

Enabling the use of Environmental DNA for Regulatory Policymaking in the Marine Industrial Revolution

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Abstract

New industrial activities are exacerbating anthropogenic impacts on the marine environment and imposing an unprecedented combination of stressors on ocean ecosystems. The classical methodologies for monitoring impacts on marine life, based on morphological species identification, cannot keep pace with the urgent need for more detailed information required to monitor biodiversity changes, and to promptly deliver easily-understandable data to time and budget-constrained environmental compliance and enforcement practitioners. This is particularly true for small-sized organisms that are hard to identify and that dominate in some environments, such as deep sea. Knowledge limitations affect not only the ability to remediate environmental degradation, but also the lack of adequate understanding of how the cumulative impact of human activities at sea is affecting the provision of the ocean's many ecosystem services that are fundamental for the sustainability of our planet. High-throughput sequencing (HTS) technologies applied to environmental DNA (eDNA) holds great potential to confront these knowledge gaps, although their use in marine environmental impact assessments (EIAs) is still limited.

The project *Monitoring Marine Biodiversity in Genomic Era*, funded by the Swiss Network for International Studies (SNIS), has explored the potential of the eDNA HTS approach, so called eDNA metabarcoding, for assessing the health of marine ecosystems and the impact of major human actions on them. The project concludes that 1) emerging eDNA-based technologies should be deployed alongside traditional morphological species identification techniques to provide relevant information to ocean resources managers, and environmental compliance and enforcement practitioners; 2) there are no significant legal constraints to its use; and 3) its decreasing costs open new opportunities to enhance human knowledge of complex marine ecosystems and the effects of cumulative impacts on them in both coastal and deep-ocean settings. However, the project also highlights the fact that more research is needed to improve interpretation of eDNA data for impact assessment. Particular concerns include the needs to ground truth species identities and interactions, infer abundance data and develop molecular indices of ecological status. Major challenges to overcome in eDNA data analysis are associated with signal persistence over time and in non-living material, as well as the transportation of eDNA in water over long distances.

Table of contents:

1. Introduction
2. Current challenges to comprehensive ocean resources management and conservation (*Why do we need eDNA?*)
3. Identifying avenues to enable the use of eDNA for monitoring impacts on the marine environment (*What can we do with it?*)
 - 3.1. Biodiversity surveys
 - 3.2. Species detection
 - 3.3. Ecogenomic indices
4. Promises and challenges of eDNA technology
 - 4.1. Economic and scientific tradeoffs
 - 4.2. Technical challenges
5. Regulatory and legal framework
6. Next steps
7. Conclusions
8. References

1. Introduction (*What it is about?*)

Environmental DNA (eDNA) is the sum of all DNA molecules isolated from water, sediments or any other substrate. It is released to the environment from living and decaying unicellular and small-sized multicellular organisms, but also from tissue debris, mucus or other traces of larger-sized species (Torti et al. 2015). As a result, the eDNA signal includes the dead as well as the living organisms that occupied the sampled environment, and the sequencing of eDNA at high-throughput allows the identification of these extant and former inhabitants (Corinaldesi et al. 2018, Lejzerowicz et al. 2014). Although eDNA is currently used mostly for determining the presence or absence of certain species (e.g. invasive or endangered species), its potential applications for global biodiversity surveys are very significant (Thompson et al., 2017). Multiple studies have used high-throughput sequencing of eDNA to explore the microbial diversity in the oceans, especially in the surface waters (e.g. Tara Oceans, de Vargas et al. 2015) but also at the seafloor (Pawlowski et al. 2011, Foster et al. 2016; Schulse et al. 2017). Recently, the technology is also increasingly being used to assess the environmental impact of human activities at sea, such as open water aquaculture (Pawlowski et al. 2014, Pochon et al. 2015), and oil and gas exploitation (Lanzen et al. 2016, Laroche et al. 2017, 2018). The high seas and deep seas, mostly in international waters, offer another realm for consideration.

The vastness of the ocean and the difficulty of detecting unlawful activities have acted as major barriers to the effective management of ocean resources and the sustainable conservation of the increasingly fragile global marine commons. In this context, it is especially relevant to identify technologies that allow human activities to be monitored at sea, and to do so with enough reliability to be able to inform and influence policy and regulatory decisions. These decisions may include the issuing of permits for projects or activities, establishing criteria for assessing the potential impact of new activities on the environment, monitoring the impact of currently existing activities, providing information on the positive effects of conservation measures (e.g. monitoring the recovery of ecological functions after the declaration of a new protected area), and ensuring the adequate implementation of basic principles of natural resources management, such as adopting adaptive management strategies and addressing the cumulative impact of present and former human activities on the health of the ocean.

