Marine Policy

Life in a drop: sampling environmental DNA for marine fishery management and ecosystem monitoring

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Abstract:	Science-based management of marine fisheries and effective ecosystem monitoring both require the analysis of large amounts of often complex and difficult to collect information. Legislation also increasingly requires the attainment of good environmental status, which again demands collection of data to enable efficient monitoring and management of biodiversity. Such data is traditionally obtained as a result of research surveys through the capture and/or visual identification of organisms. Recent years have seen significant advances in the utilisation of environmental DNA (eDNA) in the marine environment in order to develop alternative cost-effective ways to gather relevant data. Such approaches attempt to identify and/or quantify the species present at a location through the detection of extra-organismal DNA in the environment. These new eDNA based approaches have the potential to revolutionise data collection in the marine environment using non-invasive sampling methods and providing snapshots of biodiversity beyond the capacity of traditional sampling. Here we present a non-technical summary of different approaches in the field of eDNA, and emphasise the broad application of this approach, with value for the governance and

	management of marine aquatic ecosystems. The review focuses on identifying those tools which are now readily applicable and those which show promise but are currently in development and require further validations. The aim is to provide an understanding of techniques and concepts that can be used by managers without genetic or genomic expertise when consulting with specialists to perform joint evaluations of the utility of the approaches.
Suggested Reviewers:	Franco Biagi franco.biagi@ec.europa.eu Mr. Biagi is a senior officer at the European Commission, assigned to the task of assessing the value of genetic and genomic approaches for fisheries (and aquaculture) management under the remit of the Common Fisheries Policy.
	Devin Bartley devinmichaelbartley@gmail.com Dr. Bartley is a former FAO Officer, with extensive expertise and experience of the application of genetics and genomic for the management of marine living resources on a global scale. – He continues to be active in this area on behalf of the FAO)
	Jennifer Ovenden j.ovenden@uq.edu.au Ms. Ovenden is an Associate Professor at the University of Queensland (Brisbane, Australia) and head of the Molecular Fisheries Laboratory. She has a deep interest and experience in introducing genetic and genomic approaches into fisheries management and policy making.
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Response to Reviewers:	

Dear sir/madam

I re-submit for your consideration our manuscript focusing on the use of environmental DNA (eDNA) to inform marine management and policy development. This work is an output from discussions in the ICES Working Group on Application of Genetics in Fisheries and Aquaculture where we felt that recent rapid developments in the field of eDNA meant that it is timely to review the state of the art in the field in order to provide an overview to managers and policy-makers. The manuscript seeks to critically analyse the field and at the same time produce a non-technical advice summary for decision makers. We feel that this work fit very nicely together with the aims of the journal.

We have revised the manuscript in line with the very helpful reviewers comments and hope we have now addressed all issues identified.

Regards
John Gilbey

We thank the reviewers for their insightful comments and hope we have addressed all points raised. This has led to some significant changes to the text and associated line number changes. To enable comparisons to be made between the original and updated versions we have provided a tracked change version based on the original submission.

Reviewer 1

I enjoyed very much your article on eDNA. A special section on the special circumstances the marine environment poses would be good. I think a main issue is that water flows much less 'contained' in marine waters than in freshwaters.
We are glad you enjoyed the manuscript and have tried to address your comments below.

Perhaps a special section on challenges would be interesting; A well written and interesting article that is needed. Comments are included on the manuscript.
Challenges are addressed in the “Considerations” section which has been expanded to include more emphasis on the need for taxonomic reference databases. We have now also added a new final paragraph to the section on Species Abundance Estimation which specifically address the significant challenges still associated with this approach.

Abstract & throughout – Why restrict to Marine?
We purposefully aimed this review at marine fishery management and ecosystem monitoring. Work in other environments is, as rightly stated by the reviewer, further advanced. However, eDNA applications are coming to the fore now in the marine sphere and as such we thought it timely to present a non-technical review here very much focused on the marine field. We have thus maintained the various references and focusses throughout. To make this point at the earliest stage we have added the marine qualifier to the title.

Line 32 - ABNJ is not the same as EU and not legally binding.
Have removed text as suggested.

Line 58 - Very important and good perspective. Could it be reduced slightly?
This section has now been rewritten and substantially shortened.

Line 59 - Can a link be made between these two paragraphs?
Linking sentence now added.

Line 80 - Very brief statement on how eDNA is collected is needed here.
Have reworded sentence to make clear the collection and filtration requirements. More detail is given later in the text and we did not want to provide too technical an overview here.

Line 125 - Some explanation of why realistic sampling and interpretation of eDNA from the marine environment may be MUCH MORE DIFFICULT than in inland waters need to be mentioned here.
Have added sentence addressing this without going into too much technical detail.

Line 138 - These are freshwater examples, which makes my point for a general review with special section on the marine environment.
Replaced with marine examples

Line 139 - these words may be unknown by non-specialists - the audience for this paper.
This is indeed true and we changed wording to non-technical examples

Line 117 - Excellent. a column on significant result of survey would be useful.
We have now added a column to all three tables of examples outlining the studies.

Line 202 - ? perhaps a better word since this is a new technology?
Have re-worded as suggested.

Line 236 - which, traditional or eDNA?
Have reworded sentence to make clear

Line 249 - Nice case study, but is a Box the right format? why not a box on the other two uses?
We agree and this was also mentioned by the other reviewer. As such we have added boxes for all three uses.

Line 347 - A comparison of the costs of a standard traditional survey vs lab time for eDNA analysis would be useful here, and noting that boat costs are increasing whilst genetic analyses costs are decreasing.
We did not want to address directly absolute costs here as these are rapidly changing as new approaches and technologies are developed. However, the changes in boat v genetic costs pointed out is a good one and we have added this now by adding in the sentence "This is especially relevant as ship-based survey costs increase while genetic screening costs are decreasing"

Reviewer 2

General

I'm recommending that the authors make a major revision of this review before acceptance for publication. The document needs to engage with the needs of non-specialists and address in more detail how the science of eDNA can address those needs.
We hope the revisions undertaken following both reviewers very helpful comments have addressed this point.

- The use of worked examples, perhaps in the form of boxes, would be welcome.

We agree and this was also mentioned by the other reviewer. We have thus added a box example for each of the three general application classes referred to

The information about the science of eDNA needs to be presented with more attention to organisation and accuracy. For example, authors need to explain
1) that species identification using eDNA can be done with routine PCR and Sanger sequencing in addition to qPCR
This has now been added (Line 174)
2) the distinction between eDNA and community DNA
This has been expanded where first mentioned (Line 64) and has a new section added under Fig. 1 (Lines 124-127)
3) the essential nature of reference sequences from taxonomically identified organisms.
This was mentioned previously, but now has been changed to explicitly refer to taxonomically identified organisms in the considerations section.

Specific
16: Non-specialists (here you are referring to fisheries scientists and managers without genetics, genomics or molecular expertise) would be unlikely to rely on their knowledge to "make informed

decisions". Instead, the role of this review should be to provide an understanding of techniques and concepts that can be used when consulting with specialists to perform joint evaluations of the quality of the underlying science.
This is indeed the intention of this review. We have found in a number of different fields that managers, policy makers and many other stakeholders were coming across mention of eDNA and associated applications and were interested in its utility. We want to give an introduction and overview of the subject and approaches together with their state of development. We have followed your and the other reviewers' suggestions throughout and think we better meet this objective now after the helpful comments. Specifically, in this section, we have reworded the final part of the abstract to reflect these objectives as suggested.

20: "Identifying" rather than "disentangling" would be more satisfactory.
Reworded as suggested.

30: How does "supranational" differ from "international"? Suggest use the latter term only.
Removed term.

31 – 34: Reword sentence for clarity.
Reworded.

41 – 58: This paragraph contains information that is not directly related to the review topic. I suggest it is removed or substantially shortened.
This section has now been rewritten and substantially shortened such that it specifically addresses the rationale behind the review.

59 – 69: eDNA is able to characterise species, but taxonomic identification is completely independent of this process. The point is that eDNA relies on, but does not contribute to, taxonomy. Modify the wording to reflect this.
Reworded paragraph to make this point clear.

Fig. 1. Modify last column to split into two columns; one column for the sampling of whole organisms (those in plain text currently) and second column for eDNA sampling (currently in bold italics). It's an important distinction that needs to be emphasized here.
Figure and legend modified as suggested.

131: The definition of community DNA needs to be placed higher in the text where the term is first used.
The first mention of community DNA is on (new) line 64, and the definition has now been expanded to: "In contrast to DNA extracted from tissue samples, or community DNA – where DNA is extracted from communities of whole organisms - eDNA does not require sampling the target organisms themselves, but instead the sampling of the environment they live in".

130 – 135: Revise this section to clarify any overlap between community DNA and eDNA. Are they the same? This is essential, as on line 138 the reader is expected to understand they are two different sources of DNA.
This paragraph has been re-written to clarify this difference.

141: I would expect that community DNA would be influenced by those factors listed here as affecting eDNA. If not, clarify this.
This section has been rewritten to try to make the similarities and differences in influences clearer.

172: Real time PCR is not the only tool used; regular PCR and either Sanger or genomic scale sequencing is also used. Please modify and update.
This section has been updated and genomic sequencing introduced as suggested.

183 - 186: It is optimistic to give non-specialists the notion that eDNA can replace monitoring using trawls, nets, traps and visual surveys. Some examples (or at least citations) are needed here,
We did not want to give the impression that in every case eDNA could replace traditional methods (and indeed go into this in more detail towards the end of the review). We have rewritten this section to try to make this clearer. We have given examples of the various applications in Table 1. This has now been expanded with an extra column to make clear what was being examined. We did not want to make this an exhaustive review of all studies, but rather an overview of applications with some selected examples which we hope are now clearer in the table/s.

183 - 186: with a list of necessary validation steps required to move from traditional to DNA methods. For example; comparison between eDNA and visual survey data in context, controls for type I and type II errors, validation of experimental results in the laboratory, scaling up versus one-off sample collection, temporal and spatial replicates.
We have expanded this section to include reference to these required steps some of which are address in more detail in the section on Considerations later in the text.

183 - 186: Furthermore, marine monitoring probably does not belong in a section entitled “Targeted species detection”.
We think that monitoring programmes have and are often using targeted species detection to detect invasives, pathogens, cryptic species etc so have retained this wording here.

233 – 247: The use of eDNA to estimate abundance for marine species in the wild is awaiting agreement from the scientific community that it is a valid method. As you say, quantification of eDNA is highly variable under controlled conditions (240 – 241), so I think it’s necessary to be a lot more circumspect about the potential of this method to estimate abundance in the sea. At this point, and particularly in light of information in Box 1, the document risks becoming an argument in favour of eDNA for abundance estimates rather than a balanced evaluation of techniques that can inform non-specialists.
We agree that abundance applications are very much in the development stage and have tried to make this clear especially in the final paragraph of this section in the original manuscript draft. However, we agree that this fact needs making even stronger as you suggest, and so have added a new paragraph at the end of the section to try to address this point strongly and directly.

272: Table 3 is a list of possible applications. It does not provide evidence of the ‘increasing reliability’ of the use of eDNA for abundance estimates.
Wording removed/replaced as suggested

291 – 328: This is a suitable description of issues surrounding the use of eDNA. From my point of view, lack of DNA sequences from verified reference samples from taxonomically identified species/groups is the largest problem.
We have now added specific mention of taxonomically identified reference sequences to the section.

