaceuticals, PAHs, and PCBs through processes such as binding, oxidation, volatilization, and immobilization (Malla et al., 2018).

Traditional bioremediation studies rely on "treatability studies" to estimate microbial consortia's metabolic activities but provide little insight into specific microbes responsible for bioremediation (Head et al., 2003). Molecular-based approaches, such as denaturing gradient gel electrophoresis, initially profiled microbial community diversity but had limitations (Green et al., 2010).

Advancements in next-generation sequencing (NGS) offer detailed insights into biodegradative pathways (Malla et al., 2018; Ali et al., 2020). Omics methods (genomics, proteomics, transcriptomics, metabolomics, fluxomics) and in silico analyses help understand microbial communities in contaminated environments, leading to the discovery of novel bacteria (Maphosa et al., 2010).

For example, Genome Canada's Genomic Applications Partnership Program (GAPP) funds a project to demonstrate anaerobic bioaugmentation cultures' efficacy in benzene-contaminated sites, incorporating metagenome-enabled analysis and groundwater modeling (Genome Canada, n.d.). Another pilot study by Stantec and SiREM evaluates Enhanced In-Situ Bioremediation (EISB) for degrading VOCs in groundwater using microbial injections (Stantec, unpublished data).

Research on Alcanivorax borkumensis, a bacterium effective in degrading petroleum products, shows promise for decontaminating oil spills (INRS, 2018). Studies following the Deepwater Horizon oil spill used metagenomic analysis to examine microbial community responses and identify hydrocarbon-degrading strains (Kimes et al., 2013; Mason et al., 2014).

Ecological restoration aims to renew and restore degraded ecosystems through active intervention. Conventional monitoring includes visual surveys, tissue sampling, and chemical analysis. eDNA sampling can improve monitoring by detecting elusive species, genetic diversity, and microbial communities, offering a less invasive method suited for sensitive species. For example, Yan et al. (2018) used eDNA to track fungal community changes at a restoration site, while Gellie et al. (2017) monitored soil bacterial communities following revegetation. Fernandes et al. (2019) used eDNA to assess invertebrate composition at mine restoration sites, finding community composition a better indicator of restoration success than biodiversity alone.

Stantec's pilot project with Canadian Natural used eDNA to assess fish species presence in Horizon Lake, corroborating conventional findings and documenting additional species (Murdoch et al., 2019). eDNA techniques can also prepare for reintroducing endangered species, as demonstrated by Kamoroff and Goldberg (2018) in distinguishing restored sites' status.

Restoration programs may also provide opportunities for invasive species. eDNA sampling can monitor and eradicate invasive species during restoration, ensuring successful ecosystem recovery.

Without monitoring restoration programs, scientists and conservation managers will not know whether attempts to restore a degraded ecosystem were successful. The use of eDNA sampling to measure success shows great promise and provides access to bioindicator species that are difficult to sample by conventional monitoring. An understanding of organism interactions within a given ecosystem, as well as applicable eDNA methodological limitations are required for well-designed monitoring programs. eDNA restoration monitoring programs may be a valuable technique for stakeholders, who are required to demonstrate successful restoration to regulators.

Like early detection of invasive species, bioremediation is another industry-specific application of eDNA techniques that has gained a lot of attention from researchers and industry. eDNA techniques to monitor success of restoration programs, still requires much study, as depending on the approach for measuring success, it must overcome the many limitations of eDNA surveying discussed throughout this report (e.g., biodiversity surveys have more limitations than targeted species surveys). Currently there are few studies that have assessed its potential and/or have applied it in monitoring programs but with continued application in tandem with conventional methods, acceptance and application will lead to its incorporation into restoration programs.

2.2 Sampling Procedure & Analysis Pipeline

The following sections outline key concepts/terminology as well as sampling procedures and analysis, providing examples and associated benefits/limitations of study designs, sample collection and preservation techniques, extraction methods, DNA detection and data interpretation. Figure 2-2 illustrates an example of a generic eDNA workflow, from sample collection through to analysis, although specific workflows will vary (e.g., preservation may sometimes occur prior to filtration).

2.2.1 Sampling Design

Effective eDNA study design must consider the target species' ecology, habitat, season, budget, and accessibility. Spatial and temporal sampling should account for habitat (Koziol et al. 2019) and species behavior. For instance, marine vertebrate studies may sample various water column depths to capture vertical distributions (Andruszkiewicz et al., 2017), while invertebrate studies may focus on bottom water or sediment sampling (Everett and Park, 2018; Lejzerowicz et al., 2014). Timing should consider abiotic factors such as dilution from runoff and life history events like spawning and migration (Pilliod et al., 2013a). Biological replicates are crucial due to the heterogeneous distribution of DNA in the environment, ensuring a representative sample area (Andruszkiewicz et al., 2017). Rarefaction curves help determine the necessary sample number for accurate species richness representation by plotting the expected number of species against the number of samples (Gotelli et al., 2001; Grey et al., 2018; Valdivia-Carrillo et al., 2019).

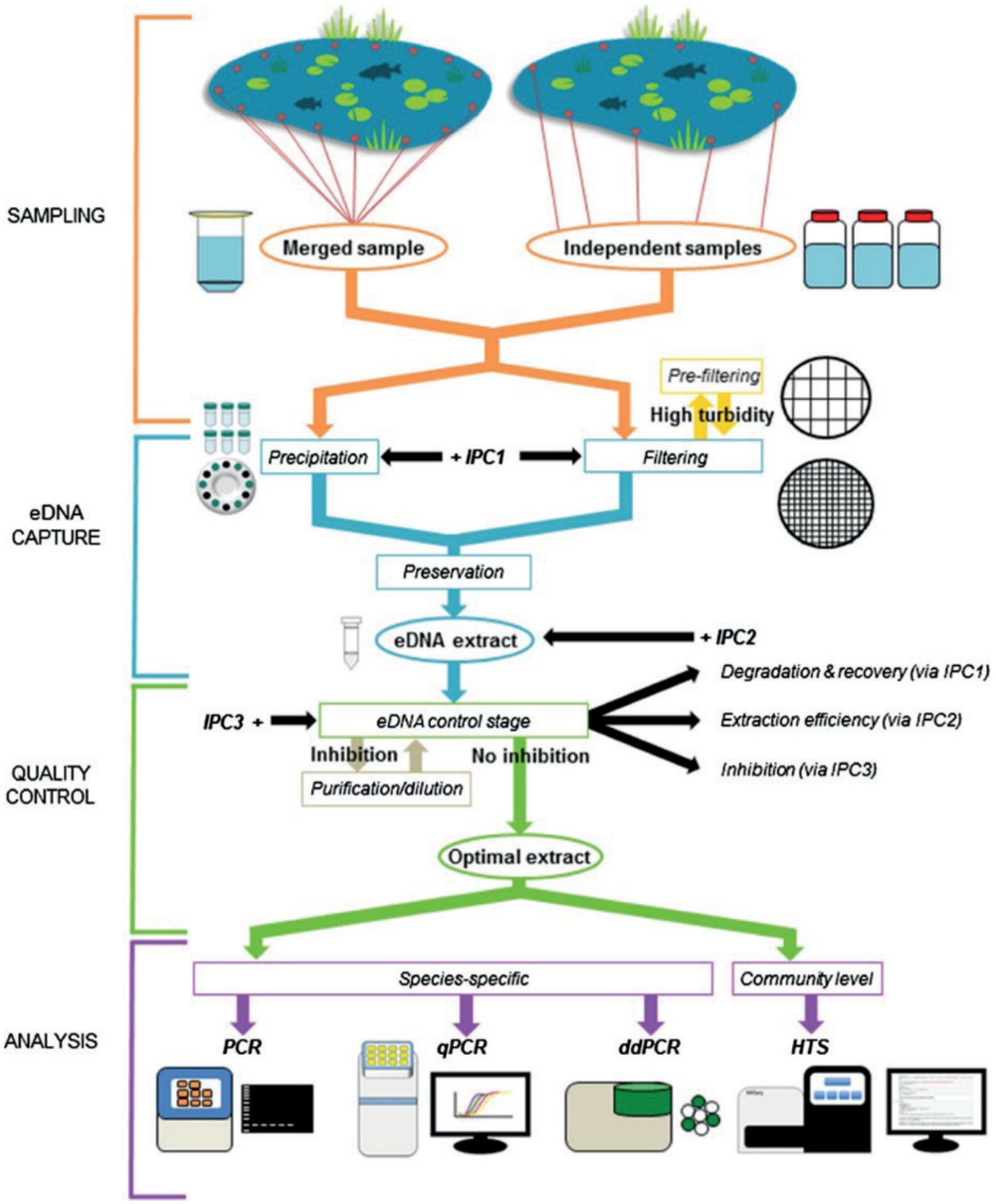

Figure 2 - Schematic of a generic eDNA workflow (Harper et al., 2019). IPC = Internal Positive Control. Field and laboratory controls (both blanks and IPCs) are crucial for quality assurance of an eDNA program.

Field and Laboratory Controls

Technical replicates, or repeated measurements of the same sample, improve reproducibility and accuracy by accounting for intra-sample variation and small-scale spatial heterogeneity (Zhan et al., 2014; Lanzén et al., 2017). Negative controls are essential to test for potential false positives from contamination during sampling, handling, and DNA extraction (Pilliod et al., 2013a). Internal positive controls (IPCs) are additionally used to assess quality assurance. The IPC can help identify issues pertaining to false negatives that may arise due to DNA degradation or inhibitors. IPCs can be universal source DNA from a taxon expected in all samples or spiked foreign DNA. Low IPC yield may indicate DNA degradation or inhibitors, necessitating sample clean-up or re-sampling (Hobbs et al., 2019; Veldhoen et al., 2016; Harper et al., 2019).

eDNA Persistence

DNA persistence varies by medium; sediment retains DNA longer than water, affecting species composition accuracy. Sediment samples may reflect community composition from centuries prior due to reduced DNA degradation under anoxic conditions (Turner et al., 2015; Holman et al., 2019; Willerslev et al., 2003). In water, eDNA degrades within days to weeks, influenced by UV radiation, temperature, and microbial activity (Thomsen et al., 2012; Barnes et al., 2014). Salinity also affects eDNA persistence, with higher degradation rates in inshore environments compared to offshore (Collins et al., 2018). Freshwater systems show eDNA persistence up to 30 days, while marine systems report up to seven days (Dejean et al., 2011; Thomsen et al., 2012). Controlled experiments are needed to further understand salinity's role in eDNA persistence (Skinner et al., 2020).

eDNA Dispersal

eDNA dispersal varies by environment. Rivers can transport eDNA over long distances, with invertebrate eDNA detected 20 km downstream from its source (Deiner and Altermatt, 2014). Ocean currents are likely to disperse DNA over large distances. However, marine eDNA samples appear to be quite restricted in their spatial representation of species presence, with significant differences among depth zones and habitats that are spatially close together (Jarman et al. 2024). It is hypothesized that the while DNA may disperse widely in the ocean, currents and the sheer volume of water involved may mean that at any distance from the source, only traces of DNA are present, which are easily swamped during sequencing by DNA from locally present species (Alexander et al. 2024, but also see O'Donnell et al., 2017; Jeunen et al., 2019). However, as sequencing depth increases, or specific primers are developed, detections of trace amounts of DNA at distance from the source may become more common.

2.2.2 Collection and Preservation of eDNA Samples

Sediment and Soil

The method for sediment collection and preservation depends on the environmental medium, study purpose, budget, time, and sampling location. Clean tools are essential to prevent DNA contamination.