Environmental genomics constitute a promising, timely, and practical tool for policymaking, especially since DNA sequencing costs have decreased very substantially in the last decade, with different technologies being optimized and combined. The dominant environmental genomics approach is based on the enrichment and sequencing of taxonomic gene markers (metabarcoding) from environmental DNA and RNA. Here, we review possible ways in which these methods can be used in marine impact studies, how they might fit into existing regulatory and legal frameworks, and the steps necessary to develop these approaches further. Other omics approaches not discussed in this review include less-biased, direct sequencing of total eDNA (metagenomics) or total eRNA (metatranscriptomics), as well as the analysis of proteins (metaproteomics) or metabolites (metabolomics). Together, these rapidly-advancing fields provide rich functional information that regulatory agencies can use to better assess the impact of human activities on marine biodiversity.

2. Current challenges to comprehensive ocean resources management and conservation *(Why do we need eDNA?)*

The fast growth of offshore industrial activities constitutes an unprecedented opportunity for economic development while at the same time posing a threat to the integrity of ocean ecosystems (Mengerink et al. 2014). This exceptional situation has been labeled a “Marine Industrial Revolution” (see e.g. <http://seagrant.gso.uri.edu/marine-industrial-revolution-human-impacts-on-the-ocean/> or “Industrialization of the Deep Ocean” and can only be confronted through the development of effective assessment methods and regulatory tools. In this context, the implementation of adaptive management and the accurate appraisal of cumulative impacts is paramount to sustainability. These two actions can only be effectively conducted by significantly raising the level of information available and the rate of updating and refinement of that information, which must also be comparable across management agencies and national jurisdictions. Traditional methodologies do not adequately fill this need and, in addition, the regulatory frameworks often fail to adequately reflect the specific requirements needed to ensure that the ocean’s basic ecological functions are secured. Overall, regulatory protections for the oceans have not kept pace with the potential for impacts and ocean management decisions are usually based on limited data. This predicament, combined with the ever-expanding human exploitation of the seas, has resulted in a situation in which our lack of knowledge limits our capacity to understand the current health status of marine biodiversity and how new activities conducted at sea are affecting key strategic elements for life on Earth, such as the provision of marine ecosystem services. With the increasing number and complexity of human activities at sea comes a higher risk of environmental disaster. Cases of the use and abuse of marine living resources by transnational criminal networks, pollution from the Deepwater Horizon oil spill (2010), or the sinking of the Sanchi tanker (2018), prove that technological progress and enhanced capacity to reach previously inaccessible marine areas constitute a tangible threat to marine life. In addition, the “shifting baselines effect” (Knowlton and Jackson, 2008) severely limits our understanding of what a healthy ocean ecosystem should look like, and negatively influences the views of ocean managers on how healthy is “healthy enough” in the context of the ocean ecosystems.

There is an urgent need to develop assessment tools that can provide baseline information and rapidly identify environmental change to managers, policymakers, and enforcement officers. Only through ample and accessible scientific information, and a set of legal instruments that adequately capture that influx of scientific knowledge, will environmental protection frameworks and institutions be able to exercise meaningful oversight of human activities in marine spaces.

3. Identifying avenues to enable the use of eDNA for monitoring impacts on the marine environment (*What can we do with eDNA?*)

In recent years, progress in eDNA-based technologies has enabled its wider use in biomonitoring and bioassessment (Bohmann et al. 2014, Valentini et al. 2016, Taberlet et al. 2018). The three main types of eDNA applications are (1) single-species detection, (2) biodiversity surveys, and (3) biotic indices. These applications differ according to the type of technology necessary (PCR for species detection, HTS metabarcoding for biodiversity surveys and bioindication), and the methods used to analyse the resulting molecular data.

Here, we briefly review eDNA studies that have been applied to the assessment of marine industry impacts, focusing on those that have been developed in the present project.