Highlights

- Non-technical summary of environmental DNA use for management of marine ecosystems
- Marine community characterisation using environmental DNA
- Marine abundance estimates using environmental DNA
- Integration of DNA based approaches into routine ecosystem monitoring

Life in a drop: sampling environmental DNA for marine fishery management and ecosystem monitoring

Abstract

Science-based management of marine fisheries and effective ecosystem monitoring both require the analysis of large amounts of often complex and difficult to collect information. Legislation also increasingly requires the attainment of good environmental status, which again demands collection of data to enable efficient monitoring and management of biodiversity. Such data is traditionally obtained as a result of research surveys through the capture and/or visual identification of organisms. Recent years have seen significant advances in the utilisation of environmental DNA (eDNA) in the marine environment in order to develop alternative cost-effective ways to gather relevant data. Such approaches attempt to identify and/or quantify the species present at a location through the detection of extra-organismal DNA in the environment. These new eDNA based approaches have the potential to revolutionise data collection in the marine environment using non-invasive sampling methods and providing snapshots of biodiversity beyond the capacity of traditional sampling. Here we present a non-technical summary of different approaches in the field of eDNA, and emphasise the broad application of this approach, with value for the governance and management of marine aquatic ecosystems. The review focuses on identifying those tools which are now readily applicable and those which show promise but are currently in development and require further validations. The aim is to provide an understanding of techniques and concepts that can be used by managers without genetic or genomic expertise when consulting with specialists to perform joint evaluations of the utility of the approaches.

Title Page (with Author Details)

Life in a drop: sampling environmental DNA for marine fishery management and ecosystem monitoring

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Declarations of interest: none

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Abstract

Science-based management of marine fisheries and effective ecosystem monitoring both require the analysis of large amounts of often complex and difficult to collect information. Legislation also increasingly requires the attainment of good environmental status, which again demands collection of data to enable efficient monitoring and management of biodiversity. Such data is traditionally obtained as a result of research surveys through the capture and/or visual identification of organisms. Recent years have seen significant advances in the utilisation of environmental DNA (eDNA) in the marine environment in order to develop alternative cost-effective ways to gather relevant data. Such approaches attempt to identify and/or quantify the species present at a location through the detection of extra-organismal DNA in the environment. These new eDNA based approaches have the potential to revolutionise data collection in the marine environment using non-invasive sampling methods and providing snapshots of biodiversity beyond the capacity of traditional sampling. Here we present a non-technical summary of different approaches in the field of eDNA, and emphasise the broad application of this approach, with value for the governance and management of marine aquatic ecosystems. The review focuses on identifying those tools which are now readily applicable and those which show promise but are currently in development and require further validations. The aim is to provide an understanding of techniques and concepts that can be used by managers without genetic or genomic expertise when consulting with specialists to perform joint evaluations of the utility of the approaches.

Keywords: Environmental DNA, eDNA, management, ecosystem, fisheries

1. Introduction

Globally, it is increasingly acknowledged that our future depends on the maintenance of good environmental status and the conservation of biodiversity, both within defined regional and global standards [1, 2]. The broad consensus is endorsed by such global initiatives as the UN Sustainable Development Goals [3]. Moreover, international and national policies and legislation require the protection of the environment and ecosystems [4-6]. For example, this is explicitly aimed at under the remit of the development of an international instrument on marine biodiversity in areas beyond national jurisdiction (ABNJ) and stipulated in the European Union Marine Strategy Framework Directive [7], and also the Common Fisheries Policy (CFP). The implementation of such legal requirements requires commitment of the member states to carry out extensive monitoring in time and space, preferably in real-time. The development of tools to assess impacts such as invasive species introduction and spread, climate change, contaminants, eutrophication, fishing activities and marine litter on populations and ecosystem interactions remains a high priority. This is an increasingly challenging undertaking, to which state-of-the-art technological and scientific developments can and should contribute.

Effective ecosystem monitoring, the sustainable exploitation of aquatic living resources, sustainable fisheries management and associated policy development should be, as in the case of the CFP, a legally enshrined requirement, based on the best available scientific advice. The integration of scientific advice into governance and policy development and implementation is often challenging, particularly the communication of scientific approaches from specialists to managers and policy makers in a rapidly developing and specialised field. This review seeks to address this issue with regards to new genetic based techniques in the fields of species identification and community characterisation and thus facilitate more effective development of marine fishery management and monitoring approaches.

Effective fishery and ecosystem management rely on the identification and quantification of the species living a certain environment, that is, characterising its biodiversity. There are two significant limitations in gathering such information using traditional techniques: how to representatively sample the biodiversity in an ecosystem and how to identify individuals to species level? Sampling requires complicated logistics, is costly, is biased in its sampling coverage, and is especially difficult for species with low abundance and/or elusive species. Identification also requires taxonomic expertise, which is often lacking and difficult to apply in some cryptic species. The requirement to overcome such impediments has stimulated the search for new tools and approaches to integrate the various environmental dimensions in decision making into an evidence-based policy approach [8]. One such approach is utilisation of DNA collected from the environment to identify and/or quantify the species present in the ecosystem.

Environmental DNA (eDNA) stems from individual organisms which release DNA into the environment through waste products, skin/tissue, scales, gametes, mucus, blood and carcasses [9-12]. This extra-organismal DNA is termed environmental DNA (eDNA) [13]. In contrast to DNA extracted from tissue samples, or community DNA – where DNA is extracted from communities of whole organisms - eDNA does not require sampling the target organisms themselves, but instead the sampling of the environment they live in [14, 15]. The development of new ways of monitoring marine ecosystems and marine biodiversity using eDNA has advanced over recent years and has revolutionised the ability to track invasive species, monitor endangered species, assess the health of fish stocks, and explore the world of marine biodiversity [16]. The seeming simplicity and cost-effectiveness of eDNA-based approaches, together with the interest from wider stakeholder groups, has made such applications highly attractive [17].

The development of genetic technologies to identify species and characterise whole communities through the collection and filtration of water and/or sediment sample is both a potentially invaluable tool for managers and an irresistible story for the popular press. Press articles focusing on such tools range from the very small, such as “New Nano Strategy Fights Superbugs” [18],

to the very large (and improbable) “Loch Ness Monster Hunters to Try DNA Search?” [19]. Disentangling fact from fiction, and hyperbola from reality, is thus not a simple task for the manager striving to understand the field. As such this raises two opposing issues which could each negatively affect the ability to manage fisheries and monitor ecosystems using the most appropriate available scientific tools: the pre-emptive uptake of unproven approaches versus the failure to take advantage of robust new techniques. Stories in the press, together with questions from stakeholders, about new potential approaches that have been developed are often powerful incentives for major funding and uptake of these tools in practice [20]. Whilst in some cases this uptake may be justified, in others, especially in rapidly developing fields, such reliance may be potentially premature. However, each investment requires an accessible, robust and balanced evidence base as deriving management decisions on unproven and/or unreliable techniques brings obvious dangers and potential lack of trust in novel molecular technologies. Further, focusing effort and especially funding on such approaches means that other, perhaps more proven techniques with higher TRL (technology readiness levels) will be starved of resources. It is thus of particular importance that managers and policy makers can distinguish with confidence among approaches that although show promise, are at an early stage of validation.

The converse of the dangers of using unproven tools is avoiding the utilisation of effective proven tools due to uncertainties about their efficacy. As scientific technologies develop it is often the case that some areas progress further and faster than others. Proven approaches emerge and begin to be utilised in limited applications. In order to take full advantage of such developments in a wider context, managers need a straightforward guideline explaining the potential of each molecular tool and its state of readiness for routine applications in order to navigate in the various information streams and stakeholder drivers they are exposed to.

In order to bridge the information gap between the specialist and the manager, we provide here a non-technical synthesis of the evidence surrounding the use of eDNA based monitoring techniques for management of fisheries and ecosystems in the marine environment. It is not intended to be an

exhaustive overview of the growing number of studies that have been carried out. Indeed, there are other reviews which attempt to do this [13, 17, 21-23]. Rather, we focus on key areas of interest, encompassing an overview of approaches with practical applications and priority needs. The focus here will be (i) to cover the different areas of interest to managers, (ii) to provide a brief overview of eDNA-based methods and strategies and (iii) to outline their state of development, practical uses, and development requirements, together with their limitations and factors which need to be addressed when integrating these tools into the management of marine resources.

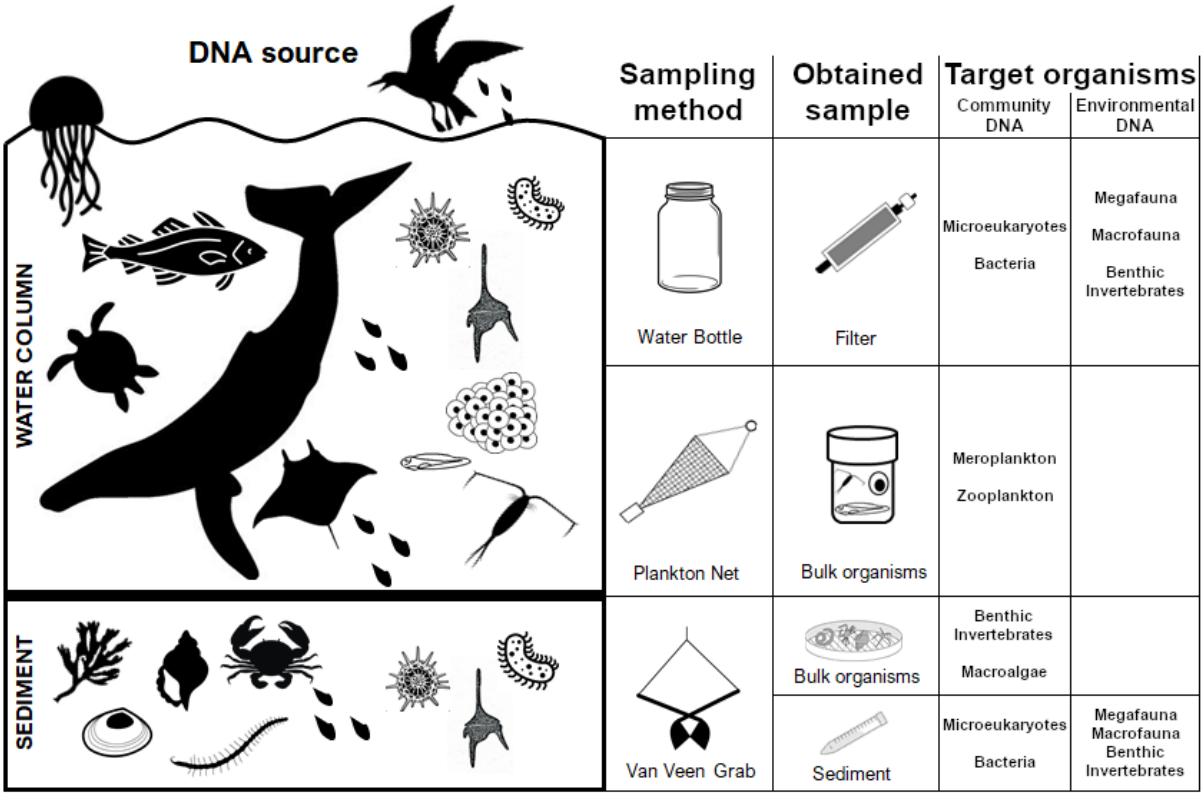

Fig. 1. Different methods for sampling marine ecosystems associated with their DNA source, type of sample obtained and target organisms. Target organisms are shown based on the source of the DNA collected.

2. Environmental DNA in a fisheries context

The marine environment harbours a huge diversity of species [24], ranging from large and charismatic whales to tiny worms and unicellular plankton (Fig. 1). Compared to the sampling of eDNA in freshwater it also poses its own set of, often difficult to address, issues when trying to obtain unbiased samples, especially in relation to factors such, tides, currents, great depths and rapid movements of individuals in three dimensions. Thus, depending on the habitat and taxa of interest, various sampling methods are needed to collect the full range of target species present at a given site so that, when possible, visual identification and quantification of the species is done to study, monitor, and provide information of relevance to the management of marine communities (Fig. 1).