Nearshore Marine Sediments: Often sampled with a sediment grab (e.g., Van Veen grab) from a small boat (Chariton et al., 2015; Stoeck et al., 2018a) or a spade in intertidal locations (Aylagas et al., 2018). Offshore ocean studies typically use a corer or large grab (Kimes et al., 2013; Laroche et

al., 2016; Yeung et al., 2011). Subsamples from the top 1-2 cm are taken using pre-sterilized tools or those soaked in 70% ethanol and rinsed in sterile water (Mason et al., 2014). Samples are frozen at -20°C or -80°C, stored in 95% ethanol, or exposed to a desiccant until analysis (Lear et al., 2018).

Freshwater Sediments: Collected using a hand corer, with surface sediment placed in cetyl trimethylammonium bromide (CTAB) or stored on ice before freezing at -20°C or -80°C (Turner et al., 2015).

Terrestrial Soils: Collected using a shovel or corer, stored temporarily at 4°C, and frozen for long-term storage at -20°C or -40°C (Andersen et al., 2012; Leempoel et al., 2020; Li et al., 2020). Soil samples can also be sieved and stored at -70°C (Lloyd-Jones and Hunter, 2001) or dried with desiccant. Wildlife snow tracks have also been used for eDNA collection, kept frozen until analysis (Franklin et al., 2019).

Water and Ice

Water Samples: Collection and preservation vary by water depth, vessel capabilities, location, budget, time, and field conditions. Deep-sea water collection often uses Niskin bottles attached to a CTD-Rosette or a remotely operated vehicle (ROV) (Yeung et al., 2011; Everett and Park, 2018). Immediate filtration reduces storage requirements, decreases PCR inhibition, and prevents DNA degradation (Takahara et al., 2015; Thomsen et al., 2012). Filtration is more efficient than direct amplification, yielding higher DNA concentrations and detecting rare fragments (Deiner et al., 2015; Renshaw et al., 2015). Ice samples are collected as cores or blocks, stored at -20°C, then thawed and treated as water for eDNA extraction (Bayless et al., 2015).

Sampling Equipment: The Smith-Root ANDeTM sampler backpack standardizes sampling and reduces contamination risk. It includes a portable pump, pole extension with remote pump controller, custom filter housings, and onboard sample storage (Thomas et al., 2018; Skinner et al., 2020).

Filter Types: Various filter membranes (e.g., cellulose nitrate, polyethersulfone, polycarbonate track-etch, polyvinylidene difluoride, glass microfibre) affect results. Mixed cellulose ester (MCE) filters yield consistent metazoan community composition and recover more DNA copies than polyethersulfone (PES) filters (Majaneva et al., 2018; Renshaw et al., 2015). Polyvinylidene difluoride (PVDF) filters yield higher eDNA concentrations than MCE and PES filters (Capo, 2019). Smaller pore sizes generally capture more DNA but may clog, affecting efficiency (Turner et al., 2014; Capo, 2019). Ethanol precipitation is an alternative to filtration but requires high eDNA concentration (Doi et al., 2015).

Filter Preservation: Methods include freezing at -20°C, storing in >90% undenatured ethanol, lysis buffers, or silica gel (Majaneva et al., 2018). Filters preserved dry in silica gel or lysis buffer at room temperature provide consistent community composition. Ethanol preservation is common but may result in lower taxa detection and variable community composition (Minamoto et al., 2016; Spens et al., 2017; Pilliod et al., 2013a; Majaneva et al., 2018). For microbial communities, 95% ethanol is effective (Song et al., 2016). CTAB and Longmire's buffers are recommended for freshwater samples (Renshaw et al., 2015).

2.2.3 Laboratory Processing

DNA Extraction

DNA extraction and amplification can be affected by biotic and abiotic inhibitors (Pedersen et al., 2015). Extraction kits that remove PCR inhibitors are available. Inhibition can be reduced by diluting DNA extracts, additional purification steps, or using enzyme facilitators (Lear et al., 2018). Verification steps, such as spiking samples with foreign DNA, ensure effective inhibition removal.

Soil and Sediment: Extraction protocols are well established, with resources like the Earth Microbiome Project recommending DNeasy PowerSoil® and PowerMax® kits (Qiagen) (Lear et al., 2018). Sample quantities vary, with 1 g providing stable DNA extraction efficiency (Wei et al., 2018).

Water and Ice: Extraction is routinely accomplished with commercial kits like DNeasy Blood and Tissue, DNeasy PowerWater-DNA Isolation, QIAamp DNA, and FastDNA Spin (Lear et al., 2018). Kit choice may depend on the target taxa, with DNeasy Blood and Tissue kits commonly used for macro-organisms and PowerSoil® and PowerMax® for micro-organisms (Laramie et al., 2015; Pilliod et al., 2013b; Takahara et al., 2013; Thomsen et al., 2012; Lejzerowicz et al., 2014; Pawlowski et al., 2011). Manual methods are also used, highlighting the need for further research and standardization (Barberán and Casamayor, 2014; Callejas et al., 2011; Everett and Park, 2018).

eRNA Co-extraction

Co-extraction of eDNA and eRNA can reduce false positives from DNA persistence, as RNA reflects current living organisms. However, RNA processing can introduce errors, necessitating comparison with eDNA to improve accuracy. eRNA must be converted to complementary DNA (cDNA) using reverse transcriptase, which can introduce point mutations (Ellefson et al., 2016; Houseley and Tollervey, 2010). Analyzing both eDNA and eRNA datasets together can improve overall sensitivity and accuracy by removing OTUs not shared by both (Laroche et al., 2017). However, this process may produce false negatives, affecting results depending on the specific data and research question. For baseline assessments, eRNA and eDNA/eRNA trimming may not provide a complete environmental characterization due to potential false positives and missed detections. For impact monitoring, this trade-off may be acceptable depending on the monitoring hypotheses and research questions (Laroche et al., 2017).

Genetic Markers and Laboratory Analyses

Genetic Markers

Genetic markers are essential for targeting specific taxa or characterizing entire communities. The choice of markers depends on the research question, sampling logistics, target species' life history, and eDNA availability in samples (Díaz-Ferguson and Moyer, 2014). Detection methods include PCR, qPCR, ddPCR, and high-throughput sequencing (HTS) (Zaiko et al., 2015a; Doi et al., 2015; Shokralla et al., 2012). Advances in NGS/HTS, such as Illumina (MiniSeq, MiSeq, NovaSeq) and Ion Torrent sequencing, combined with ddPCR, enhance rare species detection, abundance estimates, and multi-species detection from complex samples (Singer et al., 2019).

Barcoding

Barcoding targets a single taxon using specific markers to differentiate it from similar taxa. Reference databases like the Barcode of Life Data Systems (BOLD) and GenBank assign sequence results to taxa. Short eDNA barcode fragments (<150 bp) provide sufficient phylogenetic resolution, unlike traditional barcodes requiring >500 bp sequences (Pedersen et al., 2015). Primers designed to bind unique sequence regions of the target taxon enable exponential DNA fragment amplification, producing detectable amplicons (Darling and Mahon, 2011).

Commonly used barcodes include the mitochondrial COI gene for animals (Hebert et al., 2003a), with alternatives like 18S rRNA, 28S rRNA, and 12S rRNA for species-level identification (Machida and Knowlton, 2015; Machida and Tsuda, 2010; Markmann and Tautz, 2005). Plant genes include rbcl and matK, while fungi and protists use the Internal Transcribed Spacer Region (ITS) (BOLD, 2014-2020). DNA barcoding involves sequencing the barcode region (~500+ bp) for identification and reference library placement, requiring tissue samples for confirmation. eDNA identification relies on unique short sequences within the barcode region, with PCR assays developed to detect the target taxon in environmental samples. Quantification can be achieved using qPCR or ddPCR, though abundance estimate accuracy varies across studies (Hebert et al., 2003b; Creer et al., 2010).

Metabarcoding

Metabarcoding characterizes communities or conducts biodiversity surveys, using universal primers to target multiple sequences simultaneously. It can identify specific taxa, but barcoding or species-targeted qPCR are more efficient for this purpose. Metabarcoding primers include COI, cytochrome b, and 12S rRNA (Thomsen et al., 2012; Miya et al., 2015). Universal primers, such as those designed by ECOPRIMER software, amplify multiple taxa and detect unexpected taxa (Pedersen et al., 2015).

However, universal primers may preferentially amplify certain taxa while others go undetected due to interspecific differences in tissue and DNA decay, primer-binding bias, PCR inhibition, and stochasticity (Pedersen et al., 2015). Research continues to improve universal primers, such as 18S rRNA for eukaryotic biodiversity studies in marine sediment, which has proven effective and can be standardized (Hadziavdic et al., 2014; Lekang et al., 2018). Metabarcoding HTS provides community-level data, enabling comprehensive community characterization (Pedersen et al., 2015).

2.2.4 Data Interpretation and comparability

Some of the major limitations of eDNA analysis occurs during the data interpretation step because programs and packages used are not standardized, making it difficult to compare results across studies. This section outlines how eDNA can be interpreted through the use of biotic indices; current packages and modeling being used; and probability of detection reported for various studies.

Post-Amplification Quantification of PCR Products

Quantification of PCR products is essential for confirming target DNA presence, estimating species abundance, and quantifying amplified sequences. PCR products amplify exponentially, creating a linear relationship between amplification cycles and the logarithm of molecule numbers. Methods for quantifying eDNA concentration include NanoDrop spectrophotometry, fluorometric methods (Picogreen, Invitrogen, Qubit) (Zaiko et al., 2015a; Lear et al., 2018), and qPCR-related methods.

These methods estimate eDNA concentration relative to a standard curve derived from tissue-derived DNA samples (Barnes et al., 2014; Pilliod et al., 2013b; Strickler et al., 2015). Limits of Detection (LOD) and Quantification (LOQ) are determined from these curves (Tréguier et al., 2014). However, qPCR accuracy and reproducibility are limited by the need to run all samples on the same plate to minimize technical error (Taylor et al., 2015; Doi et al., 2015).

Droplet Digital PCR (ddPCR), a third-generation DNA detection method, offers absolute quantification without a standard curve and is more accurate, cost-effective, and time-efficient than qPCR (Doi et al., 2015; Nathan et al., 2014; Taylor et al., 2015). ddPCR uses emulsion chemistry to distribute PCR reactions into thousands of nanodroplets, with statistical analysis determining target DNA concentration (Doi et al., 2015). Despite its promise, further understanding of field sampling effects on eDNA occupancy and abundance estimates is needed (Foote et al., 2012; Pilliod et al., 2013a; Pilliod et al., 2013b).

Sequence Analysis Pipeline

Post-amplification, data processing involves clustering sequences into Operational Taxonomic Units (OTUs) corresponding to ecological or biological species (Cristescu, 2014). Sequence analysis pipelines use various tools for data pre-processing, processing raw sequence reads, and interpreting data in the context of existing sequences (Cristescu, 2014; Flynn et al., 2015). Bioinformatics software packages include QIIME2, UCLUST, Greengenes, PyNAST, R with Vegan package, BioEdit, UPARSE, CD-HIT, ESPRIT, BLAST, MacVector 9.0, and PRINSEQ. The large selection of packages necessitates a comprehensive evaluation of OTU generation methods and best practices in quality filtering, clustering, and OTU definition (Cristescu, 2014).

Factors to consider when choosing a sequencing package include sequence quality, number of sequences, cost, and sequence length (Lear et al., 2018). Aylagas et al. (2016a) found that a 313 bp barcode provided better taxonomic resolution than the traditional 658 bp barcode for metazoan metabarcoding, likely due to fewer sequencing errors in shorter barcodes.