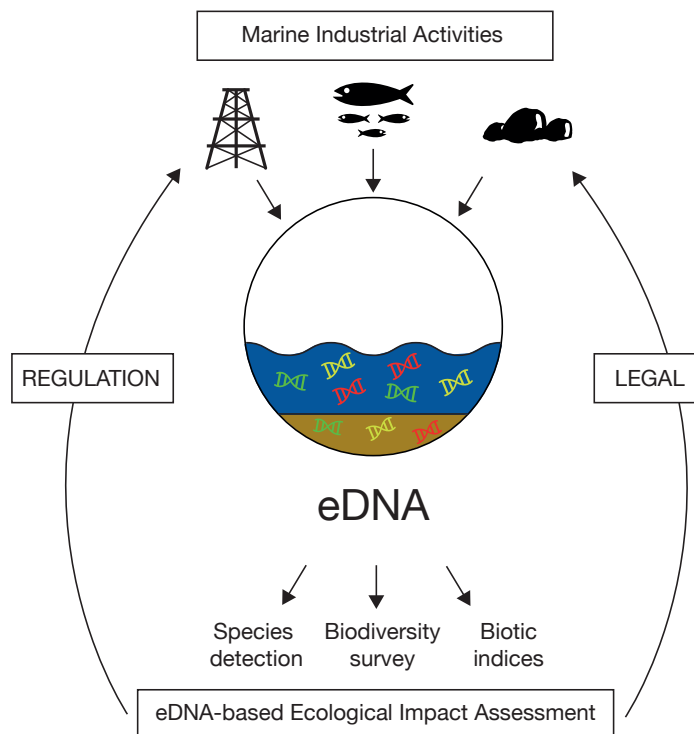


Fig.1. Potential applications of eDNA for Environmental Impact Assessment of marine industry

3.1. Species detection

Single-species detection is one of the most straightforward ways to use the eDNA for biodiversity monitoring. The approach is based on PCR amplification and uses species-specific primers mainly to detect rare, endangered, or invasive species in aquatic environments. The method is widely used to target freshwater invasive or threatened species of fish, amphibians and

some aquatic invertebrates (e.g. zebra mussels or crayfish), using their DNA traces present in environmental samples. The use of eDNA for the detection of marine species is less common, and its application to the assessment of marine industry impacts is relatively limited. Nevertheless, the number of studies using eDNA to detect the occurrence of alien or harmful species that are related to industrial activities, such as invasive species transported in ballast water or parasites infesting fish farms, is rapidly growing.

3.2. Biodiversity surveys

Compared to eDNA-based single-species detection, the high-throughput sequencing (HTS) of eDNA samples provides information about the whole community of species targeted by PCR amplification. This approach, also called **eDNA metabarcoding**, is widely used in studies of marine biodiversity, especially to explore microbial diversity and gather information about the biogeography and ecology of planktonic (de Vargas et al. 2015, Logares et al. 2014, Massana et al. 2015) and benthic (Lecroq et al. 2011, Forster et al. 2016) microbes. Metabarcoding is also used to explore the diversity of marine meio- and macrofauna (Fonseca et al. 2010, 2014), revealing immense gaps in our taxonomic knowledge of some communities, such as deep-sea benthos (Sinniger et al. 2016).

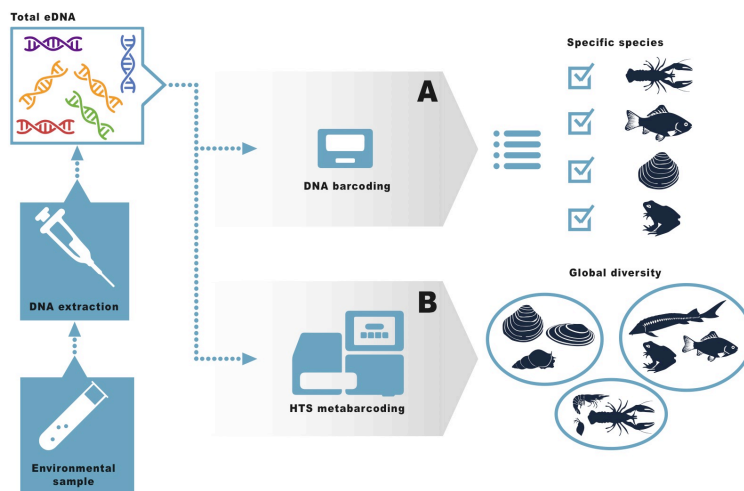


Fig. 2. Use of eDNA for single species detection (A) or biodiversity surveys (B) (courtesy L. Apothéloz-Perret-Gentil)

During recent years, metabarcoding of marine biodiversity has also been used increasingly for the assessment of anthropogenic impacts. Several studies use metabarcoding data for the monitoring of various marine ecosystems, including the estuaries (Chariton et al. 2010, 2015; Lallias et al. 2014), deep-sea canyons (Guardiola et al. 2015), and tropical seas (Stat et al. 2017). Most of these studies target microbial prokaryotes and eukaryotes, but many metabarcoding studies analyse the diversity of meiofauna (e.g. nematodes, Dell'Anno et al. 2015, Avo et al. 2017), macrobenthos (Cewart et al. 2015, Lobo et al. 2017) as well as marine vertebrates (Foote et al. 2012, Thomsen et al. 2012, 2016, Bakker et al. 2017).