Identification and characterization of these samples can be accelerated using genetic techniques. These will differ depending on the source of the DNA obtained. In the first case, community DNA can be collected. This refers to the collection of whole communities of organisms in the sample from which DNA is extracted from the cells of the sampled individuals. Such analysis results in highly comparable results for monitoring and impact assessment, compared to traditional morphological analyses [25, 26] and at a fraction of the time and cost [25]. In the second case, organisms are not directly sampled, rather extraorganismal DNA in the environment (eDNA) is collected and used to infer a species presence. The use of eDNA in this way may even further simplify sampling and increase throughput, decreasing the costs and allowing for large scale surveys of marine ecosystems.

Traces of DNA in the water column and in the sediment can be used to identify species and characterize communities [e.g. 27], to investigate their distribution [e.g. 28], and to determine their abundance [e.g. 29]. Both community DNA and eDNA data are affected by technical (e.g. laboratory assay choices, incomplete reference databases) and biological (e.g. size of the organisms) biases, which should be taken into account when interpreting the data for fisheries management and ecosystem monitoring [30]. While the distribution of the entire organisms collected during community DNA surveys is, of course, affected by environmental parameters, extracellular eDNA is especially sensitive to such factors. eDNA data is thus influenced by environmental factors such as water

temperature, organic matter, pH, UV radiation, and water currents, and by the type and amount of material used during sampling [17]. Further, as eDNA is used as a proxy for species presence, any biases in the transport and persistence of eDNA can result in its distribution being significantly different from that of the actual ornagisms. Careful evaluation of these biases is needed for the correct interpretation of eDNA results in the framework of fisheries management and conservation.

3. From water to results - the eDNA workflow and approaches

Identifying the presence of a particular species or characterizing the entire community from eDNA samples requires a series of steps that often need to be adjusted to each case study and fully understood in order to derive sound conclusions from the data obtained [30]. Sampling eDNA in the marine environment is possible through water or sediment [31]. It is however usually done by collecting water that is subsequently passed through variable pore size filters, generally < 1 µm pore size. It is also often common practice to add a prefiltering step (e.g. with a 3 µm prefilter) to avoid clogging the filtering process with large pieces of tissue or small animals such as zooplankton [32]. Water samples from the marine environment can be collected using procedures that span from the simple act of using a bucket to collect surface samples to a more sophisticated procedure involving the use of Niskin bottles [33] or rosette samplers [34] to capture samples at greater depths. In all cases, strict procedures to avoid cross-contamination between samples are needed along with proper preservation and storage for filters containing eDNA prior to laboratory analysis. While applications are diverse, approaches using eDNA can be categorised into three groups based on their main objectives: *1) Targeted Species Detection*, to detect the presence or absence of a single or a limited number of defined targeted species at a location; *2) Community Characterisation*, to produce an inventory of the biodiversity of an ecosystem; and *3) Species Abundance Estimation*, to inform on absolute and/or relative abundance of species at the sampling location. An overview of the three groups is presented below, detailing their objectives, strengths and limitations. Selected examples of each technique are also outlined in Tables 1-3 to show typical situations where they have been utilised.

3.1. Targeted species detection

Perhaps the most developed and utilised eDNA application is the detection of individual species and/or small groups of targeted species of interest in an ecosystem. Targeted species detection from eDNA involves the development of genetic probes designed to match explicitly the target species DNA, and distinguish the target from other species potentially present in a sample using classical genomic Sanger sequencing [13, 35, 36] and/or quantitative real time PCR (qPCR) [37]. Marker amplification is achieved by the use of DNA probes, which allow the genetic code of specific sections of the genome to be examined, and resulting unique species-specific genetic sequences. qPCR is based on detection and quantification of a fluorescent light signal produced by binding of a dye-labelled species-specific probe, during amplification, to the target species DNA sequence present in a sample [38]. Detection of small groups of species using qPCR can be achieved by combining (multiplexing) probes for these species, labelled with different fluorescent dyes, in a single reaction.

Table 1

Selected applications of targeted species detection using marine eDNA.

Application	Example study outline	Example
Detection and mapping of the spread of invasive or non-native species	Invasive slipper shell on the European Atlantic coast	[39]
Identification and monitoring of rare/endangered species	White sharks in the open ocean	[34]
Detection of cryptic species	Cryptic seahorse species off western Australia	[40]
Biosecurity during import/export	Ornamental fish imports	[41]
Investigating spawning activity	Spawning ecology of the Japanese eel	[42]
Monitoring of hard to access environments	Deep-sea octocorals using remote submersibles	[43]

Applications are varied and are detailed with examples in Table 1. It can be observed from these examples that targeted species detection has shown its usefulness across many and varied situations of fishery management and ecosystem monitoring. Marine monitoring using traditional methods such as individual capture (with e.g. trawls, nets and traps) and visual surveys are time consuming, costly to carry out and in some cases simply impossible. Investigations using eDNA have shown that in numerous situations the approaches have the potential to add to the available information to inform a variety of management questions. Adding value to traditional programmes is, perhaps, the most cost-effective way to integrate eDNA screening into routine management and monitoring programmes (see below). However, in some specific situations the use of eDNA has the potential to replace traditional monitoring. For this to occur a number of technical and validation steps are required such as comparisons between eDNA and visual survey data in context, controls for type I (false-positive) and type II (false negative) errors, validation of experimental results in the laboratory, scaling up versus one-off sample collection, temporal and spatial replicates (see below). If such steps are successful, targeted species detection using eDNA has shown that it can fulfil the requirements of fishery and ecosystem monitoring programmes and can be used as an alternative approach to answer relevant questions for managers.

Box 1. Case study – Targeted species detection – eDNA and ecology of commercially important food species [42]

The catadromous Japanese eel *Anguilla japonica* is an important food fish in East Asia, where after spawning at sea and migrating to freshwater it is raised in aquaculture ponds. Intensive research including sampling with large plankton and trawl nets, genetic species identification of eggs and newly hatched larvae, and direct observations using deep-tow camera systems has led to the discovery of the eel's spawning area. Such approaches have provided useful information on the spawning area of Japanese eels. However, their precise spawning sites and ecology still remain largely unknown, in part due to the significant depths and vast scale of the possible survey areas and the need to narrow down the search areas.

In order to address these issues, species-specific genetic probes were developed and tested in the laboratory by filtering and extracting eDNA from tank water containing eels. This showed that the probes could identify the Japanese eel from a minute amount of eDNA. Samples were collected at varying depths during an ocean survey on the southern West Mariana Ridge in the general spawning area of the eel. eDNA positive signals were detected for *A. japonica* from 3 of the 108 samples.

This first attempt to detect Japanese eel eDNA suggests the approach has the potential to provide information in near real-time about the spawning aggregations in a deep-water environment which is very challenging to survey using traditional techniques.

3.2. Community characterisation

Community characterisation, often referred to as community metabarcoding, is a technique used to characterise either the species composition or a selected subset of species, whose eDNA is represented in a water sample [44, 45]. Using this approach, a region of DNA conserved within a species and diverse across a wide range of taxa is specifically targeted and many targets are captured simultaneously in a single reaction. Amplified products are sequenced, revealing unique species-specific signatures (i.e. a barcode for that species) within a sample and sequences are compared to reference sequences within a database. As such, each unique sequence match between the sample and the reference database will identify DNA from a specific species in the sample [46]. Metabarcoding has been utilized in a variety of settings, showing a broad potential application for biodiversity monitoring (Table 2).

Table 2

Selected applications of community characterisation using marine eDNA.

Application	Example study outline	Example
Fish diversity	Fish community composition in a large (120,000 km^2) area of the NE Atlantic	[47]

Identification of new species in an area	Detection of a number of invasive, cryptic and observations of species for the first time in the North Sea	[48]
Connection of life stages	Linking distributions of adult and immature stages of South African marine fish species	[49]
Clarification of feeding behaviour	Characterisation of prey species of invasive lionfish through gut content analysis in the Mexican Caribbean	[50]
Ecosystem food-web structure and dynamics	Characterisation of community structure of Japanese coastal waters	[51]
The impact of aquaculture on benthic communities	Comparison of benthic Foraminifera communities at different distances from aquaculture sites	[52]
Identification of non-indigenous species in ballast/harbour water	Detection of the transfer of North Sea molluscs across tropical waters in ballast water	[53]
Monitoring of marine vertebrates	Distribution in space and water column of marine vertebrates in Monterey Bay	[54]
Habitat preference	Fine-scale geographic and temporal mapping of marine fish populations in the Hudson River estuary	[55]
Characterisation of non-indigenous species	Detection of introduced and newly observed resident marine species around southern Britain	[27]
Biodiversity assessment- marine sanctuaries	Characterisation of pelagic and benthic eukaryotic biodiversity in the Florida Keys National Marine Sanctuary	[56]

eDNA metabarcoding is well established in providing unique insights into the diversity and functioning [57] of aquatic ecosystems. Such applications have allowed the characterisation of fish communities in freshwater [e.g. 58] and marine [e.g. 59] environments, including pelagic [e.g. 60] and benthic communities [e.g. 61]. Together with such an often-unique ability to characterise entire communities, metabarcoding has also been used in a more applied way to answer specific questions of interest to managers and policy makers. These include investigations of the impact of aquaculture on local bottom communities, the transfer of non-indigenous and invasive species in ballast and harbour water, and monitoring of marine vertebrates (Table 2). Where targeted species detection using eDNA allows specific species to be examined, aquatic eDNA metabarcoding allows the cost-

effective characterisation of entire communities, and therefore it is especially useful in ecosystem monitoring scenarios.

Box 2. Case study – Community characterisation – fish biodiversity assessment using eDNA over large oceanic areas [47]

Traditional methods of monitoring marine fish diversity rely on trawling surveys. These are costly, time-consuming and, especially in complex environments, may be biased in the species they capture with only a sub-set being targeted. Community characterisation using eDNA has the potential to address some of these shortcomings by, in theory, being able to identify all species in an area using the eDNA they shed into the environment.

In order to test this hypothesis, an eDNA based metabarcoding approach was used to characterise the species present across a 120,000 km^2 area of the Northeast Atlantic using eDNA filtered from water samples. Species specific genetic sequences were obtained from the eDNA which were identified through matches in reference databases. The results of this analysis were compared to traditional trawl surveys carried out simultaneously to the water sampling.

It was found that trawl and eDNA samples resulted in the same most abundant species (European anchovy, European pilchard, Atlantic mackerel, and blue whiting), but eDNA metabarcoding resulted in more detected bony fish and elasmobranch species (116) than trawling (16). The eDNA metabarcoding approach was thus seen to capture the biodiversity present in the area at least as good, and with some groups of species better, than traditional techniques. The findings support the integration of eDNA metabarcoding for broad-scale marine fish diversity monitoring in the context of Directives such as the Common Fisheries Policy or the Marine Strategy Framework Directive.

3.3. Species Abundance Estimation

Together with the identification of both individual and ecosystem-based biodiversity, eDNA can be used to estimate either the relative abundance of multiple species using metabarcoding [62], or the absolute abundance of individual species using qPCR [63]. At its simplest, such approaches involve quantifying the amount of eDNA from a species represented in a sample and using that as a simple proxy for abundance [64]. Such information may be used to estimate numbers of individuals and/or biomass. The use of eDNA-based tools to quantify stocks of species of interest is of course of great interest to fishery managers and policy makers, as population or stock assessment is a central component of any management and/or conservation programme. Estimating absolute counts and/or biomass, relies on the establishment of a robust correlation between DNA concentration and living biomass whereas relative biomass estimates assume that the relative amounts of DNA measured in the sample are representative of the relative abundance of the different species in the ecosystem. While both approaches may seem to rely on fairly simple calculations and indeed are beginning to be used (Table 3), in practice, there are many factors which interact to make the relationships upon which the assumptions about the correlations are made very complex to disentangle and to obtain robust estimates.