Customized computational pipelines, such as those for Illumina sequencing platforms, address specific issues like overestimation of species richness due to PCR artifacts (Pawlowski et al., 2014). Standardizing eDNA sequence analysis is crucial to avoid misreporting rare species and misleading management applications (Cristescu, 2014).

Standardization Efforts

A study by Stein et al. (2018) compared results from four laboratories using different taxonomic databases, clustering algorithms, and quality control approaches. Initial results showed substantial differences in taxonomic composition, but reconciliations in demultiplexing, OTU data exclusion, taxonomic identification, and error correction led to similar taxa composition across pipelines. Applying the reconciled pipeline to a novel dataset yielded consistent results, demonstrating that standardizing best practices can achieve comparable outputs (Stein et al., 2018).

Collaborative comparisons and guided standardization can increase the reliability and acceptance of eDNA data, potentially leading to regulatory acceptance and industry application in biomonitoring studies. Porter and Hajibabaei (2018) emphasized the need for improved bioinformatic algorithms and robust software tools for policy and management decisions.

Biotic Indices

Biotic indices range from simple univariate indices, measuring metrics such as diversity, richness, and evenness, to complex multivariate indices that compare expected values to reference conditions to evaluate ecological status (Pawlowski et al., 2018). These indices are used in both terrestrial and aquatic systems, such as assessing soil quality using nematode communities (Urzelai et al., 2000) and evaluating ecological quality status with marine benthic macrofauna (Moreno et al., 2011; Ni et al., 2019; Umehara et al., 2019).

Species abundance is a common metric in biotic indices, but quantifying species abundance using eDNA presents challenges. Relative abundance or metrics not requiring abundance can be used as alternatives (Stein et al., 2019). Functional metrics, such as feeding types of infaunal invertebrates (Maurer et al., 1999) or preferred spawning habitats for fish, are also valuable (Hering et al., 2006).

Pawlowski et al. (2018) categorize the incorporation of eDNA data into biotic indices into three approaches: using metabarcoding data to identify taxa and infer morphotaxonomy-based indices, exploring new bioindicator taxa, and developing new molecular indices. The latter two approaches are promising for future bioassessment applications due to the current limitations of reference databases.

Aylagas et al. (2014) analyzed benthic marine invertebrates to implement a genetic-based AZTI marine biotic index (gAMBI). They found that species-level identification, presence/absence data, and targeting frequently occurring species were necessary for accurate gAMBI calculations. Studies comparing conventional and eDNA-derived biotic indices have shown good correlation (Apothéloz-Perret-Gentil et al., 2017; Aylagas et al., 2016a, 2018, 2014; Lejzerowicz et al., 2015).

Metabarcoding provides a holistic view of taxonomic diversity, capturing both macrofaunal and meiofaunal diversity (Lejzerowicz et al., 2015). This approach is beneficial for bioassessment using small bioindicator taxa. Metabarcoding can identify taxa sensitive to environmental disturbance that are difficult to detect by conventional means (Stoeck et al., 2018a, 2018b; Chariton et al., 2015; Pawlowski et al., 2014; Lanzén et al., 2016).

Reducing taxonomic resolution and focusing on the phylogenetic species concept is another method for interpreting ecological change using eDNA (Dunthorn et al., 2014). However, species-level identification may still be required for certain taxa, such as bacteria, which exhibit variable responses to stressors and rapid adaptations (Borja, 2018; Haggerty and Dinsdale, 2017).

A taxonomy-free molecular index approach, assigning ecological values to molecular operational taxonomic units (MOTUs), can overcome limitations of insufficient DNA reference libraries (Apothéloz-Perret-Gentil et al., 2017). This approach showed higher correlation between morphological and molecular indices without taxonomic assignment and utilized a larger proportion of MOTUs.

Supervised machine learning (SML) has been proposed to develop predictive models for biotic index values from eDNA metabarcoding data without taxonomic assignments (Cordier et al., 2017). SML approaches have produced accurate ecological status predictions and more accurate models of ecological status than traditional metabarcoding (Cordier et al., 2018).

Genetic-based biotic indices provide similar conclusions about environmental status as conventional methods and can address identification issues. Incorporating metabarcoding data into existing indices is the first step towards developing genetic-based indices and SML predictive models (Pawlowski et al., 2018).

2.2.5 Reporting

Communication and Collaboration

Effective communication and collaboration are crucial for aligning industry, academic, and regulatory protocols in environmental genomics. The study by Stein et al. (2018) exemplifies collaboration that fosters comparability across laboratories and may lead to standardized sequence analysis methods.

The Barcode of Life Data Systems (BOLD) is an online workbench and database for DNA barcode records, facilitating global research and collaboration (Ratnasingham and Hebert, 2007). BOLD standardizes approaches, documents morphology-genetic sequence matches, and provides voucher samples for each species. It supports the Canadian Barcode of Life Network's campaign to barcode all eukaryotic life in Canada. BOLD transfers sequence and specimen data to the International Nucleotide Sequence Database Collaboration (INSDC) and its repositories (DDBJ, ENA, GenBank), enhancing the potential for metabarcoding as the reference database grows.

2.2.5.1 Accuracy of Results

It is important to recognize when and how a result may be inaccurate, incomplete or misleading. In addition to the reference databases lacking or lagging, they are not infallible and could contain incorrectly assigned species identifications to sequences due to inaccurate morphological identifications (Lejzerowicz et al., 2015; Pawlowski et al., 2018). There are many other factors that influence the accuracy of results via introduction of false negatives and/or false positives. This section will outline potential causes of false negatives and false positives when using eDNA techniques and the corresponding step in the sampling and/or analysis protocol that will need refinement in order to reduce the occurrence of the error, which is also illustrated in Figure 2-4.

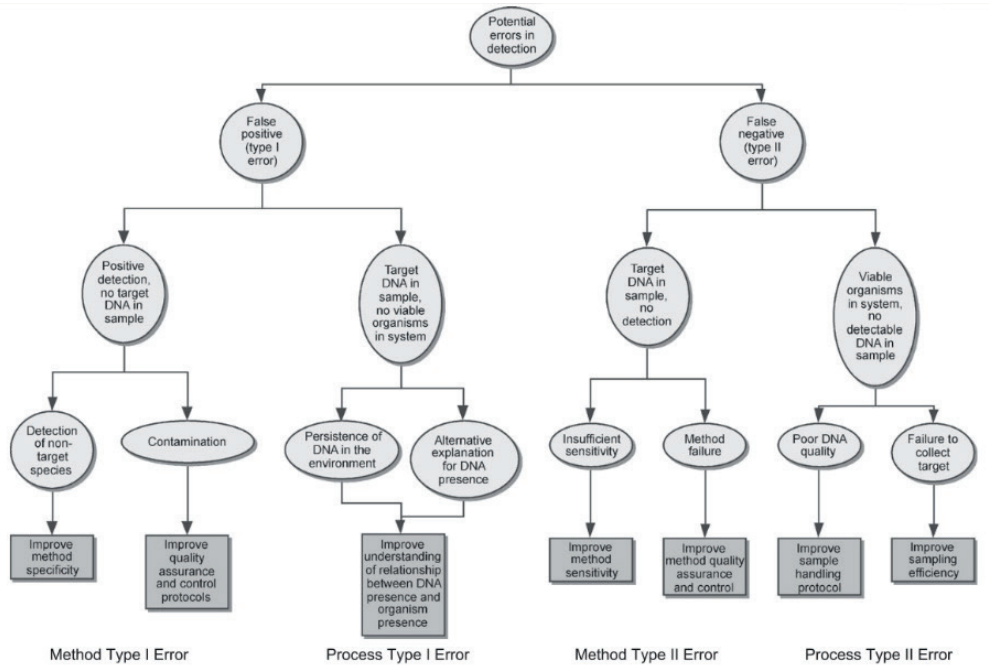

Figure 2.4 - Potential sources of error in DNA-based aquatic invasive species monitoring protocols. Errors can be attributed specifically to the detection method employed ("method" error) or to the overall monitoring process ("process" error) including, for instance, sampling design, and sample handling (Darling and Mahon 2011).

2.2.5.2 Key Considerations

An overview of False Positive and False Negatives

False negatives occur when a species is present but its eDNA remains undetected due to inadequate sampling techniques, poor sampling design (Process Type II Error), low DNA extraction efficiency, PCR inhibition, or contamination (Method Type II Error) (Darling and Mahon, 2011; Goldberg et al., 2015). High concentrations of filter feeders, such as bivalves, can reduce local eDNA sources, obstructing target species detection and potentially resulting in false negatives (Friebertshauser et al., 2019). Holman et al. (2019) found that bioinformatic parameter settings could lead to false-negative eDNA detections. To prevent false negatives, Cristescu and Hebert (2018) recommend increasing sample numbers per locality, analyzing multiple DNA extracts per sample, increasing PCR cycles, and including positive controls, such as mock communities.

False positives occur when a target species is absent but its DNA is detected erroneously, suggesting its presence (Method Type I Error). Causes include contamination, technical error, incorrect primer binding, or exogenous DNA persistence despite the absence of the source organism (Process Type I Error) (Darling and Mahon, 2011). This can lead to erroneous conclusions about community composition and biodiversity. False positives can also result from sample contamination, poor primer design, inadequate probe specificity, or sequence processing ambiguities (Cristescu and Hebert, 2018). Accurate assay design and verification with tissue samples from target taxa are crucial to minimize false positives.

Molecular Assay Design

Accurate molecular assay design and specificity are essential for reliable results. Assay sensitivity (Method Type II Error) represents the smallest amount of target DNA required for detection. Sensitivity can be tested using spiked samples with known target DNA quantities. Detection limits should be calculated using ecologically relevant sample volumes to avoid misleading results (Darling and Mahon, 2011). Assay specificity ensures correct identification of the target species and exclusion of non-target species. Specificity can be tested using statistical methods to identify potential mismatches and tools like BLAST to compare probe design to sequence databases (Darling and Mahon, 2011). Sequencing all positive detections and testing probes against non-target species from the same family are recommended during assay development.

MOTU Clustering to Estimate Species Richness

Accurate taxonomic assignment using metabarcoding is limited by reference DNA databases. Researchers are building robust DNA databases (e.g., BOLD, GenBank) and using molecular operational taxonomic units (MOTUs) determined by de novo clustering to avoid species assignment. UniFrac, a distance metric for comparing biological communities, can improve accuracy in defining distinct MOTUs, though it depends on phylogenetic alignments (Lanzén et al., 2016).

MOTU clustering can overestimate species richness due to intraspecific or intragenomic variation, often addressed using divergence thresholds or cut-offs. Commonly accepted divergence thresholds for species-level resolution are around 3%, but group-specific or multiple cut-offs are recommended for certain taxa (Brown et al., 2015). For prokaryotes, OTUs and 3% species-level thresholds have been succeeded by amplicon sequence variants (ASVs) and 99% clustering thresholds. Narayan et al. (2020) recommend ASV error correction with a 99% identity cut-off for Illumina sequences and OTU clustering with a 96% identity cut-off for other platforms.

Despite potential overestimation, MOTU clustering applied in biotic indices minimally affects final interpretation (Lanzén et al., 2016; Tapolczai et al., 2019).

DNA Extraction Methods and Sample Processing

DNA extraction methods and sample processing can affect results (Lekang et al., 2015). A homogenized-sampling method designed to characterize macroinvertebrate communities may favor larger specimens and amplify non-target taxa (Aylagas et al., 2016b; Elbrecht and Leese, 2015; Aylagas et al., 2018). Significant variation in eDNA yield and purity has been observed between different extraction methods, especially in sediments with high silt and clay content (Lekang et al., 2015; Simonelli et al., 2009).