Metabarcoding has been applied in marine industries principally to assess the impact of finfish aquaculture and offshore oil and gas platforms. In the first case, the impact of organic enrichment associated with the activities of salmon farms has been analysed by targeting benthic communities of bacteria (Dowle et al. 2015, Stoeck et al. 2018a), protists (Pawlowski et al. 2014, 2016, Pochon et al. 2015, Stoeck et al. 2018b), and metazoans (Lejzerowicz et al. 2015). In the case of the oil and gas industry, the metabarcoding approach has been used to assess the impact of oil spills (e.g. Deepwater Horizon oil spill, Bik et al. 2012; Smith et al. 2015, Xie et al. 2018) as well as other type of pollution related to oil and gas drilling and production activities (Lanzen et al. 2016, Laroche et al. 2016, 2017).

In this project, we focused on the baseline study of benthic diversity in an area of possible future deep-seabed mining. We selected the Clarion-Clipperton Zone (CCZ) in Pacific where polymetallic-nodule mining may present challenges for the sustainable exploitation of the deep seafloor (REF). We analysed metabarcoding data from 316 samples collected in the CCZ as well as sites in the NW Pacific, Atlantic and Southern Ocean. Our results revealed very different assemblages of benthic foraminifera and meiofauna in different ocean basins, suggesting global-scale patterns in distribution of small-sized organisms. However, at local scales, the benthic community is relatively homogenous with high variability between sampling sites due to the micro-patchiness in distribution of benthic organisms (Lejzerowicz et al. in prep).

3.3. Ecogenomic indices

Another potential application of eDNA in routine EIAs of marine industries is related to the development of eco-genomic indices. Currently used indices (e.g. AMBI, BENTIX, NSI) provide the assessment of sea-bottom ecological status based on the taxonomic composition and abundance of manually sorted and morphologically identified benthic macrofaunal species, which are assigned to particular ecological groups.

Several recent studies proposed the development of benthic indices based on metabarcoding data. These studies used various molecular markers that target either the DNA of zoobenthos, in which case sequences could be assigned to a reference database (Lejzerowicz et al. 2015), or the DNA of different groups of microbial organisms, such as bacteria (Stoeck et al. 2018a), ciliates (Stoeck et al. 2018b) or foraminifera (Pawlowski et al. 2014, 2016). The results of these studies show relatively good congruence between conventional and molecular indices in the case of marine aquaculture (Pawlowski et al. 2014, 2016, Lejzerowicz et al. 2015, Pochon et al. 2015) and the offshore oil and gas industry (Lanzen et al. 2016, Laroche et al. 2018). However, several issues appeared in relation to the limited reference database and different biases related to the taxonomic assignment of sequences.

In this project, we proposed a new solution to overcome these limitations. We used an innovative taxonomy-free approach to compute biotic indices without taxonomic assignment of sequences. This approach was first introduced by our group in the case of benthic diatoms used to assess the ecological status of rivers and streams (Apothéloz-Perret-Gentil et al. 2017). Further development of this approach involved the use of Supervised Machine Learning (SML) algorithms to predict values of benthic indices in the case of fish farming (Cordier et al. 2017).

The application of SML methods allow the development of predictive models, using different molecular markers for various taxonomic groups, including bacteria, protists and metazoans (Cordier et al. 2018). Our studies show a high correlation between conventional and molecular indices. Following these results, the new approach to predict benthic indices based on SML analysis of eDNA metabarcoding data has been proposed as a replacement or a complement for the routine assessment of impacts associated with salmon farming.