Table 3

Selected applications of abundance estimation using marine eDNA.

Application	Example study outline	Example
Seasonal fish abundance	Seasonal relative fish species abundance in the Hudson River estuary	[55]
Marine vertebrate abundance	Vertebrate relative abundance in a kelp forest off the Monterey Peninsula	[65]
Monitoring pathogen abundance in aquaculture	Relative abundance of two parasite species on salmon farms	[66]
Monitoring deep water species	Relative abundances of Subarctic, deep water fish species from the continental slope off Southwest Greenland	[62]

Invasive species abundance	Temporal abundance of invasive Codium seaweed in the Bay of Biscay	[67]
Stock assessment	Biomass estimation of Atlantic cod in oceanic waters around the Faroe Islands	[29]

Applications of using eDNA to assess abundance in the aquatic environment are at present most advanced in freshwater [62]. Abundance estimation using traditional methods such as gillnet data and trawling provides a relative index assumed to be directly proportional to density/absolute abundance [29, 64, 68]. Such traditional non-genetic methods are the most common to estimate fish abundance in lakes for fisheries management [69] and biodiversity characterisation [70], although they are often expensive, time consuming and destructive. Initial results from experimental aquaria and ponds show positive correlations between species abundance and eDNA concentration [71, 72]. However, even in controlled tank situations, it has been found that "…quantification of eDNA samples can be highly variable even when sampling from the same individual under controlled conditions" [72]. Approaches have now moved from the experimental set-up to the field. The abundance of individual targeted species has been characterised using eDNA in freshwater fish species including lake trout (*Salvelinus namaycush*) [64], common carp (*Cyprinus carpio*) [73] and Atlantic salmon (*Salmo salar*) [74]. Similarity between relative and absolute abundance has been reported in communities including both amphibians [75] and fish [55, 76], including commercially important species such as Atlantic cod (*Gadus morhua*) [29].

Box 3. Case study – environmental DNA and quantitative assessment of commercial fish species [29]

Traditionally, standardised trawl surveys are used as an effective monitoring tool for management of commercial fisheries, providing valuable estimates of quantity (biomass) and spatial distribution of fish stocks. Such surveys, however, are costly and have other associated biases and drawbacks such as gear and ground selectivity and negative impact on habitats.

In order to determine the utility of eDNA for assessing commercial stocks a quantitative eDNA survey of Atlantic cod was compared to results from a standardised demersal trawl survey. Important stock metrics such as regional cod biomass and Catch Per Unit Effort (CPUE) were determined using traditional assessment analysis of trawl data. At 35 trawl stations water samples were also collected 4 m above the seafloor and eDNA analysed in the laboratory using cod-specific DNA probes.

There was an overall 80 % concordance between trawl and eDNA cod detection, with good spatial conformity between the two approaches. Nearly 70 % of all discrepancies in the detection of Atlantic cod were at the sampling stations where actual or predicted Atlantic cod catch rates were very low (≤ 3 fish h^{-1}). Similarly, there were also significant positive correlations between the regional integrals of cod biomass (kg) and eDNA quantities (copies) and between sampling effort-normalised CPUE and eDNA concentrations.

This study shows that eDNA monitoring can provide valuable spatial and abundance information which is comparable to traditional standardised trawl data but less costly and with less impact on the environment. The findings reinforce the opportunities for the incorporation of approaches utilising eDNA into stock biomass assessments of commercially important fish stocks.

In the marine environment, abundance estimates using eDNA, while inherently more difficult than a relatively enclosed freshwater ecosystem, are starting to be examined (Table 3). Approaches are developing rapidly and, while at present robust relationships between abundance quantification using eDNA and more traditional methods are sometimes weak [62, 77, 78], in some cases the approach seems to be comparable to that of other quantitative methods [29, 79]. The inherent uncertainty in the robustness of biomass quantification when utilising eDNA approaches is due to both the assumptions on which the technique rests and the impact of extraneous factors on such assumptions. eDNA abundance quantification relies on the assumption that local population numbers may be inferred by measuring the concentration of eDNA at a given locality and that this estimation represents the quantitative relation between eDNA concentration and the underlying population size

[79, 80]. However, such a relationship may not be always true, or even present in most cases. The amount of eDNA at a location will vary depending on a number of biological, physical and environmental factors (see below). While these factors also have an impact on species detection, the impact of the fluctuations registered is higher if quantitative measurements are being attempted, rather than simple presence/absence results. Nevertheless, it may be possible to incorporate these impacts into modelling, to better predict how they can affect eDNA concentrations, therefore reducing the variance around such quantifications [79, 81-83]. However, due to the complexity of interacting factors, direct quantitative assessments remain highly challenging in marine ecosystems [17, 84].

Abundance estimates in the marine environment can thus be summarised to be very much in the developmental stage at the moment, notwithstanding some of the early applications being examined. Significant questions still have to be addressed to allow the amount of eDNA collected to be linked directly to either relative or absolute abundances. The three-dimensional nature of the environment, together with the many physical, chemical and environmental factors whose impacts have to be quantified means that the validity of abundance quantification using eDNA is still to be determined in most if not all situations. Significant work is, however, being undertaken around the world to determine if the method can be developed into a useful tool as, if so, it might in the future provide a very cost-effective approach. At present, however, the jury is still out if this will be possible.

4. Considerations

Analysis of eDNA allows inferences to be made about organisms, without the need to see, observe or handle them. This is the major advantage offered by this approach, but also potentially a drawback. In order to make the most informed decisions and use eDNA approaches to their fullest, managers and policy makers should be aware of the issues to be considered when seeking to understand the results of eDNA surveys. Although eDNA based applications are relatively new, especially in the context of marine management, scientists have a good understanding of the

drawbacks of this method, hence have been able to define the actions needed in order to limit errors and uncertainties [85-87].

An important consideration in any eDNA monitoring programme is the avoidance of contamination [88]. DNA molecules from many sources are everywhere around us, and if they enter eDNA samples they have the potential to produce false positives. The use of sterile equipment, gloves, and a dedicated eDNA laboratory (with strict protocols, controls and necessary separations of processes handling high and low DNA templates) are necessary measurements to be taken in order to reduce contaminations and resulting false positives [86]. It is possible to control for contamination, by taking multiple replicates (usually three) of the same samples, and by using negative controls (i.e. sterilised distilled water samples not containing any actual material) at every stage of the process (field and laboratory blanks for DNA extraction and amplification) [88]. Any DNA that results from these blanks (and there is likely to be some), is then 'subtracted' from the results of the actual samples. Thus, like in any other monitoring approach, standardization is crucial, especially when it comes to techniques of collection, essential negative control sample inclusion [89] and laboratory analysis [90], as well as the interpretation of results [91].

Another important consideration (which can be a significant drawback in certain situations) is the availability of DNA reference sequences, or a reference database of taxonomically identified species/groups [92]. Matching sequences obtained from actual eDNA samples against a reference database is the final step in the workflow, one that will tell the user what species the sampled eDNA belongs to. The reliability of such databases, together with the availability of high-quality reference sequences of previously examined and taxonomically identified organisms is crucial for robust data interpretation and to avoid false negatives and positives. There are a number of databases that can be used, with the Barcode of Life Data System (iBOL) [93] being an important example. Yet, it is advisable, when embarking on an eDNA project, to invest time assessing the reliability of the databases for the geographic area and taxa investigated, and if required, build a project-specific quality-controlled database.

Another pivotal consideration when interpreting results is that of eDNA transport. As mentioned above, eDNA offers a snapshot of the species presence in a certain habitat in a given timeframe. Environmental DNA sampled might indeed come from the organisms that live in the sampled area at that time, but it might also originate from degrading tissue, eggs and sperm and, depending on environmental conditions, it might have simply been transported from elsewhere with the currents or tides. Many researchers are now concentrating their efforts into understanding how long these molecules can persist in the environment and remain detectable [reviewed in 17].

5. Integration into existing management and monitoring programmes

The development of new approaches to gather information of relevance to fisheries and ecosystem monitoring through the use of eDNA sampling methods, and the associated novel insights such approaches generate, has the potential to revolutionise the information available to managers. However, together with the requirement for the new methods to be able to provide robust results, there is also a need to investigate the practicalities and cost-benefit of incorporating the new techniques into standardised monitoring surveys [94, 95]. In some situations, for example, the requirement for targeted detection of specific species, it may be necessary to develop novel surveying programmes. However, by far the most preferred situation would be if the added value could be embedded into existing survey programmes, through the addition of the collection of eDNA samples, potentially requiring relatively little extra cost/effort on top of that already being invested. This is especially relevant as ship-based survey costs increase while genetic screening costs are decreasing. Trawl surveys may be able to be supplemented by simultaneous eDNA collection from water samples, and benthic sediment monitoring by eDNA collection from grab samples. Indeed, in many if not most, often costly, traditional fishery and ecosystem monitoring surveys there would seem to be an ideal opportunity to collect such samples and add value in this way. It seems, therefore, that the design of future surveys, together with that of existing programmes, should be evaluated in the light of the

developments in eDNA approaches outlined above and the added value that the integration of these approaches could bring.

6. Conclusion

Rapid developments in the field of eDNA analysis have provided a range of new tools for research scientists, and fishery and ecosystem managers. With such developments, it is not straightforward for the manager to disentangle which tools can provide robust evidence to incorporate into policy development discussions, and which are still in the developmental phase. In tandem, reports about such advances in the mainstream media drive stakeholders to question managers about the utility of the toolkits, including specific questions that might be difficult to answer for a non-specialist. Here, we have attempted to provide a topic-based overview which goes some way to address this problem, and thus can be of use to inform managers of the strengths and weaknesses of the various approaches currently available.

Environmental DNA-based tools have, for a number of years now, been providing reliable evidence in areas such as single species detection, and the characterisation of ecosystem biodiversity. As such, they represent a robust, cost-effective, and in an increasing number of cases a more sensible option for managers and monitors for incorporation into their standard scientific toolkits. While significant advances have been, and continue to be, made in the use of eDNA to quantify both relative and absolute abundance, such analyses are less well developed and still suffer from uncertainties associated with various environmental, biological and methodological challenges of these techniques [17]. As these influences are studied and their impacts better understood such uncertainties will be reduced. However, at present their application is likely to be more limited.

Every scientific monitoring method has uncertainties and the field of eDNA research is no exception. However, in many cases such uncertainty is well understood and as such, and considering the potential significant benefits and potential cost-savings of the new tools available, managers and monitors should consider the integration of these approaches in their management planning

discussions along with the more traditional techniques. The different approaches can work together to provide complementary information. In the end they will allow enhanced scientific understanding, resulting in improved science-based policy development in view of ecosystem-based management.