Other factors affecting results include eDNA extraction volumes and sample storage. PCR inhibition is associated with lower extraction volumes and previously frozen samples (Takahara et al., 2015). Preservation techniques can cause varying effects depending on the targeted amplicon. For example, freezing produced more OTUs for the 18S rRNA amplicon compared to Longmire's preservation method, while no significant differences were observed for the COI amplicon (Holman et al., 2019).

Using Mock Communities as a Control

Mock communities, defined synthetic communities of known species and quantities, are used as controls to refine eDNA methods and estimate false positive/negative rates (Cristescu, 2014). For example, Zizka et al. (2019) used macroinvertebrate mock communities to test a fixative-based metabarcoding approach, identifying limitations in detecting molluscs. Comparisons between conventional and eDNA sampling approaches are valuable for refining methods, though they can be expensive (Roussel et al., 2015).

Probability of Detection

eDNA techniques do not have perfect detection, and sampling design affects study outcomes (Díaz-Ferguson and Moyer, 2014; Pilliod et al., 2013b). Statistical methods, such as site occupancy models, are important for interpreting eDNA results and verifying study designs relative to traditional field methods (Pilliod et al., 2013b; Schmidt et al., 2013). Studies should include estimates of false negative detection errors. For example, Tréguier et al. (2014) found only 38.5% convergence between conventional and eDNA methods for detecting freshwater crayfish. In contrast, Janosik and Johnston (2015) showed eDNA was more effective than traditional sampling for detecting the Slackwater darter. Valentini et al. (2016) reported higher detection probabilities with eDNA metabarcoding compared to conventional methods for amphibians and fish.

Occupancy models provide important information about detection probabilities, allowing for method refinement and informed decisions for environmental managers designing baseline or monitoring programs.

2.2.5.3 Community Representation

Community representation or composition is often assessed to establish baseline conditions for monitoring environmental changes due to development, restoration, remediation, or invasive species detection, thereby evaluating the overall health of an ecosystem.

Studies have shown that eDNA metabarcoding offers multiple benefits over conventional sampling techniques for characterizing community composition. These benefits include increased taxonomic resolution, comparability across regions, detection of early life stages and specimen fragments, and identification of a wider range of taxonomic groups that are difficult to identify by conventional means (Pawlowski et al., 2018). Improvements in taxonomic resolution using eDNA require robust reference DNA databases such as BOLD and GenBank, which match curated and verified species to their genotypic sequences (Cowart et al., 2015; Thomsen et al., 2016). Although many species are currently underrepresented, reference libraries are growing and will improve over time (Ratnasingham et al., 2007; Vitecek et al., 2017).

eDNA sampling has successfully detected multiple terrestrial forest mammals in a zoological garden (Ishige et al., 2017) and a natural forest ecosystem in northern Japan (Ushio et al., 2017). Similarly, Andersen et al. (2012) found that eDNA in soil accurately reflected the taxonomic richness of terrestrial vertebrates in safari parks, zoological gardens, and farms. Snow sampling in arctic and temperate regions has also detected rare carnivorous mammals using eDNA (Franklin et al., 2019).

In coral community studies, eDNA results matched or corrected visual ROV survey identifications, identifying some corals to species level that were indistinguishable visually (Everett and Park, 2018). Cowart et al. (2015) found that molecular barcodes revealed a more diverse invertebrate seagrass community than morphological surveys, with both methods agreeing on beta-diversity. Baker et al. (2018) used eDNA to confirm killer whale individuals and increased taxonomic resolution to a "southern resident community" ecotype, with detection up to two hours after visual and acoustic identifications.

Early Life Stages

Morphological identification of early life stages and specimen fragments is challenging, making metabarcoding or barcoding valuable for baseline assessments and community characterization (Valentini et al., 2009). Detecting early life stages provides a more complete community overview and early warning of invasive species spread (Besansky et al., 2003).

Microbial Community

eDNA methods can study microbial communities, which are difficult to assess using conventional tools. Metabarcoding can detect prokaryotes, protists, and metazoan meiofauna, providing a comprehensive ecosystem picture (Pawlowski et al., 2018). Sawaya et al. (2019) used metabarcoding to survey eukaryotic diversity along a coral reef, detecting a wide range of species across multiple trophic levels and establishing ecosystem baseline conditions.

Metabarcoding has been used to monitor anthropogenic impacts by detecting sensitive taxa such as protists, which respond quickly to environmental changes (Lanzén et al., 2016; Pawlowski et al., 2014; Stoeck et al., 2018a, 2018b; He et al., 2019). For example, He et al. (2019) assessed benthic impacts of marine finfish aquaculture by linking geochemical parameters to foraminifera diversity and composition based on eDNA analysis. Results were consistent with previous studies on salmon aquaculture impacts (Pawlowski et al., 2016a; Pochon et al., 2015).

Estimating Species Richness

Metabarcoding reveals underlying biodiversity within and among species groups, particularly in poorly understood taxa. However, challenges include overestimating taxa richness due to intraspecific and intragenomic variability. MOTU clustering and fixed thresholds can address these

biases (Pawlowski et al., 2018). Despite these challenges, species richness is one of the easiest parameters to assess using metabarcoding.

Disagreement between morphotaxonomic inventories and molecular datasets is often due to incomplete or inaccurate DNA reference libraries (Pawlowski et al., 2018; Cowart et al., 2015; Taberlet et al., 2012a). To overcome this, the BOLD database facilitates research and global collaboration, improving reference libraries (BOLD Systems, 2014-2019).

Community representation assessment using eDNA is also affected by sampling scale, presence of "ghost" MOTUs, differential primer efficiency, and DNA extraction methods (Pawlowski et al., 2018; Aylagas et al., 2018).

2.2.5.4 Species Abundance

Species abundance is challenging for metabarcoding because the number of sequences generated by HTS does not necessarily represent specimen numbers or biomass (Carew et al., 2013; Elbrecht and Leese, 2015). Factors affecting this include variations in biomass, DNA shedding rates, primer efficiency, DNA extraction methods, sample storage, and PCR bias (Pawlowski et al., 2018; Shaw et al., 2016; Takahara et al., 2015).

Despite these challenges, some studies show potential for quantification using metabarcoding. Aylagas et al. (2018) found a positive correlation between the number of reads generated for each taxon and the abundance and biomass determined using morphology. Carew et al. (2013) reported a strong quantitative relationship between 454 sequence reads and species abundance, though sequence reads did not perfectly represent species occurrence frequency.

Quantification of a single targeted species' abundance can be achieved using ddPCR, which offers higher accuracy and efficiency than qPCR (Doi et al., 2015; Nathan et al., 2014; Taylor et al., 2015). Studies have shown positive correlations between eDNA concentrations and species abundance or biomass (Minamoto et al., 2017; Hernández-López et al., 2019; Skinner et al., 2020; Lacoursière-Roussel et al., 2016; Salter et al., 2019).

Mock communities have provided evidence that relative abundance can be determined using eDNA. Klymus et al. (2017) demonstrated that careful primer design allows for reliable relative abundance estimates using eDNA. Further research is needed to validate and refine quantitative methods for multiple species.

2.2.5.5 Species Distribution

eDNA has shown promise for assessing and mapping species distribution via presence/absence detection to determine the spatial extent of a species. However, caution is necessary when inferring geographical extent or distribution, especially in aquatic environments where moving water masses can carry eDNA far from the source organism. Some studies provide evidence of limited dispersal in marine environments (Jeunen et al., 2019; O'Donnell et al., 2017).

Many studies have investigated eDNA techniques for freshwater invasive species distribution (Cai et al., 2017; Klymus et al., 2017; Parrondo et al., 2018; Takahara et al., 2013), but fewer have applied this approach in marine environments. Andruszkiewicz et al. (2017) identified distinct vertical and horizontal distributions of marine vertebrate communities in Monterey Bay National Marine Sanctuary using metabarcoding. Although not verified with traditional methods, the known habitat preferences matched those estimated from eDNA.

Yamamoto et al. (2016) found a positive correlation between eDNA concentration and the estimated distribution and biomass of jack mackerel based on sonar survey echo intensity. Minamoto et al. (2017) demonstrated a positive correlation between visual descriptions and eDNA surveys of temporal and spatial jellyfish distribution.

Occupancy models using eDNA data have shown great promise by accounting for imperfect detection at spatio-temporal scales (Pilliod et al., 2013b; Schmelzle and Kinziger, 2016; Smith and Goldberg, 2019). Higher detection probabilities using eDNA water sampling compared to traditional methods have extended the known distribution of rare species, such as the tidewater goby (Schmelzle and Kinziger, 2016). Smith and Goldberg (2019) showed that multi-scale occupancy modeling with eDNA data improved monitoring of spotted frogs, incorporating false positive detection rates to assess model influence.

Aquatic hydrodynamics can complicate species distribution interpretation using eDNA. Wood et al. (2019) found that eRNA is more stable than previously thought, with no significant difference in decay rates between eDNA and eRNA. Both persisted up to 13 hours after organism removal, potentially leading to misinterpretation. Persistence in biofilms was much longer, detectable up to 21 days, complicating the distinction between living and dead organisms.

Integrating quantitative eDNA measurements, hydro-geomorphological scaling, and ecological models is a promising avenue for future research to map species distributions in complex aquatic environments. Carraro et al. (2018) demonstrated high potential for mapping sessile and mobile organisms in fluvial ecosystems, an approach that could be valuable in marine ecosystems if validated with conventional methods.

2.2.5.6 Species Viability

The persistence of genetic material in the environment can result in eDNA detection of an organism despite its physical absence. Differentiating viable (living) from non-viable (dead) organisms is essential for accurately characterizing existing communities, especially in baseline assessments and biomonitoring. eRNA, due to its labile nature compared to eDNA, has been proposed to overcome persistence limitations (Laroche et al., 2017). However, rRNA/DNA ratios can sometimes misclassify active populations as dormant, leading to misinterpretation (Steven et al., 2017).

Brescia et al. (2009) developed a propidium monoazide (PMA) pre-PCR treatment that blocks amplification of DNA from dead Cryptosporidium oocysts, allowing detection of viable oocysts. This method also distinguished between infectious and noninfectious enteric viruses in water samples using RNA extraction (Parshionikar et al., 2010). Viability dyes like PMA and ethidium monoazide (EMA) have limitations, such as assuming all intact cells are living, which may not always be the case (Fittipaldi et al., 2012).

Viability dyes are photoactivatable and cannot permeate intact cell membranes. Exposure to light irreversibly modifies DNA-bound dyes, inhibiting amplification of non-living cells. PMA is recommended for bacterial cells or mixed species samples due to its lower efficiency in permeating damaged membranes compared to EMA (Fittipaldi et al., 2012). Increasing amplicon length or applying pre-treatments to enhance cell membrane destabilization can improve exclusion of dead cells (Fittipaldi et al., 2012; Lee and Levin, 2009; Shi et al., 2011).

Distinguishing between extracellular DNA from living or dead sources is complex. Kamoroff and Goldberg (2018) found that sampling and analytical methods influence false positive detection from dead individuals. Surface water sampling reduces detection chances of dead individuals, and quantification-based detection can help differentiate between living and dead organisms.

In summary, eDNA persistence can confound results due to the inability to differentiate between viable and dead individuals. Methods such as combining eRNA/eDNA, using viability dyes, adjusting filter type, sampling location and time, and employing qPCR instead of conventional PCR techniques may help overcome this limitation. Further research is needed to validate these approaches for specific research areas and questions.

2.2.5.7 Real-Time On-Site Measurement and Analysis

Handheld and portable devices for real-time quantitative PCR (qPCR) analysis have been developed, showing results that correlate with traditional bench qPCR (Nguyen et al., 2018). New eDNA technologies, such as MinION and Biomeme, have the potential to revolutionize biomonitoring by enabling on-site eDNA extraction and analysis.