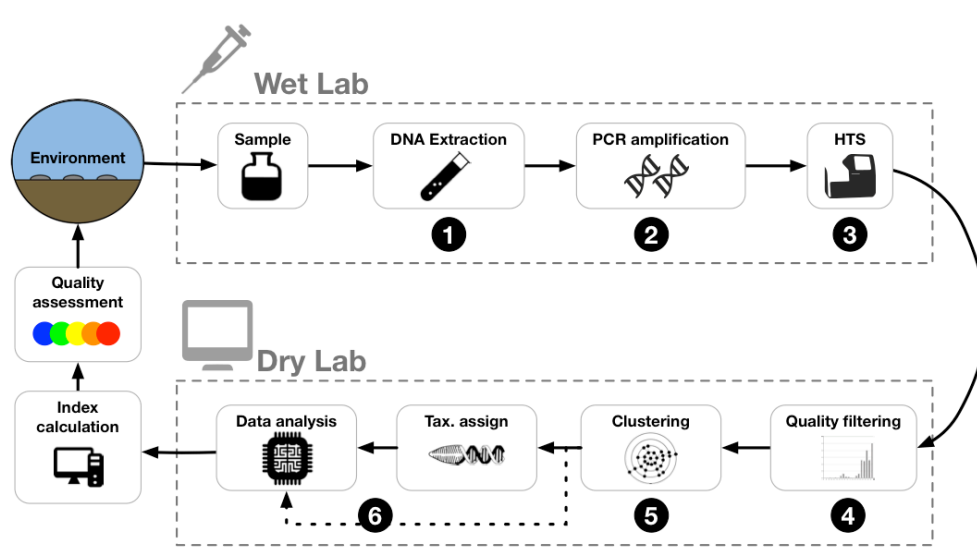


Fig. 3. Key steps in eDNA metabarcoding applied to calculation of biotic indices (after Pawlowski et al. 2018)

4. Promises and challenges of eDNA technology applied to EIA

4.1. Economic and scientific tradeoffs

Environmental monitoring and assessment are essential to ensure a healthy ocean, especially in the face of increasing human activity and use of natural resources. However, traditional biodiversity measurements can be costly as they often employ traditional morphology-based taxonomy (MBT), which requires a taxonomic expert to manually identify individual organisms found in an environmental sample (e.g. sediment, water) (Baird & Hajibabaei, 2012). Molecular techniques, such as metabarcoding, provide an additional tool for environmental assessment and monitoring especially in cases when traditional methods are impractical. In particular, deep-sea biodiversity may be amenable to these molecular approaches due to the dominance of small taxa and rare species that are difficult to identify morphologically (Schewew 2001). Here, we evaluate the economic and scientific tradeoffs between metabarcoding of eukaryotes and morphology-based taxonomy (more specifically, of meiofauna) in the context of deep-sea benthic environmental assessment and monitoring. Either methodology can be incorporated into

a network of decisions that influence the economic and scientific outcomes. As a result, there may be scenarios in which one approach is more appropriate than the other. However, that is not to say the approaches are not complementary.

One significant tradeoff to consider between metabarcoding and MBT is economic costs (Table 1), which can be influenced by several factors. Costs associated with MBT are largely driven by the amount of time required for the taxonomic identification of organisms, which varies with target taxa, vertical fractionation of sediment cores, size fraction, and level of taxonomic identification. Metabarcoding costs are dependent on factors such as chemical kits and primers. Costs associated with bioinformatics, not included here, can also comprise a significant cost.

Table 1. Comparison of consumable and labor costs (nominal USD) between one 2ml sample for metabarcoding and one 78.5 cm³ sample for morphology-based taxonomy of meiofauna. Labor costs are based on an average graduate student stipend in the U.S. (=\$29,500 USD annually).

	Metabarcoding	Morphology-based taxonomy
Consumables	\$49.45	\$26.94
Labor	\$208.49	\$324.78 - 2,876.25

In addition to differences in cost, there are also scientific tradeoffs that must be considered while comparing metabarcoding and MBT (Table 2). These tradeoffs have significant effects on what data are generated, how to interpret them, and ultimately what questions can be addressed. In the context of DSM, many deep-sea organisms are small and rare. This may favor taking a molecular approach, especially as there is relatively limited deep-sea taxonomic expertise in many countries (e.g. nematodes, soft-bodied foraminifera). However, the deep sea is severely undersampled (Ramirez-Llodra et al., 2010; Sinniger et al., 2016) and, therefore, molecular databases required for species-level identification (e.g. GenBank, Barcode of Life) are very inadequate. Barcodes need to eventually be linked to species and morphological descriptions because, otherwise they only provide a series of nucleotides without any biological or ecological information. This highlights the need to use both methodologies in conjunction in order to better understand deep-sea diversity, and the ecosystem functions and services that arise from it.

Table 2. Advantages and disadvantages associated with metabarcoding and morphology-based taxonomy.

	Metabarcoding	Morphology-based taxonomy
Small size-classes (< 0.5mm)	Better suited	Possible but requires specialist expertise

Rare & cryptic species	Better suited	Poorly suited
Expertise required	Weeks of training	Years of training
Time to results	Days	Weeks to months
Biases	Technical (e.g. PCR primers)	Human error
Abundance	Relative	Absolute
Biomass	Cannot provide	Can provide
Morphological data	Cannot provide	Can provide
Demographic information	Cannot provide	Can provide
Behavior	Cannot provide	Cannot provide
Species	OTUs used as proxy	Identified by experts

There are both economic and scientific tradeoffs that should be taken into account when comparing molecular and morphology-based methods. While the former can be expensive with respect to chemical kits and primers, the latter is expensive with respect to labor and may require expertise that is not available. However, because molecular databases required for species-level identification are currently lacking, there may be a need to use both metabarcoding and MBT together in order to assign taxonomy to barcodes. Barcodes associated with a species are more useful because they provide information about how that particular organism might be interacting with its environment. Rather than substitutes, MBT and molecular techniques can be seen as complementary methodologies.