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Life in a drop: sampling environmental DNA for marine fishery management and ecosystem monitoring

Abstract

Science-based management of marine fisheries and effective ecosystem monitoring both require the analysis of large amounts of often complex and difficult to collect information. Legislation also increasingly requires the attainment of good environmental status, which again demands collection of data to enable efficient monitoring and management of biodiversity. Such data is traditionally obtained as a result of research surveys through the capture and/or visual identification of organisms. Recent years have seen significant advances in the utilisation of environmental DNA (eDNA) in the marine environment in order to develop alternative cost-effective ways to gather relevant data. Such approaches attempt to identify and/or quantify the species present at a location through the detection of extra-organismal DNA in the environment. These new eDNA based approaches have the potential to revolutionise data collection in the marine environment using non-invasive sampling methods and providing snapshots of biodiversity beyond the capacity of traditional sampling. ~~The rapid developments in the field provide an oftentimes bewildering suite of novel tools. However, it is often difficult for a non-specialist to make informed decisions as to the utility of the different approaches. In order to bridge this information gap, here~~Here we present a non-technical summary of different approaches in the field of eDNA, and emphasise the broad application of this approach, with value for the governance and management of marine aquatic ecosystems. The ~~paper~~review focuses on ~~disentangling~~identifying those tools which are now readily applicable and those which show promise but are currently in development and require further validations. The aim is to provide an understanding of techniques and concepts that can be used by managers without genetic or genomic expertise when consulting with specialists to perform joint evaluations of the utility of the approaches.

Keywords: Environmental DNA, eDNA, management, ecosystem, fisheries

1. Introduction

Globally, it is increasingly acknowledged that our future depends on the maintenance of good environmental status and the conservation of biodiversity, both within defined regional and global standards [1, 2]. The broad consensus is endorsed by such global initiatives as the UN Sustainable Development Goals [3]. Moreover~~, supranational~~, international and national policies and legislation require the protection of the environment and ecosystems [4-6]. ~~This is for~~For example, this is explicitly aimed at under the remit of the development of an international ~~legally binding~~ instrument on marine biodiversity in areas beyond national jurisdiction (ABNJ) and stipulated in the European Union Marine Strategy Framework Directive [7], and also the Common Fisheries Policy (CFP). The implementation of such legal requirements requires commitment of the member states to carry out extensive monitoring in time and space, preferably in real-time. The development of tools to assess impacts such as invasive species introduction and spread, climate change, contaminants, eutrophication, fishing activities and marine litter on populations and ecosystem interactions remains a high priority. This is an increasingly challenging undertaking, to which state-of-the-art technological and scientific developments can and should contribute.

Effective ecosystem monitoring, the sustainable exploitation of aquatic living resources, sustainable fisheries management and associated policy development should be, as in the case of the CFP, a legally enshrined requirement, based on the best available scientific advice. ~~The integration of scientific advice into governance and policy development and implementation is dependent on the mutual exchange of information on possibilities, opportunities, limits and needs between scientists, managers and policy makers. Crucial is the clarity and directness of communication across diverse players, supported by a fully validated, robust and accessible evidence base. Generating and extending such resources require standardisation and comparability, thereby allowing ongoing information~~

~~retrieval and analyses [8, 9]. Although there are often very good communication channels between scientists and managers, science coverage in the mass media is the major channel that bridges the gap between science and the general public [10]. Most people, including managers and policy makers, acquire their information about scientific progress firstly, or for some stakeholder groups exclusively, from the mass media. Accordingly, it is argued that media coverage contributes strongly to the image and understanding of new developments and, importantly, influences their legitimation, public support, and funding [10]. It is thus vital that the bidirectional information flow between managers and scientists is well established and a mutual understanding assured [11]. This is often challenging, particularly the communication of scientific approaches from specialists to managers and policy makers in a rapidly developing and specialized field~~The integration of scientific advice into governance and policy development and implementation is often challenging, particularly the communication of scientific approaches from specialists to managers and policy makers in a rapidly developing and specialised field. This review seeks to address this issue with regards to new genetic based techniques in the fields of species identification and community characterisation and thus facilitate more effective development of marine fishery management and monitoring approaches.

Effective fishery and ecosystem management rely on the ~~taxonomic~~ identification and quantification of the species living a certain environment, that is, characterising its biodiversity. There are two significant limitations in gathering such information using traditional techniques: how to representatively sample the ~~organisms~~biodiversity in an ecosystem and ~~then~~ how to ~~taxonomically~~ identify ~~them~~individuals to species level? Sampling requires complicated logistics, is costly, is biased in its sampling coverage, and is especially difficult for species with low abundance and/or elusive species. Identification ~~then~~also requires ~~often lacking~~ taxonomic expertise~~-~~, which is often lacking and difficult to apply in some cryptic species~~ are especially difficult to identify~~. The requirement to overcome such impediments has stimulated the search for new tools and approaches to integrate the various environmental dimensions in decision making into an evidence-based policy approach ~~[12].~~ ~~One such approach is utilisation of DNA collected from the environment to characterise~~[8]. One such

approach is utilisation of DNA collected from the environment to identify and/or quantify the species present in the ecosystem.

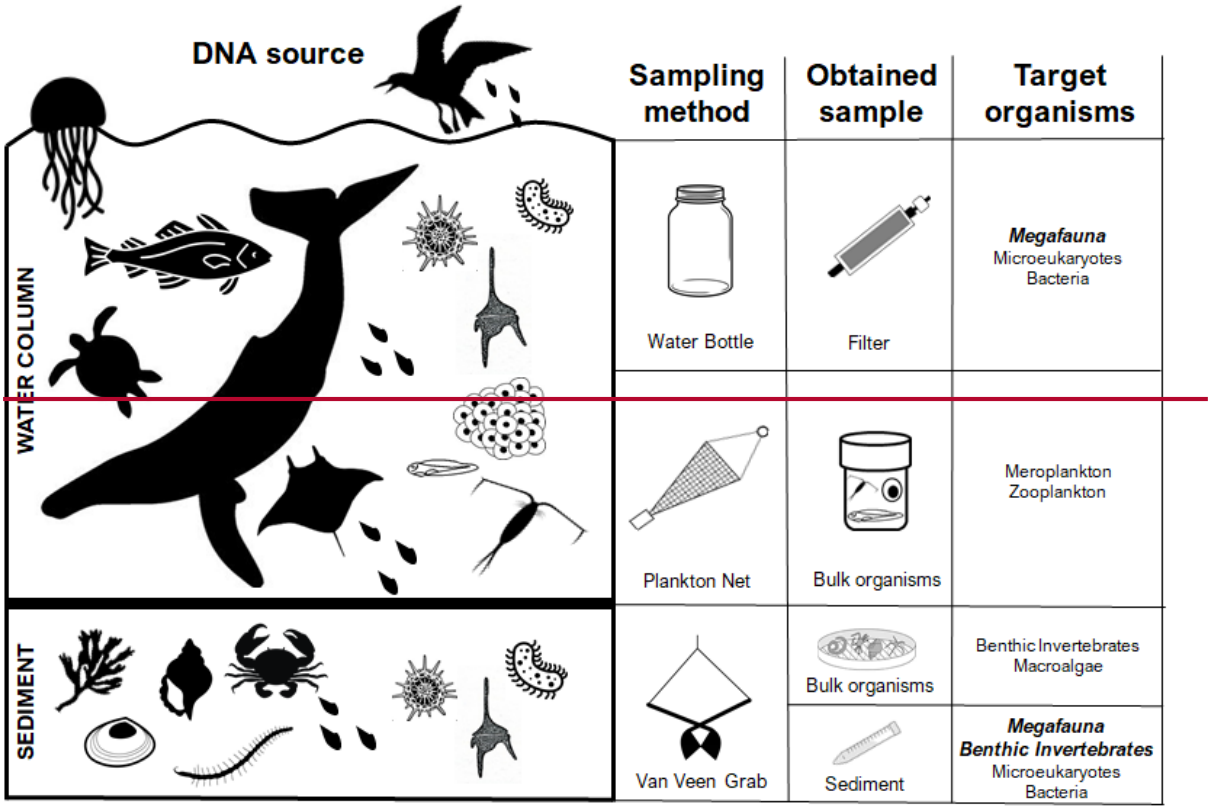
Environmental DNA (eDNA) stems from individual organisms which release DNA into the environment through waste products, skin/tissue, scales, gametes, mucus, blood and carcasses [~~13-16~~9-12]. This extra-organismal DNA is termed environmental DNA (eDNA) ~~[17].~~[13]. In contrast to DNA extracted from tissue samples, or community DNA – where DNA is extracted from ~~the~~communities of whole organisms - eDNA does not require sampling the target organisms themselves, but instead the sampling of the environment they live in [~~18, 19~~14, 15]. The development of new ways of monitoring marine ecosystems and marine biodiversity using eDNA has advanced over recent years and has revolutionised the ability to track invasive species, monitor endangered species, assess the health of fish stocks, and explore the world of marine biodiversity ~~[20].~~[16]. The seeming simplicity and cost-effectiveness of eDNA-based approaches, together with the interest from wider stakeholder groups, has made such applications highly attractive ~~[21]~~[17].

The development of genetic technologies to identify species and characterise whole communities through the ~~utilisation of a filtered~~collection and filtration of water and/or sediment sample is both a potentially invaluable tool for managers and an irresistible story for the popular press. Press articles focusing on such tools range from the very small, such as “New ~~nano-strategy fights superbugs~~Nano Strategy Fights Superbugs” ~~[22]~~[18], to the very large (and improbable) “Loch Ness Monster Hunters to Try DNA Search?” ~~[23].~~[19]. Disentangling fact from fiction, and hyperbola from reality, is thus not a simple task for the manager striving to understand the field. As such this raises two opposing issues which could each negatively affect ~~their~~the ability to manage fisheries and monitor ecosystems using the most appropriate available scientific tools: the pre-emptive uptake of unproven approaches versus the failure to take advantage of robust new techniques. Stories in the press, together with questions from stakeholders, about new potential approaches that have been developed are often powerful incentives for major funding and uptake of these tools in practice ~~[10].].~~[20]. Whilst in some cases this uptake may be justified, in others, especially in rapidly developing

fields, such reliance may be potentially premature. However, ~~such~~each investment requires an accessible, robust and balanced evidence base as deriving management decisions on unproven and/or unreliable techniques brings obvious dangers and potential ~~future~~ lack of trust in novel molecular technologies. Further, focusing effort and especially funding on such approaches means that other, perhaps more proven techniques with higher TRL (technology readiness levels) will be starved of resources. It is thus of particular importance that managers and policy makers can distinguish with confidence among approaches that although show promise, are at an early stage of validation.

The converse of the dangers of using unproven tools is avoiding the utilisation of effective proven tools due to uncertainties about their efficacy. As scientific technologies develop it is often the case that some areas progress further and faster than others. Proven approaches emerge and begin to be utilised in limited applications. In order to take ~~a~~ full advantage of such developments in a wider context, ~~the~~ managers need a straightforward guideline explaining the potential of each molecular tool and its state of readiness for routine applications in order to navigate in the various information streams and stakeholder drivers they are exposed to.

In order to bridge the information gap between the specialist and the manager, we provide here a non-technical synthesis of the evidence surrounding the use of eDNA based monitoring techniques for management of fisheries and ecosystems in the marine environment. It is not intended to be an exhaustive overview of the growing number of studies that have been carried out. Indeed, there are other reviews which attempt to do this [13, 17, 21~~, 24-26~~-23]~~,~~. Rather, we focus on key areas of interest, encompassing an overview of approaches with practical applications and priority needs. The focus here will be (i) to cover the different areas of interest to managers, (ii) to provide a brief overview of eDNA-based methods and strategies and (iii) to outline their state of development, practical uses, and development requirements, together with their limitations and factors which need to be addressed when integrating these tools into the management of marine resources.