MinION

The MinION is a portable device that uses an array of protein nanopores embedded in a polymer membrane to measure ionic current changes as DNA passes through. The DNA bases are sequenced based on characteristic current changes associated with each nucleotide. A consumable flow cell, inserted into the MinION, contains a sensor that detects the nanopore signal as the DNA molecule is analyzed. Each flow cell can sequence ultra-long read lengths (hundreds of kb) and generate up to 30 Gb of DNA sequence data or 7-12 million reads for RNA analysis (Oxford Nanopore Technologies, 2008-2019).

Advantages of the MinION over short-read sequencing technologies, such as Illumina, include real-time reads, rapid library preparation, the ability to sequence DNA molecules of any length, and its small, portable size, making on-site sequencing possible (Rang et al., 2018). However, the current sequencing accuracy for MinION is reported to be 85%-95% (Rang et al., 2018; Jain et al., 2018; Stancu et al., 2017; Jain et al., 2017; Tyson et al., 2017), compared to the higher accuracy of Illumina, with PhiX quality scores for MiSeq and HiSeq systems showing 95.2%-97.0% of bases with scores > Q30 (equivalent to 99.9% base call accuracy) (Illumina, 2011).

Tyler et al. (2018) reported a sequencing alignment accuracy of 97% using R9.4 model flow cells with 2D sequencing and 94% with 1D sequencing. Truelove et al. (2019) compared MinION to Illumina MiSeq, finding that a 3.5-hour MinION run produced a small number of accurate reads with high quality and similar confidence to Illumina sequencing. Laver et al. (2015) reported an error rate of 38.2% for MinION, but more recent studies using new MinION Detection Software have shown improved accuracy (Deshpande et al., 2019).

Biomeme

Biomeme Inc. has developed handheld devices, such as the Two3 and the newer Franklin (one9, two9, or three9), which are thermocyclers capable of performing on-site PCR, reverse transcription (RT)-PCR, qPCR, and isothermal tests (Biomeme, 2018). The Franklin allows for multiplex, real-time detection of up to 27 targets from one sample or nine samples for up to three unique targets each, with three fluorescence channels detecting FAM/SYBR, TexasRedX, and ATTO647N/CY5. Skinner et al. (2020) noted error rates of up to 26% for the Franklin compared to 10% for the MIC thermocycler bench qPCR platform. Nguyen et al. (2018) found a significant correlation between Biomeme and traditional bench qPCR in a microcosm experiment. Sepulveda et al. (2018) found that Biomeme's Two3 was more prone to inhibition and less sensitive than lab-based approaches, increasing the potential for false negatives.

Loop-Mediated Isothermal Amplification (LAMP)

LAMP, initially developed for clinical use, has shown promise in ecological studies (Centeno-Cuadros et al., 2016; Arunrut et al., 2016). LAMP rapidly amplifies DNA at one temperature, resulting in a color change within reaction tubes. It operates under isothermal conditions based on autocycling strand displacement (Lee et al., 2017). Arunrut et al. (2016) reported that LAMP significantly reduced time, difficulty, and cost for eDNA-based detection of acute hepatopancreatic necrosis disease in shrimp, with detection limits comparable to other PCR methods.

Environmental Sample Processor (ESP)

The ESP is a near real-time fully automated platform that can be attached to a mooring, suspended in the water column, to detect multiple targeted species on a single probe array via robotized seawater sample collection. It has been tested with probes targeting microorganisms responding to oil, successfully detecting all targeted taxa with good correlation to bench qPCR assays (Baussant et al., 2017). The third-generation (3G)-ESP, fitted to an Autonomous Underwater Vehicle (AUV), can collect eDNA samples and provide real-time acoustic backscatter data, enabling identification of pelagic marine animals at precise locations within the water column (Zhang et al., 2020).

Considerations

Although handheld eDNA analysis devices are not yet as sophisticated as benchtop equivalents, they offer the convenience of rapid, less labor-intensive sampling and analysis. They can complement conventional sampling methods and eDNA analysis. However, care must be taken in interpreting results and making management decisions due to lower accuracy, yield, and differences in sequencing depth associated with handheld devices.

2.3 Consultation

2.3.1 Methods

A two-phase consultation process was conducted to evaluate the potential applications of environmental genomics for regulatory purposes:

Phase 1 involved a digital questionnaire sent to approximately 60 professionals from academic, regulatory, and industrial backgrounds with expertise in environmental genomics. The questionnaire included 18 questions covering:

- Demographics (Name, Role, Organization, Experience)
- Ecosystem/application of interest
- Identification and qualification of the most pressing knowledge gap/study area to enhance regulatory uptake, including:
  - Technology Readiness Level (TRL; European Commission definition)
  - Estimated cost and timelines to complete the study
  - Challenges to advancing the study area

Follow-up telephone interviews were conducted to gather detailed information and discuss ongoing research initiatives not publicly available.

Phase 2 involved telephone interviews with representatives from International Offshore Petroleum Environment Regulators (IOPER) member countries to assess the current and planned applications of environmental genomics in environmental permitting and operational monitoring. Interviews were conducted with representatives from:

- Australia - National Offshore Petroleum Safety and Environmental Management Authority (NOPSEMA)
- Norway - Norwegian Environment Agency
- The United Kingdom - Offshore Petroleum Regulator for Environment and Decommissioning (OPRED)
- Guyana - Environmental Protection Agency
- United States of America - Southern California Coastal Water Research Project (SCCWRP)

2.3.2 Results

Phase 1: Digital Questionnaires

The response rate for the digital questionnaire was 25% (15 of 60). Responses were received from representatives of various organizations, including:

- Norwegian Environment Agency
- Fisheries and Oceans Canada (DFO)
- L'Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER)
- Southern California Coastal Water Research Project
- BP
- Repsol SA
- AZTI
- Bureau Veritas Laboratories
- Shell Oil Company
- Canada's Ocean Supercluster
- Applied Genomics Ltd.
- NORCE AS
- Rio de Janeiro Federal University
- University of South Florida
- Université Laval

Interviews lasted 60 to 90 minutes and covered topics such as current studies, identified knowledge gaps, and experiences with regulatory implementation of genomic methods.

Respondents were predominantly from government organizations, with the remainder equally distributed among academia, industry, and service providers. Most respondents (80%) were very familiar with the technology, while the rest were moderately familiar.

All respondents were concerned with marine habitats, with two-thirds focusing on aquatic habitats (marine and freshwater) and the remaining third incorporating terrestrial habitats.

The questionnaire identified several study areas of interest in environmental genomics, including:

- Methodological development
- Sampling design
- Data analyses/bioinformatics
- Species abundance
- Community representation
- Species distributions

Seven respondents indicated interest in all study areas, with minor variations in preferences. Additional concerns included the design and validation of robust eDNA assays and temporal variability.

Respondents also indicated relevant ecological monitoring applications, such as:

- Baseline assessments
- Habitat delineation
- Detection of key species
- Rapid assessment of invasive species
- Population status and dynamics
- Monitoring environmental effects of energy industry activities
- Monitoring other anthropogenic effects or ecological change
- Remediation/restoration

The most favored applications were the detection of key species, baseline assessments, and rapid assessment of invasive species.

The primary knowledge gaps identified for regulatory uptake were methodological standardization and regulations. Respondents classified the current status of priority studies between TRL 4 and TRL 7, with most at TRL 5. Estimated costs for studies exceeded $1 million (USD) and required one to five years to complete.

Perceived challenges included funding and regulatory interest. Additional challenges noted were management funding issues and data accessibility for regulators.

Secondary knowledge gaps focused on standardization and methodological development, including:

- Regulator understanding of technique strengths and weaknesses
- Autosampling technologies
- Standardization of speciation analysis and taxonomic databases
- Incorporation of eDNA data into environmental indices
- Adoption of eDNA techniques varying by application and field of use
- Ensuring standards do not stifle innovation
- Scoring ecological status based on eDNA multi-marker target analysis
- Developing and evaluating phylogenetic microarrays for in situ analysis
- Building reference libraries and taxonomic competence

This comprehensive consultation process highlights the current state and future potential of environmental genomics in regulatory applications.

3. Regulatory Acceptance & Application

Disclaimer: This information was representative at the time the study was undertaken (2020), however, regulatory acceptance and application may have advanced in some jurisdictions in the interim.

Aside from OSPAR, the principal international commissions and conventions related to monitoring environmental compliance and development of best practices for the offshore energy industry are devoid of any specific consideration of environmental genomics. The International Offshore Petroleum Environment Regulators (IOPER) is a collaborative group of national regulators established to increase environmental performance standards within the offshore petroleum exploration and production industry, including normal operations and environmental management (IOPER, 2013). Member nations include Australia, Brazil, Canada, Ireland, Israel, Guyana, Mexico, Netherlands, New Zealand, Norway, Suriname, United Kingdom, and United States. IOPER members are currently investigating the development of a common set of Environmental Performance Indicators (EPIs) to measure the environmental performance of offshore petroleum industries and regulatory regimes in member counties. These EPIs will be developed in consideration of existing schemes in place such as the International Regulators' Forum and the Global Reporting Initiative. Although there is no specific mention of environmental genomics applications, the current scientific and regulatory communities continue to research and develop these techniques. Based on the previously described studies that have assessed the use these tools to monitor the influence of offshore energy industry activities on the environment, the path forward will likely see environmental genomics more commonly integrated into environmental management and assessment plans in certain jurisdictions.

The Barcelona Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean consists of 22 Mediterranean countries which aim to protect the Mediterranean marine and coastal environment while achieving sustainable development (European Commission, 2019). Although it does not appear that environmental genomics have been implemented to address these ecological objectives, many of the objectives could be addressed with applications discussed throughout UNEP-MAP (2012) report. Notably, monitoring or assessments of biological diversity, invasive species (non-indigenous species), population status of commercial species, and impact assessments of "resource extraction" would be most amenable to environmental genomics techniques.

The Convention for Cooperation in the Protection, Management and Development of the Marine and Coastal Environment of the Atlantic Coast of the West, Central and Southern Africa Region provides a legal framework for marine-related programs along approximately 14,000 km of the African coast from Mauritania to South Africa. After recommendations from UNEP, an Action Plan focused on the marine and coastal environment of West, Central and, later, Southern Africa, was designed to link assessment of the quality of the marine environment and the causes of its deterioration with activities for the management and development (Abidjan Convention, 2019). The Nairobi Convention is a partnership between governments, civil society, and the private sector, intended for sustainable development of the Western Indian Ocean Region with healthy rivers, coasts and oceans. The Contracting Parties include Comoros, France, Kenya, Madagascar, Mauritius, Mozambique, Seychelles, Somalia, Tanzania and the Republic of South Africa and the program is part of the 18 Regional Seas initiatives that is a global initiative with more than 143 countries (Nairobi

Convention, n.d.). These conventions, which are establishing and developing environmental policy, provide opportunity for the incorporation of new technologies, such as environmental genomics in assessments, baseline studies and ecosystem-based management. While the Abidjan and Nairobi Conventions are not engaged in dedicated environmental genomics initiatives, they could provide valuable existing data and/or future collaboration opportunities for comparisons with conventional sampling methods and also increase genomic reference database volumes.

3.1 International Jurisdictions

While all of the regulatory agencies consulted were aware of environmental genomics and the potential application to resource development decision-making, some were more advanced as a function of the maturity of the energy industry within each jurisdiction as well as the willingness of agencies to collaborate on technologic advances. We have classified these jurisdictions along a gradient of regulatory appetite potential of increasing to decreasing acceptance: *Genomic Affirmative; Genomic Permissive*; or *Genomic Emergent*.