4.2. Technical challenges

Compared to the conventional biodiversity monitoring, the eDNA approach is characterized by high technical complexity and is prone to multiple technical biases at all stages of eDNA workflow, from sample processing to data analysis (reviewed in Pawlowski et al. 2018).

During this project, we have identified and addressed several biases that are particularly important from the point of view of eDNA-based biomonitoring. One of the most important is the fact that eDNA is usually extracted from relatively small volumes of water or sediments and therefore the eDNA samples contain only traces of macro- and megafauna. They are represented through their developmental stages, tissue fragments, cellular organelles or extra-cellular molecules, which provide limited information about their occurrence and abundance. This information can be complementary to the conventional monitoring methods based on visual observation, but it can hardly replace them. On the other side, the eDNA samples contain large

number of small-sized organisms, including microbes and meiofauna, and their analysis can offer a very detailed insight into their taxonomic composition and abundance. Consequently, metabarcoding provides a much more accurate analysis of the micro- and meiofaunal domains of biodiversity compared to the analysis of macro- and megafauna. However, these limitations of eDNA monitoring can be overcome to a certain degree by analyzing bulk samples composed of specimens isolated from water or sediment samples through filtration, elutriation (Brannock and Halanych 2015), or sieving.

The fact that the DNA extracted from environmental samples do not only represent living organisms can also induce important biases in eDNA-based biomonitoring. Several studies show that DNA can be preserved in marine sediments over long periods of time (up to 150'000 yrs). It is impossible to distinguish between DNA of living and dead organisms, which may lead to a misinterpretation of some eDNA datasets in the context of anthropogenic impacts. To overcome this problem, some authors propose to use eRNA rather than eDNA for biomonitoring (Laroche et al. 2017). Although the biomonitoring based on eRNA data shows better correlation with conventional methods (Pawłowski et al. 2014, Visco et al. 2015), high costs related to processing of RNA samples impede its wide acceptance for routine biomonitoring.

Another set of important technical biases is related to PCR amplification. The metabarcoding approach is based on high-throughput amplicon sequencing, which means that the eDNA samples are enriched in genome fragments targeted by PCR primers. The primer specificity is the main factor that alters the taxonomic composition of eDNA datasets, often impeding the detection of some taxa or leading to the over-representation of others. PCR primer efficiency is also considered as the principal source of quantitative biases that hamper the inference of species abundance from eDNA data. As the abundance and taxonomic composition are the main factors used in calculation of biotic indices, the development of new ecogenomic indices needs to take into consideration these PCR-induced biases. A solution proposed in the case of freshwater biomonitoring, which involves the introduction of correction factors assigned to particular taxa (Vivien et al. 2015) could also be applied to marine biomonitoring. Alternatively, PCR-free approaches, such as metagenomics, might provide a less biased view of marine biodiversity.

Last but not the least, the use of eDNA in marine biomonitoring can be limited by gaps in DNA barcoding reference databases. Conventional biodiversity surveys provide a list of morphologically identified species present in a given environment. In the case of eDNA surveys, many sequences remain unassigned. This is particularly an issue in poorly explored areas such as abyssal plains, where most of the diversity remains undescribed. However, even in extensively studied coastal areas, only the common macrofaunal species have been barcoded, while the majority of small-sized meiofauna and microbes have never been genetically documented. To overcome these limitations, we proposed a taxonomy-free approach to predict benthic indices used for coastal aquaculture monitoring (described in chapter 3.3.). However, a similar approach is difficult to apply in the case of deep-sea baseline studies, the objective of which is to inventory the diversity that could be potentially impacted by mining activities.

5. Regulatory and legal framework

5.1. Generally, existing legal frameworks enable the use of eDNA

To understand the suitability of using eDNA for conducting environmental impact assessments (EIAs), we conducted an analysis of various legal instruments that manage open-water aquaculture and deep-seabed mining internationally, as well as on the domestic legal frameworks in three case studies: Scotland, Norway, and Chile. Most legal instruments analyzed present similar, broad definitions of the conditions for environmental compliance on seabed mining and marine aquaculture. When setting the requirements for conducting EIAs on ocean resources exploration or exploitation activities, statutes tend to use undefined legal concepts like the duty to base regulatory decisions on “the best available science,” “recognized methodology,” “best environmental practices” or “recognized scientific methods.” (Warner et al. submitted, Mar. Policy). This approach enables management agencies to design subordinate legislation or implement regulations that establish more specific requirements for EIA. Consequently, a key element in the regulatory implementation of eDNA lies in the way agencies interpret the concept of “best available science” and echoes that interpretation in policies, guidance documents, and operating procedures.