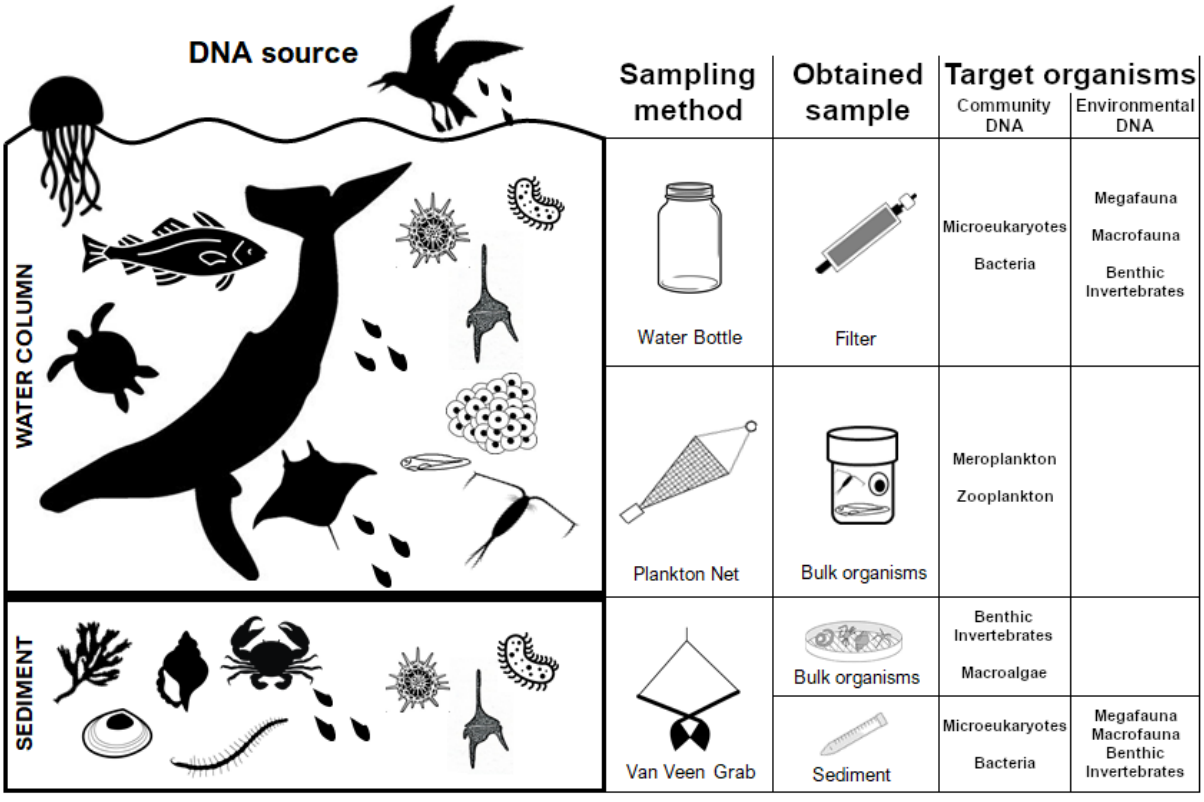


Fig. 1. Different methods for sampling marine ecosystems associated with their DNA source, type of sample obtained and target organisms. ~~Bold/italics indicate that the corresponding target~~Target

organisms are ~~sampled in~~ shown based on the source of the ~~form of environmental~~ DNA ~~and unformatted text sampled as whole organisms~~collected.

2. Environmental DNA in a fisheries context

The marine environment harbours a huge diversity of species [~~27~~24], ranging from large and charismatic whales to tiny worms and unicellular plankton (Fig. ~~1). Depending~~1). Compared to the sampling of eDNA in freshwater it also poses its own set of, often difficult to address, issues when trying to obtain unbiased samples, especially in relation to factors such, tides, currents, great depths and rapid movements of individuals in three dimensions. Thus, depending on the habitat and taxa of interest, ~~different~~various sampling methods are needed ~~to study, monitor, and provide information of relevance to the management of marine communities (Fig. 1). These sampling methods aim~~ to collect the full range of target species present at a given site so that, when possible, visual identification and quantification of the species is done~~.~~ to study, monitor, and provide information of relevance to the management of marine communities (Fig. 1).

Identification and characterization of these samples can be ~~either~~ accelerated ~~through~~using genetic techniques. These will differ depending on the source of the DNA ~~extraction of all~~obtained. In the first case, community DNA can be collected. This refers to the collection of whole communities of organisms in the sample ~~(referred to as community DNA) with~~from which DNA is extracted from the cells of the sampled individuals. Such analysis results in highly comparable results for monitoring and impact assessment, compared to traditional morphological analyses [~~28, 29~~25, 26] and at a fraction of the time and cost ~~[28]. Or the use of eDNA~~[25]. In the second case, organisms are not directly sampled, rather extraorganismal DNA in the environment (eDNA) is collected and used to infer a species presence. The use of eDNA in this way may even further simplify sampling and increase throughput, decreasing the costs and allowing for large scale surveys of marine ecosystems.

Traces of DNA in the water column and in the sediment can be used to identify species and characterize communities ~~[e.g. 30]~~[e.g. 27], to investigate their ~~movement~~distribution ~~[e.g. 31]~~[e.g.

28], and to determine their abundance [e.g. 32][e.g. 29]. Both community DNA and eDNA data are affected by technical (e.g. DNA marker choice, DNA primer choicelaboratory assay choices, incomplete reference databasedatabases) and biological (e.g. size of the organisms) biases, which should be taken into account when interpreting the data for fisheries management and ecosystem monitoring [3330]. In addition,. While the distribution of the entire organisms collected during community DNA surveys is, of course, affected by environmental parameters, extracellular eDNA is especially sensitive to such factors. eDNA data are alsois thus influenced by environmental factors such as water temperature, organic matter, pH, UV radiation, and water currents, and by the type and amount of material used during sampling [21].[17]. Further, as eDNA is used as a proxy for species presence, any biases in the transport and persistence of eDNA can result in its distribution being significantly different from that of the actual ornagisms. Careful evaluation of these biases is needed for the correct interpretation of eDNA results in the framework of fisheries management and conservation.

3. From water to results - the eDNA workflow and approaches

Identifying the presence of a particular species or characterizing the entire community from eDNA samples requires a series of steps that often need to be adjusted to each case study and fully understood in order to derive sound conclusions from the data obtained [3330]. Sampling eDNA in the marine environment is possible through water or sediment [34].[31]. It is however usually done by collecting water that is subsequently passed through variable pore size filters, generally < 1 µm pore size. It is also often common practice to add a prefiltering step (e.g. with a 3 µm prefilter) to avoid clogging the filtering process with large pieces of tissue or small animals such as zooplankton [35].[32]. Water samples from the marine environment can be collected using procedures that span from the simple act of using a bucket to collect surface samples to a more sophisticated procedure involving the use of Niskin bottles [36][33] or rosette samplers [37] to capture samples at greater depths.[34] to capture samples at greater depths. In all cases, strict procedures to avoid cross-contamination between samples are needed along with proper preservation and storage for filters containing eDNA

prior to laboratory analysis. While applications are diverse, approaches using eDNA can be categorised into three groups based on their main objectives: *1) Targeted Species Detection*, to detect the presence or absence of a single or a limited number of defined targeted species at a location; *2) Community Characterisation*, to produce an inventory of the biodiversity of an ecosystem; and *3) Species Abundance Estimation*, to inform on absolute and/or relative abundance of species at the sampling location. An overview of the three groups is presented below, detailing their objectives, strengths and limitations. Selected examples of each technique are also outlined in Tables 1-3 to show typical situations where they have been utilised.

3.1. Targeted species detection

Perhaps the most developed and utilised eDNA application is the detection of individual species and/or small groups of targeted species of interest in an ecosystem. Targeted species detection from eDNA involves the development of genetic probes designed to match explicitly the target species DNA, and distinguish the target from other species potentially present in a sample using classical genomic Sanger sequencing [13, 35, 36] and/or quantitative real time PCR (qPCR) ~~[38]. Real time PCR~~[37]. Marker amplification is achieved by the use of DNA probes, which allow the genetic code of specific sections of the genome to be examined, and resulting unique species-specific genetic sequences. qPCR is based on detection and quantification of a fluorescent light signal produced by binding of a dye-labelled species-specific probe, during amplification, to the target species DNA sequence present in a sample ~~[39].~~[38]. Detection of small groups of species using qPCR can be achieved by combining (multiplexing) probes for these species, labelled with different fluorescent dyes, in a single reaction.

Table 1

Selected applications of targeted species detection using marine eDNA.

Application	Example study outline	Example

Detection and mapping of the spread of invasive or non-native species	~~[43]~~Invasive slipper shell on the European Atlantic coast	[39]
Identification and monitoring of rare/endangered species	~~[39]~~White sharks in the open ocean	[34]
Detection of cryptic species	~~[45]~~Cryptic seahorse species off western Australia	[40]
Biosecurity during import/export	~~[40]~~Ornamental fish imports	[41]
Investigating spawning activity	~~[47]~~Spawning ecology of the Japanese eel	[42]
~~Monitoring of high diversity (multispecies) environments~~		~~[48]~~
Monitoring of hard to access environments	~~[49]~~Deep-sea octocorals using remote submersibles	[43]

~~Table 1.~~ ~~Selected applications of targeted species detection using marine eDNA.~~

Applications are varied and are detailed with examples in Table 1. It can be ~~seen~~observed from these examples that targeted species detection has shown its usefulness across many and varied situations of fishery management and ecosystem monitoring. Marine monitoring using traditional methods such as individual capture (with e.g. trawls, nets~~,~~ and traps ~~etc~~) and visual surveys are time consuming, costly to carry out and in some cases simply impossible. Investigations using eDNA have shown that in numerous situations ~~this tool~~the approaches have the potential to add to the available information to inform a variety of management questions. Adding value to traditional programmes is, perhaps, the most cost-effective way to integrate eDNA screening into routine management and monitoring programmes (see below). However, in some specific situations the use of eDNA has the potential to replace traditional monitoring. For this to occur a number of technical and validation steps are required such as comparisons between eDNA and visual survey data in context, controls for type I (false-positive) and type II (false negative) errors, validation of experimental results in the laboratory, scaling up versus one-off sample collection, temporal and spatial replicates (see below). If such steps are successful, targeted species detection using eDNA has shown that it can fulfil the requirements of fishery and ecosystem monitoring programmes and can be used as an alternative approach to answer relevant questions for managers.

Box 1. Case study – Targeted species detection – eDNA and ecology of commercially important food species [42]

The catadromous Japanese eel *Anguilla japonica* is an important food fish in East Asia, where after spawning at sea and migrating to freshwater it is raised in aquaculture ponds. Intensive research including sampling with large plankton and trawl nets, genetic species identification of eggs and newly hatched larvae, and direct observations using deep-tow camera systems has led to the discovery of the eel's spawning area. Such approaches have provided useful information on the spawning area of Japanese eels. However, their precise spawning sites and ecology still remain largely unknown, in part due to the significant depths and vast scale of the possible survey areas and the need to narrow down the search areas.

In order to address these issues, species-specific genetic probes were developed and tested in the laboratory by filtering and extracting eDNA from tank water containing eels. This showed that the probes could identify the Japanese eel from a minute amount of eDNA. Samples were collected at varying depths during an ocean survey on the southern West Mariana Ridge in the general spawning area of the eel. eDNA positive signals were detected for *A. japonica* from 3 of the 108 samples.

This first attempt to detect Japanese eel eDNA suggests the approach has the potential to provide information in near real-time about the spawning aggregations in a deep-water environment which is very challenging to survey using traditional techniques.

3.2. Community characterisation

Community characterisation, often referred to as community metabarcoding, is a technique used to characterise either the species composition or a selected subset of species, whose eDNA is represented in a water sample [~~41, 42~~44, 45]. Using this approach, a region of DNA conserved within a species and diverse across a wide range of taxa is specifically targeted and many targets are ~~then~~ captured simultaneously in a single reaction. Amplified products are ~~then~~ sequenced, revealing unique species-specific signatures (i.e. a barcode for that species) within a sample and sequences are ~~then~~ compared to reference sequences within a database. As such, each unique sequence match between

the sample and the reference database will identify DNA from a specific species ~~being present~~ in the sample ~~[43].~~[46]. Metabarcoding has been utilized in a variety of settings, showing a broad potential application for biodiversity monitoring (Table 2).