3.1.1 Genomic Affirmative

We defined *Genomic Affirmative* regulators as those which actively cultivate the advancement of environmental genomic methods in regulatory decision-making for external proponents. This class of regulatory acceptance functions above and beyond the conventional approach of the majority of regulators which do not generally rely on this technology for assessment of applications by industrial proponents yet will readily utilize environmental genomics to satisfy internal mandates related to biodiversity surveys, invasive species detection, or effects assessments. The 2015 NEA environmental monitoring guidelines (NEA, 2015), do not discuss the use of DNA-based approaches other than to assess DNA damage to fish, the government does fund genomic research to determine its potential for future monitoring programs. The research into the use eDNA as a tool for environmental management in Norway includes projects that compare conventional and DNA-based approaches and build reference databases for future monitoring programs. Funding is also allocated to other genomic research and training technicians in sample collection and analysis (Aagaard, 2017). A three-year project (Metamon Project), funded by the Norwegian Research Council, Equinor and TOTAL, aims to address outstanding issues for use of metabarcoding in environmental monitoring of petroleum activities on the Norwegian Shelf. The main objectives of the study are to compare analyses of morphology- and metabarcoding-based diversity data; work towards standardization of sediment metabarcoding sampling and extraction; and assess and archive reference database gaps for COI and 18S barcodes, to guide efforts in barcoding of additional species.

Additionally, NEA commissioned NTNU Science Museum, as head of the Norwegian Barcode of Life network, to review standardization criteria for the use of DNA barcoding and environmental DNA. The report1, published in Norwegian with the English title "Criteria for depositing eDNA samples and data, including vouchered specimens", aims to ensure the necessary reproducibility when reporting data and metadata, as well as storing samples from projects where data are generated from environmental DNA analysis. While the document does not address technical requirements for the design of studies or technical details around laboratory protocols and analyzes, it involves setting requirements for:

- An agreed minimum standard for reference to and use of publicly available reference material
- A common platform for reporting and making publicly available DNA sequences and associated metadata
- Retention of collected samples, which are not analyzed immediately, over a long period of time to ensure that these are available for future research and management, regardless of the original contracting institution.

The impetus for this report was a public risk assessment which upon review lacked an eDNA-specific reporting template, documentation of reference materials, reference sequences, or a data storage facility.

From NEA's perspective, there is a long lead time on the science required to verify new technologies before use in enforcement monitoring in terms of reproducibility, detection, and so on. Some indicator species, if shown to be easily and reliably detectable with eDNA, may be considered for a paradigm shift in monitoring regulations. However, eDNA is not currently specified in regulations in Norway and there are no immediate plans to move in that direction as Norwegian regulations do not usually specify technologies that shall be used. As an example, there are ongoing discussions between NEA and Equinor to use eDNA as a supplement to traditional monitoring to add more information; however, this is not a regulatory requirement.

While environmental genomics may not be codified in Norwegian regulations, there is institutional support to develop the ability to utilize this technology. In addition to the standardization guidance report mentioned above, NEA via Innovation Norway with funding from the Norwegian Research Council recently released a pre-commercial procurement aimed at interested suppliers, research groups and users for potential pilot project development of an automatic environmental monitoring of freshwater systems using environmental DNA2. Via engagement with these stakeholders, the aim is to assess the technical possibility and viability as well as costs of such an automated solution.

Early detection, warning and modelling of dispersal of foreign organisms are also high priority issues in relation to the research requirements for the Norwegian Ministry of Climate and Environment. Additionally, the document indicates other relevant uses of such technology may include water quality monitoring as mandated by the EU's Water Framework Directive, early detection and delineation of pollution from petroleum operations and other future offshore industry, monitoring in terrestrial environments, as well as monitoring of dispersal of antimicrobial resistance and human pathogenic organisms.

3.1.2 Genomic Permissive

Genomic Permissive regulators as those which are methodologically agnostic and as a matter of principle do not specify which assessment techniques are employed by industrial proponents in support of environmental applications or compliance monitoring. Similar to the *Genomic Affirmative* class, these regulators have established funding programs to promote environmental genomics research initiatives and actively employ such tools to satisfy internal regulatory mandates. Various agencies in Australia, North America, and Europe fit this regulatory classification.

An example of the *Genomic Permissive* class of regulators is the National Offshore Petroleum Safety and Environmental Management Authority (NOPSEMA), the Australian offshore energy regulator for health and safety, environmental management, structural and well integrity for facilities and activities in Commonwealth waters (NOPSEMA, 2019). NOPSEMA regulates in Australian

Commonwealth waters and coastal waters where a state or the Northern Territory has conferred regulatory powers to NOPSEMA. NOPSEMA has an objective-based regulatory regime, meaning responsibility to manage risk effectively rests with industry proponents or title holders. In relation to environmental management, titleholders are required to put forward a case in an environment plan (EP) that demonstrates how their proposed management measures will ensure environmental impacts and risks will be acceptable and reduced to as low as reasonably practicable (ALARP). NOPSEMA assesses the cases presented in the EP and either accepts or refuses to accept the EP. In order to be accepted, NOPSEMA needs to be reasonably satisfied that the EP meets particular regulatory decision-making criteria. A NOPSEMA-accepted EP must be in place before proposed offshore petroleum and greenhouse gas activities within NOPSEMA's jurisdiction are allowed to commence.

With respect to environmental genomics, given the objective-based regulatory regime that NOPSEMA administers, no particular approach or method is prescribed. Any industry proposal, whatever the method, needs to demonstrate it is appropriate to ensure that environmental impacts and risks will be managed to an acceptable level and reduced to as low as reasonably practicable. While NOPSEMA does not prescribe approaches, the organization recognizes there are certain benefits to standardization of any methods that are used for assessments or monitoring, including environmental genomics. NOPSEMA specifically highlighted that standardization could even facilitate comparison of multiple datasets across jurisdictions. NOPSEMA representatives further indicated they have been presented and accepted cases for application of environmental genomics for monitoring invasive species primarily, mostly for platforms serviced by vessels which transit to and from busy mainland ports. NOPSEMA has also had discussions with various titleholders to examine application of environmental genomics for monitoring of produced water or other environmental impacts of activity in the offshore setting.

NOPSEMA indicated it would encourage industry to explore standards they can define for environmental genomics and noted it also adds weight to the case for use of particular approaches/methodologies if these standards are more broadly accepted, including in other jurisdictions. If research is performed on how to apply environmental genomics standardization, NOPSEMA noted they would be keen to provide some assistance in identifying the types of environmental management outcomes NOPSEMA would find valuable in a regulatory context. In their opinion, any advances that could decrease project EIA and regulatory uncertainly has potential to increase efficiency and minimize delays in approval timelines. NOPSEMA noted extended approval timelines were mainly due to uncertainties in EIA, including in how or why methods like environmental genomics are being proposed. NOPSEMA has had discussions with universities regarding their environmental genomics activities in oil & gas activities, particularly the benefits and limitations of the methodology. To date, they have been surprised by the limitations communicated by researchers and there is a perspective that perhaps industry has not always adequately communicated these limitations to regulators. From a regulatory point of view, they feel it is important for both NOPSEMA and industry to clearly understand what environmental genomics can and cannot deliver. Regulations are underpinned by concepts of ecologically sustainable development, particularly the precautionary principle, which is directly relevant to managing scientific uncertainty. Uncertainty can result in extended assessment timeframes, requests for consideration of alternative approaches to monitoring and management and applications being denied, where legislated decision-criteria are not met.

In light of any limitations identified using environmental genomics, NOPSEMA also highlighted the value in understand how one would provide industry guidance through research outputs, such as how would proponents respond to such limitations in an adaptive management approach. For

example, understanding the level of uncertainty or risk and if any exists, i.e., is there so much uncertainty that would require a proponent to also now employ another monitoring technique to inform decisions. Or alternatively, based on the findings would a proponent now require other management responsibilities to decrease uncertainty or the risk profile. The final possible outcome could be that both regulators and proponents are clear on understanding the risk limitations and they are acceptable.

NOPSEMA is open to help advise on research that either helps identify benefits and limitations of environmental genomics or at least increases understanding of the uncertainty and associated implications for attempting to manage risks based on these technologies. NOPSEMA suggested another key principle of R&D recommendations from industry associations should be that priority be given to research objectives (and where possible, outputs) being driven by end-users, including regulators. In doing so, they feel that research outputs will have a 'home' in EIA approaches that proponents are able to readily apply so there is a clear pathway to adoption. It was noted much funding has been invested by both government and industry on research that does not fit the broader needs for efficient and effective end uses, including regulatory decision-making. Despite best intentions and excellent research, outputs commonly fall short of meeting end-user needs for adoption in their experience. End-user driven approaches, where end-users have a primary role in identifying and communicating their needs to researchers, whose primary role would be to determine how best to design and implement research to address these needs would be much more efficient to achieving these ends.

In Australia, there has been regulatory acceptance and application of eDNA-based monitoring and baseline assessments relevant to the energy industry for a decade. The coastal development and management department in Australia's Commonwealth Scientific and Industrial Research Organization (CSIRO) has been using metabarcoding since 2010. The CSIRO Molecular Ecology and Toxicology Team works with industry and regulators to aid the adoption of 'omic tools', for example, updates to the water quality and sediment quality guidelines include to eDNA techniques. CSIRO commonly uses metabarcoding/metagenomics to characterize community structure and function for monitoring and assessing the impacts of anthropogenic activities in aquatic systems. Environmental genomics project examples include routine monitoring of mine run-off, including river discharge (fresh and marine) and deep-sea tailing deposits (metabarcoding); monitoring the ecological conditions of estuaries (metabarcoding/metagenomics/metabolomics); and baseline monitoring for resource exploration (metabarcoding/metagenomics/functional profiles) (Chariton, 2016).

In Canada, a growing acceptance and interest in eDNA applications exists within the federal government. In advance of broad-scale regulatory acceptance and application, the Canadian government is transitioning from funding individual studies to more cohesive R&D initiatives. Genome Canada a not-for-profit organization, funded by the Government of Canada, providing economic and social benefits for Canadians, by developing and applying genomics and genomic-based technologies. The Genomics Research and Development Initiative (GRDI) aims to assess the potential for eDNA methods and technologies for a variety of applications. This program receives approximately $20 million annually from the Government of Canada (GoC) to carry out vital genomics research. Participating GoC departments and regulatory agencies include: Agriculture and Agri-Food Canada, the Canadian Food Inspection Agency, Environment and Climate Change Canada, Fisheries and Oceans Canada, Health Canada, the National Research Council of Canada, Natural Resources Canada, and the Public Health Agency of Canada (Government of Canada, 2020). GRDI collaborates with universities and the private sector, creating economic, environmental, and social benefits for Canadians and research areas include agriculture, environment, fisheries,

forestry, and health (including developing treatments for diseases such as cancer) (Government of Canada, 2020).