The complexity of the environmental impacts caused by human activities at sea often leads to a case-by-case approach by management agencies. This case-by-case approach involves the analysis of the potential environmental impact of a proposed human action. In other words, one method can be preferred to assess mining impacts and another to assess the installation of liquefied natural gas facilities, or offshore aquaculture developments. In general, the legal frameworks regulate these activities broadly, by empowering specialized agencies to select the best scientific method to estimate the environmental impact of those activities. Agencies confront this task in two ways: either by establishing a preferred method or by acting on a case-by-case basis. Establishing a preferred method (e.g., for monitoring aquaculture facilities in Chile)¹ reduces legal uncertainty because the developers know in advance the information-gathering activities they will be required to perform. However, imposing a specific method provides a snapshot of EIA technology at a particular moment in time that might not fully reflect state-of-the-art methods. On the other hand, deciding the scientific method of EIA on a project-by-project basis may be better suited to estimating the nuances and particularities of each proposed action, but it will be a time-consuming and potentially more costly procedure. The use of different methodologies may also be problematic for the assessment of cumulative or regional impacts, for example, those associated with seabed mining in the Clarion Clipperton zone. In this later case, however, the ISA (International Seabed Authority) address the potential for eDNA to address the Art 145 from UNCLOS mandate concerning the adoption of measures necessary to ensure effective protection of the marine environment from the harmful effects which may arise from mining activities.

¹ See Chile. Subsecretaría de Pesca, Resolución que Establece Contenidos y Metodologías para Elaborar la Caracterización Preliminar del Sitio y la Información Ambiental [Order that establishes the content and methodology to prepare a Preliminary Site Description] § 4.

5.2. eDNA can help strengthen the development of environmental law concepts and requirements

eDNA metabarcoding can help ensure that two fundamental legal concepts are effectively implemented: adaptive management, and the consideration of cumulative impacts. As in the case of “best available science,” statutes tend to recognize the relevance of implementing adaptive management of ocean resources and ensuring that the cumulative impact of several human uses of the seas is adequately considered. However, to date, defining specific regulatory procedures and thresholds of compliance for the implementation of these two concepts has been challenging at best.

The drafting of Environmental Impact Assessments (EIAs) provides a good example. Regulations for EIAs require developers to conduct baseline studies that provide overall information on the characteristics of the marine ecosystem that will likely be impacted by a proposed development taking into consideration the current state of the environment, and to plan ahead of potential changes in circumstances. eDNA is potentially suitable as a scientific procedure to provide baseline information on biodiversity, delivering an in-depth picture of the state of biodiversity at a moment in time and in a specific sea area, that can be regularly updated and improved. It may ultimately also be useful as an indicator of ecosystem function and services. Indeed, some domestic-level statutes are designed to allow for the development of new methods for understanding the environmental impact of human activities at sea, providing additional opportunities for the implementation of eDNA in policymaking (e.g. Scotland Strategic Framework).

6. Next steps

This study highlights at least three key avenues for further consolidation of the use of eDNA for ocean resources management. First, the study has advanced the development of new molecular indices for the assessment of environmental impacts that in some cases, notably marine aquaculture, can be readily employed under the current regulatory framework. However, we argue that future work should not be confined to state-of-the-art eDNA-based methods such as metabarcoding. Shotgun metagenomics and complementary approaches such as metabolomics, which provide key information on the chemical dynamics and on the cues and products of species interactions in the environment, should also be considered. A combination of meta-omics approaches is emerging as a potentially powerful way to understand complex ecosystems, such as the human body (Quinn et al.). We propose tackling the methodological delay that affects the field of marine environmental research by promoting the systematic deployment of the most advanced molecular techniques, or at the very least by obtaining the samples necessary for such techniques in the future.