Table 2

Selected applications of community characterisation using marine eDNA.

Application	Example study outline	Example
Fish diversity	~~[54]~~Fish community composition in a large (120,000 km^2) area of the NE Atlantic	[47]
Identification of new species in an area	~~[55]~~Detection of a number of invasive, cryptic and observations of species for the first time in the North Sea	[48]
Connection of life stages	~~[56]~~Linking distributions of adult and immature stages of South African marine fish species	[49]
Clarification of feeding behaviour	Characterisation of prey species of invasive lionfish through gut content analysis in the Mexican Caribbean	[50]
Ecosystem food-web structure and dynamics	~~[58]~~Characterisation of community structure of Japanese coastal waters	[51]
The impact of aquaculture on benthic communities	Comparison of benthic Foraminifera communities at different distances from aquaculture sites	[52]
Identification of non-indigenous species in ballast/harbour water	~~[60]~~Detection of the transfer of North Sea molluscs across tropical waters in ballast water	[53]
Monitoring of marine vertebrates	~~[61]~~Distribution in space and water column of marine vertebrates in Monterey Bay	[54]
Habitat preference	~~[62]~~Fine-scale geographic and temporal mapping of marine fish populations in the Hudson River estuary	[55]
Characterisation of non-indigenous species	~~[63]~~Detection of introduced and newly observed resident marine species around southern Britain	[27]
Biodiversity assessment ~~of~~ marine sanctuaries	~~[64]~~Characterisation of pelagic and benthic eukaryotic biodiversity in the Florida Keys National Marine Sanctuary	[56]

~~Ballast water monitoring~~ ~~[44]~~

eDNA metabarcoding is well established in providing unique insights into the diversity and functioning [57] of aquatic ecosystems. Such applications have allowed the characterisation of fish communities in freshwater [e.g. 58] and marine [e.g. 59] environments, including pelagic [e.g. 60] and benthic communities [e.g. 61]. Together with such an often-unique ability to characterise entire communities, metabarcoding has also been used in a more applied way to answer specific questions of interest to managers and policy makers. These include investigations of the impact of aquaculture on local bottom communities, the transfer of non-indigenous and invasive species in ballast and harbour water, and monitoring of marine vertebrates (Table 2). Where targeted species detection using eDNA allows specific species to be examined, aquatic eDNA metabarcoding allows the cost-effective characterisation of entire communities, and therefore it is especially useful in ecosystem monitoring scenarios.

Box 2. Case study – Community characterisation – fish biodiversity assessment using eDNA over large oceanic areas [47]

Traditional methods of monitoring marine fish diversity rely on trawling surveys. These are costly, time-consuming and, especially in complex environments, may be biased in the species they capture with only a sub-set being targeted. Community characterisation using eDNA has the potential to address some of these shortcomings by, in theory, being able to identify all species in an area using the eDNA they shed into the environment.

In order to test this hypothesis, an eDNA based metabarcoding approach was used to characterise the species present across a 120,000 km^2 area of the Northeast Atlantic using eDNA filtered from water samples. Species specific genetic sequences were obtained from the eDNA which were identified through matches in reference databases. The results of this analysis were compared to traditional trawl surveys carried out simultaneously to the water sampling.

It was found that trawl and eDNA samples resulted in the same most abundant species (European anchovy, European pilchard, Atlantic mackerel, and blue whiting), but eDNA metabarcoding resulted in more detected bony fish and elasmobranch species (116) than trawling (16). The eDNA metabarcoding approach was thus seen to capture the biodiversity present in the area at least as good, and with some groups of species better, than traditional techniques. The findings support the integration of eDNA metabarcoding for broad-scale marine fish diversity monitoring in the context of Directives such as the Common Fisheries Policy or the Marine Strategy Framework Directive.

3.3. Species Abundance Estimation

~~**Table 2.** Selected applications of community characterisation using marine eDNA.~~

~~eDNA metabarcoding has a long history of providing unique insights into the diversity and functioning [45] of aquatic ecosystems. Such applications have allowed the characterisation of fish communities in freshwater [e.g. 46] and marine [e.g. 47] environments, including pelagic [e.g. 48] and benthic communities [e.g. 49]. Together with such an often unique ability to characterise entire communities, metabarcoding has also been used in a more applied way to answer specific questions of interest to managers and policy makers. These include investigations of the impact of aquaculture on local bottom communities, the transfer of non-indigenous and invasive species in ballast and harbour water, and monitoring of marine vertebrates (Table 2). Where targeted species detection using eDNA allows specific species to be examined, aquatic eDNA metabarcoding allows the cost-effective characterisation of entire communities, and therefore it is especially useful in ecosystem monitoring scenarios.~~

~~*3.3. Species Abundance Estimation*~~

Together with the identification of both individual and ecosystem-based biodiversity, eDNA can be used to estimate either the relative abundance of multiple species using metabarcoding [~~50~~62], or the absolute abundance of individual species using qPCR ~~[51].~~[63]. At its simplest, such approaches involve quantifying the amount of eDNA from a species represented in a sample and using that as a simple proxy for abundance ~~[52]. Such information may then~~[64]. Such information may be used to estimate numbers of individuals and/or biomass. The use of eDNA-based tools to quantify stocks of species of interest is of course of great interest to fishery managers and policy makers, as population or stock assessment is a central component of any management and/or conservation programme. Estimating absolute counts and/or biomass, relies on the establishment of a robust correlation between DNA concentration and living biomass whereas relative biomass estimates assume that the relative amounts of DNA measured in the sample are representative of the relative abundance of the different species in the ecosystem. While both approaches may seem to rely on fairly simple calculations and indeed are beginning to be used (Table 3), in practice, there are many factors which interact to make the relationships upon which the assumptions about the correlations are made very complex to disentangle and to obtain robust estimates.

Table 3

Selected applications of abundance estimation using marine eDNA.

Application	Example study outline	Example
~~Biodiversity and abundance~~	~~[53]~~	
Seasonal fish abundance	Seasonal relative fish species abundance in the Hudson River estuary	~~[62]~~[55]
Marine vertebrate abundance	~~[83]~~Vertebrate relative abundance in a kelp forest off the Monterey Peninsula	[65]
Monitoring pathogen abundance in aquaculture	Relative abundance of two parasite species on salmon farms	[66]
Monitoring ~~deepwater~~deep water species	~~[70]~~Relative abundances of Subarctic, deep water fish species from the	[62]

	continental slope off Southwest Greenland	
~~Abundance of non-indigenous~~Invasive species abundance	~~[84]~~Temporal abundance of invasive Codium seaweed in the Bay of Biscay	[67]
Stock assessment	~~[74]~~Biomass estimation of Atlantic cod in oceanic waters around the Faroe Islands	[29]
~~Spawning activity monitoring~~	~~[54]~~	

~~**Table 3.** Selected applications of abundance estimation using marine eDNA.~~

Applications of using eDNA to assess abundance in the aquatic environment are at present most advanced in freshwater [~~50~~62]. Abundance estimation using traditional methods such as gillnet data and trawling provides a relative index assumed to be directly proportional to density/absolute abundance [~~52, 55, 56~~29, 64, 68]. Such traditional non-genetic methods are the most common to estimate fish abundance in lakes for fisheries management ~~[57]~~[69] and biodiversity characterisation ~~[58]~~[70], although they are often expensive, time consuming and destructive. Initial results from experimental aquaria and ponds show positive correlations between species abundance and eDNA concentration [~~59, 60~~71, 72]~~. However, even in controlled tank situations, it has been found that "…quantification of eDNA samples can be highly variable even when sampling from the same individual under controlled conditions" [60]. Approaches have now moved from the experimental set-up to the field. The abundance of individual targeted species has been characterised using eDNA in freshwater fish species including lake trout (*Salvelinus namaycush*) [52], common carp (*Cyprinus carpio*)~~. However, even in controlled tank situations, it has been found that "…quantification of eDNA samples can be highly variable even when sampling from the same individual under controlled conditions" [72]. Approaches have now moved from the experimental set-up to the field. The abundance of individual targeted species has been characterised using eDNA in freshwater fish species including lake trout (*Salvelinus namaycush*) [64], common carp (*Cyprinus carpio*) [~~61~~73]~~ and Atlantic salmon (*Salmo salar*) [62]. Similarity between relative and absolute abundance has been reported in communities including both amphibians [63] and fish~~ and Atlantic salmon (*Salmo salar*) [74]. Similarity

between relative and absolute abundance has been reported in communities including both amphibians [75] and fish [~~64, 65~~55, 76] ~~including commercially important species such as Atlantic cod (*Gadus morhua*) [56].~~

, including commercially important species such as Atlantic cod (*Gadus morhua*) [29].

Box 3. Case study – environmental DNA and quantitative assessment of commercial fish species [29]

~~**Box 1. Case study - environmental DNA and quantitative assessment of commercial fish species** [56]~~

Traditionally, standardised trawl surveys are used as an effective monitoring tool for management of commercial fisheries, providing valuable estimates of quantity (biomass) and spatial distribution of fish stocks. Such surveys, however, are costly and have other associated biases and drawbacks such as gear and ground selectivity and negative impact on habitats.

In order to determine the utility of eDNA for assessing commercial stocks a quantitative eDNA survey of Atlantic cod ~~(*Gadus morhua*)~~ was compared to results from a standardised demersal trawl survey. Important stock metrics such as regional cod biomass and Catch Per Unit Effort (CPUE) were determined using traditional assessment analysis of trawl data. At 35 trawl stations water samples were also collected 4 m above the seafloor and eDNA analysed in the laboratory using cod-specific DNA probes.

There was an overall 80 % concordance between trawl and eDNA cod detection, with good spatial conformity between the two approaches. Nearly 70 % of all discrepancies in the detection of Atlantic cod were at the sampling stations where actual or predicted Atlantic cod catch rates were very low (≤ 3 fish h^{-1}). Similarly, there were also significant positive correlations between the regional integrals of cod biomass (kg) and eDNA quantities (copies) and between sampling effort-normalised CPUE and eDNA concentrations.

This study shows that eDNA monitoring can provide valuable spatial and abundance information which is comparable to traditional standardised trawl data but less costly and with less

impact on the environment. The findings reinforce the opportunities for the incorporation of approaches utilising eDNA into stock biomass assessments of commercially important fish stocks.

In the marine environment, abundance estimates using eDNA, while inherently more difficult than a relatively enclosed freshwater ecosystem, are ~~increasingly reliable~~starting to be examined (Table 3). Approaches are developing rapidly and, while at present robust relationships between abundance quantification using eDNA and more traditional methods are sometimes weak [~~50, 66, 67~~62, 77, 78], in some cases the approach seems to be comparable to that of other quantitative methods [~~56, 68~~29, 79]. The inherent uncertainty in the robustness of biomass quantification when utilising eDNA approaches is due to both the assumptions on which the technique rests and the impact of extraneous factors on such assumptions. eDNA abundance quantification relies on the assumption that local population numbers may be inferred by measuring the concentration of eDNA at a given locality and that this estimation represents the quantitative relation between eDNA concentration and the underlying population size [~~68, 69~~79, 80]. However, such a relationship may not be always true, or even present in most cases. The amount of eDNA at a location will vary depending on a number of biological, physical and environmental factors (see below). While these factors also have an impact on species detection, the impact of the fluctuations registered is higher if quantitative measurements are being attempted, rather than simple presence/absence results. Nevertheless, it may be possible to incorporate these impacts into modelling, to better predict how they can affect eDNA concentrations, therefore reducing the variance around such quantifications [~~68, 70-72~~79, 81-83]. However, due to the complexity of interacting factors, direct quantitative assessments remain highly challenging in marine ecosystems [~~21, 73~~17, 84].