Fisheries and Oceans Canada (DFO) is Canada's predominant regulator with respect to marine and aquatic habitats and a leader in the development and application of eDNA approaches. DFO recently released a technical report outlining national needs and priorities for eDNA and its applications to internal DFO Programs related to at-risk and aquatic invasive species, fisheries management, sustainable aquaculture, aquatic animal health, and sustainable aquatic ecosystems; highlighting challenges and technical considerations (Baillie et al., 2019a). Baillie et al. (2019a) identified current limitations for eDNA use within DFO as the need for high performance computing processing power, technical bioinformatics skills and software, and shared annotated databases of aquatic organisms; and listed the following key priorities moving forward: development of national guidelines for sampling, data analysis, reporting, and interpretation of results.Currently there are variety of scientific questions central to the mandates of several DFO Management programs that can be addressed using eDNA methods, such as describing connectivity among populations in marine protected areas (MPA) for the Oceans Management Program or characterizing biodiversity to assess impacts of aquaculture. Baillie et al. (2019b) outlined the following research needs that would lead towards providing science advice to management and client mandates:

- Guidance for sampling protocols and reporting
- Overcome species survey limitations (e.g., under ice in Arctic, deep-water species mortality)
- Surveillance of aquatic invasive species, disease/pathogens, harmful algal blooms
- Develop national-scale monitoring for MPAs, arctic shipping routes, at-risk species, fisheries and aquaculture
- Develop bioremediation technologies to consume and break down environmental pollutants
- Support for regulatory decisions for possession of prohibited species (by-catch/illegal trade)

DFO considers the next steps towards regulatory acceptance are to scale-up fundamental scientific research; move towards common Guidelines and Reporting Standards, both federally and provincially; and build momentum to plan for future informatics capacity. Baillie et al. (2019b) further discussed current projects funded by GRDI program and how they will lead to a better understanding of current limitations and best practices: modelling of eDNA dispersal and persistence in aquatic ecosystems; quantitative eDNA to estimate fish biomass through comparison with traditional multi-species stock assessment surveys (e.g., acoustic surveys and/or trawl transects) and massively parallel DNA sequencing; eDNA-based methods for detection and monitoring of rare, data-poor species in regions which are candidates for MPA designation. In a separate research initiative, DFO's national Centre for Offshore Oil, Gas and Energy Research (COOGER) is developing new sensitive, cost-effective, rapid assays. These assays will be used for bioremediation and bioaugmentation to help restore and monitor ecological health (DFO, 2018a).Abbott et al. (2019) suggests eDNA metabarcoding for broad spatial scale multi-species surveys but has a slow turn-around time from sampling to data availability. These authors suggest targeted eDNA qPCR for single species surveys as it has a fast turn-around time and small spatial scales. Finally, HTS of targeted eDNA amplicons is viewed as most suitable for few / single species and also has a slow turn-around time with large spatial scale sapling programs. Other recent research is focused on resolving challenges for regulatory implementation of DNA metabarcoding for downstream monitoring of wetlands in relation to oil sands operations(Monk et al., 2018); establishing a genomics reference library of western cod stocks to serve as baseline data, supporting management decisions of Atlantic Cod fisheries (DFO, 2018b); assessment of

handheld portable eDNA detection devices for detection of the invasive zebra mussel (DFO, 2018c); and toxicogenomic solutions for assessing exposure and effects of environmental contaminants in wildlife by characterizing mixtures from the Great Lakes and oil sands areas and contributing to screening assessment reports for evaluating toxic chemicals and their adverse outcome pathways (Government of Canada, 2019).

Environmental protection of the Northeast Atlantic is regulated under OSPAR, which includes the United Kingdom. In addition, the United Kingdom Offshore Petroleum Regulator for Environment and Decommissioning (OPRED) is responsible for regulating offshore energy industries to ensure sustainable development (Kennedy, n.d.). OPRED provides guidance to operators and enforce compliance with regulatory requirements and OSPAR recommendations during environmental assessment and monitoring. Discussions with the OPRED Environmental Management Team clarified the current and potential role of environmental genomics in UK offshore production monitoring and assessment. Any environmental monitoring method used to provide data to support an EIA application must provide a basis for assessing the degree of potential impacts while understanding any potential data limitations. As practice, OPRED does not stipulate what environmental monitoring methods should be undertaken in support of EIA applications, but methods must be relevant to what is being assessed and an application is considered on a case by case basis, considering the quality of and limitations of data used to support such an application. As things currently stand, OPRED has no intention of developing guidance on the use of environmental genomics or encouraging proponents in its use.

OPRED noted that the regulatory community is broadly aware that the development and application of environmental genomics technology has started to accelerate in the last few years as a potential option. OPRED understands the potential benefit of environmental genomics in lowering costs and reducing sampling processing times, for example, relative to use of traditional taxonomists in benthic ecology assessments. OPRED noted that the method could be beneficial in surveys targeting a particular species of conservation interest. For instance, it was highlighted that during the review process, consultees can have concerns that the selected monitoring methods may not have been the most suitable for sampling a species of conservation interest (e.g., underwater video transects failing to detect burrowing bivalves). There is therefore potential for environmental genomics to supplement traditional monitoring methods, such as video surveys under the proper circumstances to reduce such data shortfalls. OPRED also highlighted potential benefits of environmental genomics in mitigating impacts to designated conservation species that can be affected by or are difficult to sample with traditional survey methods. OPRED noted that within UK waters and throughout Europe, there are marine protected areas designated due to presence of a particular habitat type (e.g. coral reef or sandbanks) and the technology may be beneficial for some of these areas. Potential benefits aside, OPRED current understanding is that further technological development in the areas of reference libraries and ability to quantify organisms are required in order for OPRED and regulators in general, to become more comfortable with using environmental genomics data in regulatory decision making. It was also noted that the technology did not give data such as number of individuals or biomass present. Depending on the type and quality of data provided as the technology advances, OPRED anticipates there may be an opportunity and increasing interest for regulatory use in the next five to 10 years.

The European Cooperation in Science and Technology Action CA15219 on "Developing new genetic tools for bioassessment of aquatic ecosystems in Europe" - DNAqua-Net - is developing approaches to implement eDNA techniques for monitoring and environment protection with the goal is to advance the application of DNA-based tools for biodiversity assessments and develop a roadmap to

include these in standardized ecological assessments of aquatic ecosystems in Europe and beyond (Duncan 2018). Other European groups and regulators involved in the development of DNA methods and/or acceptance into legislation include the European Committee for Standardization (CEN), the United Kingdom (UK) DNA Working Group, National Environment Research Council Environmental Omics Research Program (NERC OMICS) strategy, and the Central Government Council of Europe (CoE). Although DNA-based assessments have not yet been accepted into EU/UK legislation, contributions from these groups and other research may lead the way.

The European Union (EU) Water Framework Directive (WFD) is a directive which commits EU member states to achieve good qualitative and quantitative status of all water bodies (including marine waters up to one nautical mile from shore). Ecological and chemical status of water bodies are assessed based on biological quality, hydromorphological quality, physical-chemical quality, and chemical quality of specific pollutants (i.e., concentrations of specific pollutants). Fish-based assessment methods of European rivers are generally multi-metric indices computed from traditional electrofishing (TEF) samples. Pont et al. (2019) reviewed 25 current WFD-compliant methods for river fish and found that 81% of the metrics used, measure richness or relative abundance and are therefore compatible with eDNA samples. However, metrics related to age or size structure are also used by more than half of member states, which is not measurable with eDNA sampling. eDNA methods produce higher estimations of richness, with comparable results when TEF effort is strongly increased. Due to limitations associated with TEF, relative abundance differences between the two methods vary and Pont et al. (2019) provide a series of recommendations for incorporation of eDNA sampling into the EU WFD fish assessment methods.

The United States *National Environmental Policy Act* (NEPA) promotes the enhancement of the environment and requires federal and independent agencies, such as Bureau of Ocean Energy Management (BOEM) and the Environmental Protection Agency (EPA), among others, to manage national resources and assess environmental effects of proposed projects. Under the NEPA, BOEM is responsible for the management of Outer Continental Shelf (OCS) energy industry resources and is required to evaluate potential impacts that may be associated with energy industry activities on the OCS. The Environmental Studies Program (ESP) is part of this process and enables BOEM to meet the nation's environmental research needs for Outer Continental Shelf (OCS) resource assessments (BOEM, n.d.). Several of these studies aim to utilize or optimize a variety of new technologies, including the use of eDNA analyses for species monitoring. For example, an ongoing study 2023 involves the use of eDNA for the early detection of marine invasive species in Cook Inlet, Alaska. The project's aim is to create a baseline record of planktonic, benthic, and attached organism communities near areas of current and potential future energy industry installations in Cook Inlet. This project will provide a benchmark comparison for the early detection of invasive species and the associated response and mitigation planning for containment and eradication. Planned methods include the use of settlement devices, plankton tows, substrate scrapes, and collection of open water eDNA to create a robust inventory across key substrate types. Results from this study will inform analyses under the NEPA for future lease sales and potential development of mitigation measures (BOEM, 2020). Another ESP study, titled "Facilitating Strategic Partnerships in Support of the Presidential Memo on Ocean Mapping, Exploration, and Characterization" will use a variety of interdisciplinary methods to characterize high-value deep-water habitats, including the use of eDNA for biodiversity and species distribution studies.

The United States Environmental Protection Agency (EPA) have utilized DNA-based methods for bioassessment surveys of invasive species in rivers and streams. One example of DNA-based studies informing policy and management started with a proposal to close the Chicago Sanitary

and Shipping Canal. The proposal requested the separation of the Great Lakes and Mississippi River basin due to detections of the invasive Asian carp in eDNA samples collected beyond the barrier constructed to prevent dispersal into Lake Michigan (Darling and Mahon, 2011). Despite the rejection of the proposal, the results from eDNA sampling have led to increased monitoring, demonstrating application of DNA-based methods to inform policy and environmental management in the United States. DNA Metabarcoding Surveys have also been incorporated into EPA National Assessment Programs Pilgrim (2019). The National Aquatic Resource Surveys (NARS) are collaborative programs between EPA, states, and tribes which assess the quality of United States wetlands (National Wetlands Condition Assessment [NWCA]), rivers and streams (National Rivers & Streams Assessment [NRSA]), lakes and reservoirs (National Lakes Assessment [NLA]), and coastal waters (National Coastal Condition Assessment [NCCA]).

The Environmental Security Technology Certification Program (ESTCP) is the Department of Defense's (DoD's) environmental technology demonstration and validation program. Lance et al. (2019) state the objectives listed for the ESTCP project, "Engaging a Crowd-Sourced eDNA database to enhance DoD-relevant conservation goals" were:

- Develop a framework for crowd-sourced, inter-agency eDNA sampling and data archiving
- Develop targeted eDNA analysis protocols for key taxa of concern to Southwest United States DoD installations and an efficient, multi-taxa method for parallel analysis of samples
- Test the utility of eDNA sampling for detection of terrestrial species, including birds and mammals
- Develop multi-taxa tool and protocol for parallel analysis of eDNA samples with existing qPCR assays

To reach these objectives the project aims to use eDNAtlas, a public, crowd-sourced database, which will promote collaborative sampling and archiving between other partners who manage the land and the installations, collecting samples. The eDNAtlas has an interactive ArcGIS Online (AGOL) map that allows users to view and download sample site information and lab results of species occurrence for the United States (USDA-U.S. Forest Service, n.d.). eDNAtlas is a convenient tool as each eDNA sampling location is tied to the National Hydrography Dataset (US Geological Survey), allowing users to also access environmental data from the National Hydrography Dataset and evaluate potential drivers of species distributions across the landscape. With >8,000 samples collected by dozens of Federal, state, tribal, academic, non-profit, and industry partners including Stantec, the DoD plan to build on this data. They hope to partner with other organizations, such as U.S. Forest Service, Bureau of Land Management, National Park Service, U.S. Fish and Wildlife Service, tribal governments, state agencies, and non-profit organizations, to collect samples from focal installations in southwest US, targeting high-priority/interest taxa, such as rare/cryptic, invasive, pathogenic, and terrestrial/ riparian species (Lance et al., 2019). The second iteration of the US National Conference on Marine eDNA is specifically themed on research science and regulatory uptake, planned in mid-2021. The anticipated outputs of this conference and expert workshop is a series of manuscripts identifying knowledge gaps and action plans to further transition environmental genomics into the regulatory realm. The outcomes of the first iteration of the US National Conference on Marine eDNA laid the groundwork for this planned regulatory engagement. The participants desire to initiate dialogue on the impact of marine eDNA becoming established as a credible ecosystem census indicator on existing statutes, regulations, and permitting/licensing processes. Examples include NEPA, Magnuson-Stevens Fishery Conservation and Management Act, Marine Mammal Protection Act, Endangered Species Act, and others. As marine eDNA-based analyses become increasingly available to regulators and courts, the participants deem it prudent to

begin examining how policy laws may need to be updated to accommodate this technology. Desired outcomes are establishment of eDNA standards and accreditation of reference assets. As the use of marine eDNA accelerates, stakeholders must determine priorities for collection and analysis, sector by sector, and collectively.