Second, the results of the SNIS project demonstrate that eDNA can help to provide the necessary information for drafting new legal instruments for the protection and sustainable management of small-sized species, such as those that dominate deep-sea ecosystems and are less well-known than the larger macro- and mega-faunal organisms. New DNA-sampling methods, such as environmental DNA analysis, may be particularly useful for the International Seabed Authority in

the development of a legal framework for the regulation of deep-seabed mining in abyssal settings where the lack of information on small-sized organisms is particularly acute. Here, DNA analysis can provide a strong basis for environmental impact assessment by incorporating microbes and meiofauna with potentially key roles in ecosystem function, alongside the macro- and mega-faunal species that can be better analysed using morphology-based methods. An eDNA-based ocean management approach can also strengthen other regulatory frameworks, currently under development, for the sustainable management of marine resources in areas beyond national jurisdiction. The future legally-binding agreement for the protection of marine biodiversity in areas beyond national jurisdiction (BBNJ treaty)² will include provisions for the exploitation of genetic material and an obligation to conduct EIAs. eDNA technology has a key role to play in the adequate development of the upcoming international legal obligations under the BBNJ treaty.

Finally, our current study highlights the advantages of interdisciplinary research for ocean resources management. To ensure the success of this approach, it is important to conduct similar multi- and inter-disciplinary research projects. Legal and policy mandates for the management of complex ecosystems need to be based in sound science. All actors involved in the implementation of environmental regulations, from legal drafters to environmental prosecutors, should have access to a better understanding of ecological functions and the scientific methods that help describe those functions. It is a big challenge to integrate different perspectives and identify methods that can help explain natural processes in a way that provides adequate outputs to key stakeholders, citizens, policy makers, and government officials. These stakeholders can, in turn, use eDNA data outputs to compare with, and combine with, traditional methods. The suite of information generated by eDNA analysis makes it possible to assess management effectiveness and adapt stewardship/conservation policies to the specific circumstances of countries or regions. Generalizing the use of eDNA metabarcoding will ensure that management decisions are based on a larger body of data, particularly for microbes and smaller taxa. A necessary next step in this process will be the generation of additional case studies on the use of eDNA sampling as a prelude to developing specific regulatory measures for marine resources management, EIAs, marine spatial planning, and other policy initiatives.

7. Conclusions

The current rise in new and intensive uses of the seas demands the development and implementation, in the short term, of technically more sophisticated methodologies, procedures and regulatory frameworks for understanding the impact of these actions on the marine environment, monitoring the impact, and establishing clear, comparable thresholds of action. Additionally, the tools must be fast and cost efficient, and provide parameters that environmental compliance and enforcement practitioners can use to make management decisions, for example, the issuing, suspension, or cancellation of permits. This is a task that cannot be done exclusively from a public policy or a legal perspective. On the contrary, a multidisciplinary approach is needed to help clarify knowledge gaps and identify the most adequate course of action. Law and policy practitioners in the marine resources management field need to have a better understanding of how

² http://www.un.org/depts/los/biodiversity/prepcom_files/Chairs_streamlined_non-paper_to_delegations.pdf

the scientific methodologies work and the kind of information they provide. Likewise, it is important to provide more opportunities for ‘omics’ scientists and technology developers to engage with law drafters, policymakers, managers, and also with environmental judges and prosecutors.

In the case of deep-sea ecosystems, eDNA metabarcoding is fundamental to achieving a meaningful governance system for the exploitation of mining resources in the Area. The central role of eDNA should take shape in at least three ways. First, eDNA research provides essential, previously-unavailable knowledge on the small-sized components of deep-sea and deep-seabed ecosystems. However, eDNA is a fairly new method of environmental assessment and eDNA experts have had few opportunities to conduct such research. Before any exploitation venture becomes operational, the ISA should seize the opportunity that the development of eDNA technology represents and build on eDNA metabarcoding analysis to develop detailed baseline databases on the characteristics of the myriad ecosystems under its stewardship. Second, there is currently no other monitoring tool that can provide a similar level of information on the status of deep-seabed ecosystems. As such, eDNA analysis should also inform the process, currently under way, for drafting exploitation regulations for mineral resources in the Area. It is not desirable for any management agency to enact exploitation regulations for activities posing environmental impacts we do not fully understand in ecosystems we do not know, especially if the management institution has no capacity to promptly respond to an environmental crisis if something goes wrong. The US government’s technical limitations during the 2010 Deepwater Horizon accident are a stark reminder of this (Aldy, 2011). Third, once exploitation projects become operational, eDNA metabarcoding should be embedded in the regulatory framework as a required assessment method for monitoring the environmental impact of mining procedures.

Our project has explored the potential of eDNA for providing baseline information that regulators of a range of human uses of the seas can rely on to obtain a basic understanding of the health and stability of the marine ecosystems and the impact of human activities on the functioning of the marine environment. Additional research is needed to develop specific tools that can help understand how marine ecosystems function and connect all the dots from technology development to regulatory design and implementation, to monitoring, sanctioning and/or prosecution.

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