Abundance estimates in the marine environment can thus be summarised to be very much in the developmental stage at the moment, notwithstanding some of the early applications being examined. Significant questions still have to be addressed to allow the amount of eDNA collected to be linked directly to either relative or absolute abundances. The three-dimensional nature of the

environment, together with the many physical, chemical and environmental factors whose impacts have to be quantified means that the validity of abundance quantification using eDNA is still to be determined in most if not all situations. Significant work is, however, being undertaken around the world to determine if the method can be developed into a useful tool as, if so, it might in the future provide a very cost-effective approach. At present, however, the jury is still out if this will be possible.

4. Considerations

Analysis of eDNA allows inferences to be made about organisms, without the need to see, observe or handle them. This is the major advantage offered by this approach, but also potentially a drawback. In order to make the most informed decisions and use eDNA approaches to their fullest, ~~potential~~ managers and policy makers should be aware of the issues to be considered when seeking to understand the results of eDNA surveys. Although eDNA based applications are relatively new, especially in the context of marine management, scientists have a good understanding of the drawbacks of this method, hence have been able to define the actions needed in order to limit errors and uncertainties [~~74-76~~85-87].

~~An important consideration in any eDNA monitoring programme is the avoidance of contamination [77]. DNA molecules are everywhere around us, and if they enter eDNA samples they have the potential to produce false positives. The use of sterile equipment, gloves, and a dedicated eDNA laboratory (with strict protocols, controls and necessary separations of processes handling high and low DNA templates) are necessary measurements to be taken in order to reduce contaminations and resulting false positives [75]. It is possible to control for contamination, by taking multiple replicates (usually three) of the same samples, and by using negative controls (i.e. sterilised distilled water samples not containing any actual material) at every stage of the process (field and laboratory blanks for DNA extraction and amplification) [77]. Any DNA that results from these blanks (and there is likely to be some), is then 'subtracted' from the results of the actual samples. Thus, like in any other monitoring approach, standardization is crucial, especially when it comes to techniques of collection,~~

~~essential negative control sample inclusion[78] and laboratory analysis [79], as well as the interpretation of results [80].~~

An important consideration in any eDNA monitoring programme is the avoidance of contamination [88]. DNA molecules from many sources are everywhere around us, and if they enter eDNA samples they have the potential to produce false positives. The use of sterile equipment, gloves, and a dedicated eDNA laboratory (with strict protocols, controls and necessary separations of processes handling high and low DNA templates) are necessary measurements to be taken in order to reduce contaminations and resulting false positives [86]. It is possible to control for contamination, by taking multiple replicates (usually three) of the same samples, and by using negative controls (i.e. sterilised distilled water samples not containing any actual material) at every stage of the process (field and laboratory blanks for DNA extraction and amplification) [88]. Any DNA that results from these blanks (and there is likely to be some), is then 'subtracted' from the results of the actual samples. Thus, like in any other monitoring approach, standardization is crucial, especially when it comes to techniques of collection, essential negative control sample inclusion [89] and laboratory analysis [90], as well as the interpretation of results [91].

Another important consideration (which can be a significant drawback in certain situations) is the availability of DNA reference sequences, or a reference database ~~[81].~~of taxonomically identified species/groups [92]. Matching sequences obtained from actual eDNA samples against a reference database is the final step in the workflow, one that will tell the user what species the sampled eDNA belongs to. The reliability of such databases~~ is thus~~, together with the availability of high-quality reference sequences of previously examined and taxonomically identified organisms is crucial for robust data interpretation and to avoid false negatives and positives. There are a number of databases that can be used, with the Barcode of Life Data System (iBOL~~)~~) [~~82~~93] being an important example. Yet, it is advisable, when embarking on an eDNA project, to invest time assessing the reliability of the databases for the geographic area and taxa investigated, and if required, build a project-specific quality-controlled database.

Another pivotal consideration when interpreting results is that of eDNA transport. As mentioned above, eDNA offers a snapshot of the species presence in a certain habitat in a given timeframe. Environmental DNA sampled might indeed come from the organisms that live in the sampled area at that time, but it might also originate from degrading tissue, eggs and sperm and, depending on environmental conditions, it might have simply been transported from elsewhere with the currents or tides. Many researchers are now concentrating their efforts into understanding how long these molecules can persist in the environment and remain detectable [reviewed in ~~21~~17].

5. Integration into existing management and monitoring programmes

The development of new approaches to gather information of relevance to fisheries and ecosystem monitoring through the use of eDNA sampling methods, and the associated novel insights such approaches generate, has the potential to revolutionise the information available to managers. However, together with the requirement for the new methods to be able to provide robust results, there is also a need to investigate the practicalities and cost-benefit of incorporating the new techniques into standardised monitoring surveys [~~83, 84~~94, 95]. In some situations, for example, the requirement for targeted detection of specific species, it may be necessary to develop novel surveying programmes. However, by far the most preferred situation would be if the added value could be ~~brought to~~embedded into existing survey programmes, through the addition of the collection of eDNA samples, potentially requiring relatively little extra cost/effort on top of that already being ~~employed. Pelagic trawl~~invested. This is especially relevant as ship-based survey costs increase while genetic screening costs are decreasing. Trawl surveys may be able to be supplemented by simultaneous eDNA collection from water samples, and benthic sediment monitoring by eDNA collection from grab samples. Indeed, in many if not most, often costly, traditional fishery and ecosystem monitoring surveys there would seem to be an ideal opportunity to collect such samples and add value in this way. It ~~would seem~~seems, therefore, that the design of future surveys, together with that of existing

programmes, should be evaluated in the light of the developments in eDNA approaches outlined above and the added value that the integration of these approaches could bring.

6. Conclusion

Rapid developments in the field of eDNA analysis have provided a range of new tools for research scientists, and fishery and ecosystem managers. With such developments, it is not straightforward for the manager to disentangle which tools can provide robust evidence ~~for them~~ to incorporate into policy development discussions, and which are still in the developmental phase. In tandem, reports about such advances in the mainstream media drive stakeholders to question managers about the utility of the toolkits, ~~such~~including specific questions ~~again being~~that might be difficult to answer for a non-specialist ~~to answer~~. Here, we have attempted to provide a topic-based overview which goes some way to address this problem, and thus can be of use to inform managers of the strengths and weaknesses of the various approaches currently available.

Environmental DNA-based tools have, for a number of years now, been providing reliable evidence in areas such as single species detection, and the characterisation of ecosystem biodiversity. As such, they represent a robust, cost-effective, and in an increasing number of cases a more sensible option for managers and monitors for incorporation into their standard scientific toolkits. While significant advances have been, and continue to be, made in the use of eDNA to quantify both relative and absolute abundance, such analyses are less well developed and still suffer from uncertainties associated with various environmental, biological and methodological challenges of these techniques ~~[21].~~[17]. As these influences are studied and their impacts better understood such uncertainties will be reduced. However, at present their application is likely to be more limited.

Every scientific monitoring method has uncertainties and the field of eDNA research is no exception. However, in many cases such uncertainty is well understood and as such, and considering the potential significant benefits and potential cost-savings of the new tools available, managers and monitors should consider the integration of these approaches in their management planning

discussions along with the more traditional techniques. The different approaches can work together to provide complementary information ~~to~~. In the end they will allow enhanced scientific understanding, resulting in improved science-based policy development in view of ecosystem-based management.

7. Acknowledgments

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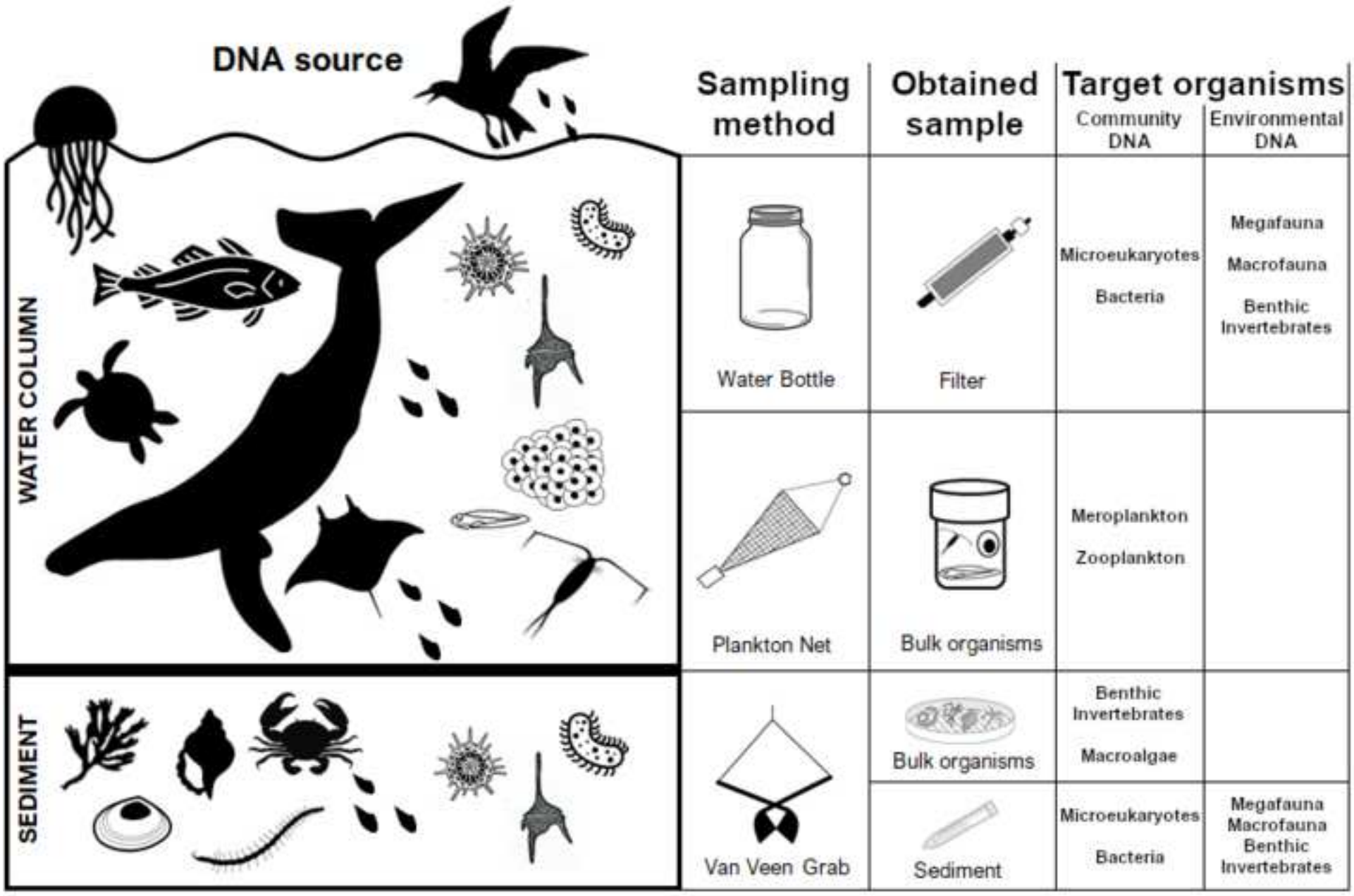
Figure1



JG led the ICES WGAGFA Terms of Reference on eDNA in Fisheries Management and Ecosystem Monitoring which resulted in the production of the manuscript. JG took the lead in writing the manuscript. All authors were involved in discussions and decisions to shape the manuscript and contributed to the writing of the final text.