3.1.3 Genomic Emergent

Genomic Emergent regulators are those which have had relatively little or no previous experience with industrial permitting requests proposing genomic tools but are generally receptive to these approaches, particularly for geographies or ecological groups with are data poor or previously unstudied.

In Brazil, energy industry exploration/production Environmental Impact Assessment Studies (EIA) and Environmental Impact Reports (RIMA) are reviewed by the Brazilian Institute of the Environment and Renewable Natural Resources (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis [IBAMA]) (Petrobras 2020). IBAMA is linked to the Ministry of the Environment as an agency of the federal government and is responsible for issuing environmental licenses, controlling the quality of the environment and overseeing the use of natural resources.

Based on discussions with IBAMA representatives in 2020, to-date only one project involving environmental genomics had been proposed for a permit application for energy industry drilling in a specific region. IBAMA indicated to the proponent that they should utilize metabarcoding to characterize a large marine area containing coral reef fauna, deepwater corals, and other sensitive benthic habitats. IBAMA did not make any recommendations on specific genomic methodology. IBAMA anticipates this environmental characterization will commence after they have reviewed and approved the study design to be submitted by the proponent. While no proponents have proposed environmental genomics approaches for permit applications related to seismic activity or production activities, IBAMA remains open to further use of the technology. An example was provided of another new project under assessment, which is intended to initiate baseline monitoring of microfauna. While there is no planned use of genomics, this project was presented as an example of where IBAMA could see utility of the approach. Beyond assessing effects from drilling and disposal of cuttings, IBAMA representatives were also interested in the potential for the use of environmental genomics in biodiversity studies related to IBAMA internal requirements. While IBAMA representatives recognized the potential benefits of this technology in fulfilling their mandate in the near term, they were ultimately concerned with understanding: 1) why this method should be chosen over others; 2) if chosen, when it should be used; and, 3) what to expect in terms of results outputs as well as limitations of the technology. Ultimately, they concluded that while they have in-house technical expertise, familiarization training for IBAMA staff on the above topics would be required in order to facilitate broader acceptance of environmental genomics in their regulatory decision-making. Moving forward, IBAMA is open to advancing discussions on the topic of methodology standardization via the existing IOPER working group mechanism as well as project-specific collaboration with potential industry and academic collaborators.

The Environmental Protection Agency (EPA) of Guyana , and specifically their Oil and Gas Unit, is responsible for authorizing, monitoring, and managing projects in the energy industry sector3. Discussions with EPA representatives indicated they had recently become aware of eDNA use in regulatory applications after attending a professional conference. Since that time, they have been primarily interested on learning more about the use of technology in other jurisdictions and how it could be applied for their purposes in Guyana. Specifically, they have been trying to also promote

the idea of inter-agency collaboration in developing this capacity for Guyana and their varying departments which manage marine resources. While there are no current environmental genomics-based regulatory surveys planned in Guyana, the representatives indicated they see value for its use in baseline survey validations. In particular, regulators generally only accept what has been actually observed and they feel actual biodiversity is being underreported by traditional survey techniques and thereby undermining management objectives. Their impression is that environmental genomics could complement those traditional surveys to help close those biodiversity gaps as very few comprehensive biodiversity studies have yet to be conducted in their jurisdiction. Another area of potential genomics application in their jurisdiction includes invasive species detection. Prior to exploring the issue further, they feel that their agency requires a broad introduction to environmental genomics, focused on topics such as sampling techniques, costs, timelines, and data outputs. They each expressed interest in capacity-building via workshops or similar interactive sessions to best understand the technology and how to interpret data.

In general, there is persistent and growing optimism for environmental genomics to advance concepts in biodiversity and ecosystem structure and function based upon a series of initial successes in moving beyond the academic sphere towards practical application within industry. As described above, limitations remain which are barriers to regulatory uptake, resulting in constrained application for supporting permitting approvals. Evaluation, prioritization and developing means of addressing these knowledge, understanding and methodology gaps are key to countering regulator uncertainty and reluctance to employ environmental genomics in decision-making processes.

4. Opportunities & Needs

The literature review and consultation identified key areas in environmental genomics requiring further development to increase the uptake and acceptance of environmental genomics in the energy industry. Five cross-cutting knowledge gaps that were relevant to many/all industry applications were highlighted.

1) Understanding environmental eDNA persistence and dispersal
2) Large-scale integration of eDNA data with different data types
3) Improvement of reference library databases
4) Molecular refinement of taxonomic indices
5) Standardization

Understanding Environmental eDNA Persistence and Dispersal

eDNA is only a proxy for the detection of a species at a specific time or location (Hinz et al. 2022). The relationship between the detection of DNA in a sample and the presence or absence of the source species is dependent upon DNA persistence and dispersal. Understanding this relationship, is, therefore, critical to the reliance on eDNA data for industry applications, some of which may need to stand up to regulatory or legal scrutiny.

Despite many significant advancements recently in our understanding of DNA persistence and dispersal in the environment (Jo et al., 2025; Zanni et al., 2025), many gaps remain, especially for frontier zones which are of focus for the energy industry such as deep sea habitats (Coté et al. 2019), and also including commonly encountered freshwater (Laporte et al., 2020) and terrestrial habitats. While there is a need to increase understanding of eDNA persistence and dispersal in a variety of environments to confidence in data, these processes are often region- or even site-specific. Therefore, considerations of DNA persistence and dispersal and their potential impact on the reliability of data, given study objectives, should be addressed in every sampling design.

Large-Scale Integration of eDNA Data with Different Data Types

Biodiversity data is collected routinely by the energy industry for a number of purposes using a range of sampling methods from observations and collections of specimens to remote sensing data, and often is summarized in terms of biological indices to track changes through time. eDNA methods produce a comprehensive assessment of assemblages, but often detect a somewhat different component to conventional methods (e.g. Alexander et al., 2022, Gibb et al., 2025). Being able to integrate eDNA data with data collected using other methods is paramount to both industry, objectives (being able to track changes through time despite different methods) and regulatory uptake (providing data that is familiar to regulators, such as in the form of biological indices).

Improvement of Reference Library Databases

The accuracy and taxonomic resolution of eDNA studies is highly dependent on the availability of reference sequences. Despite significant efforts globally, available reference databases still require substantial input from a variety of sample types (i.e., water, soil sediment, permafrost or air) and

geographical locations around the world, to improve the success of DNA-based biodiversity studies (McGee et al. 2019, Porter and Hajibabaei, 2018; Pawlowski et al., 2018; Cowart et al., 2015; Taberlet et al., 2012a). There may be opportunities for the energy industry to collaborate on reference library enhancements. Additionally, the energy industry operates and undertakes sampling in a range of environments and geographical locations, providing opportunities to collect reference material when sampling for other purposes. Industry adoption of a simple, common procedure for collection, storage, shipping and analysis of sample material may help facilitate this opportunistic reference gap filling.

Molecular Refinement of Taxonomic Indices

Ecosystem condition status (e.g. the level to which an ecosystem is impacted by human activities) is often derived from biotic index values, a concept widely used and understood by industry and regulators. Pawloski et al. (2018) suggest that, in the short term, environmental genomics data could be integrated into existing biotic indices or could supplement conventional monitoring in providing index values. However, environmental genomics goes beyond the detection of a few select species; it can be used to provide holistic data across an entire assemblage, from microbes to vertebrates, providing a potentially more powerful indicator of ecosystem condition (Suren et al., 2024; Cook et al., 2025). Additionally, indices derived from taxonomy-free sequence data are beginning to be used to provide a rapid and powerful assessment of ecosystem condition (e.g. Wilkinson et al. 2024). The use of a taxonomy-free approaches completely bypasses the issues, limitations and time associated with the taxonomic assignment of the sequences. A further advancement is the use of machine learning algorithms to look for patterns in sequence data and derive index values of ecosystem condition (Cordier et al., 2018, Pawlowski et al 2024). Environmental genomics data can also facilitate co-occurrence network analysis or holistic assessment of trophic interactions, both of which add evidence to assignment of ecosystem condition (Bush et al., 2019).

While assessment of ecosystem condition using environmental genomics methods appears promising, and techniques are advancing rapidly, conventional biotic indices and monitoring approaches have been employed by industry practitioners and regulators for decades. The energy industry can accelerate the uptake of environmental genomics methods by providing side-by-side demonstrations of the efficacy environmental genomic methods compared to conventional biotic indices in a range of habitats and locations. However, it may take time for regulators to accept environmental genomic methods that fully decouple from taxonomic-based monitoring.

Standardization

The energy industry relies on the accuracy and validity of biodiversity data to inform management decisions. Standardization of environmental genomics workflows is needed for reliable, comparable, and ecologically meaningful data (Harper et al., 2019; McGee et al., 2019). International guidance vetted by multiple regulatory jurisdictions would be ideal. Continued development of scalable laboratory methods and global efforts to achieve standardization are crucial (Porter and Hajibabaei, 2018; Leese et al., 2016). The energy industry, including the JIP, can and is playing a part to help with standardization through development of guidelines and minimum standards for environmental genomics, which can be found at https://www.iogp-edna.org/publications/.

5. Conclusion

There is growing optimism for environmental genomics to improve the speed, scale and comprehensiveness of biodiversity and ecosystem studies. Within the energy industry, initial successes have been achieved in the application of environmental genomics, but progress has been fragmented across jurisdictions and specific industry concerns, limiting technological advancement and regulatory visibility. Competing groups have independently attempted to develop environmental genomics standards, leading to some redundancy of efforts.

Significant progress has been made in the energy sector through academic-industry-regulatory networking, highlighting the need for best management practices to build confidence in environmental genomics. Consultations with regulators and scientists has identified knowledge gaps in reference libraries and methodological developments, and the need for standardized guidance on sampling, analysis, data reporting and interpretation.

Regulatory agencies are aware of environmental genomics and its potential applications, but varying levels of advancement exist. Collaborative efforts, such as those in Norway and Australia, demonstrate the benefits of engaging in research to advance decision-making. Standardization initiatives are crucial for building confidence and reliability in environmental genomics data, reducing duplication of effort, and promoting efficient use of resources.

If accepted by regulators, environmental genomics has the potential to augment or replace traditional monitoring methods within the energy industry. While some jurisdictions are more advanced in industry uptake and regulatory acceptance of environmental genomics methods, there is a widespread desire for standardization of approaches in order to increase confidence in environmental genomics data. There is considerable appetite and capacity among the scientific community, industry and regulators to engage in collaborative research initiatives, which would help bridge gaps and advance uptake and acceptance. By collaboratively addressing knowledge gaps and achieving alignment on approaches, environmental genomics can become a valuable tool for environmental management and decision-making in the energy sector.

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ENVIRONMENTAL GENOMICS RESEARCH PROGRAMME

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