

# Marine Policy

## Life in a drop: sampling environmental DNA for marine fishery management and ecosystem monitoring --Manuscript Draft--

<b>Manuscript Number:</b>	JMPO_2020_580R1
<b>Article Type:</b>	Full Length Article
<b>Keywords:</b>	environmental DNA; Fisheries; eDNA; Ecosystem; Management
<b>Corresponding Author:</b>	John Gilbey Pitlochry, Perthshire United Kingdom
<b>First Author:</b>	John Gilbey
<b>Order of Authors:</b>	John Gilbey Gary Carvalho Rita Castilho Ilaria Coscia Mark Coulson Geir Dahle Sofie Derycke Sara Francisco Sarah Helyar Torild Johansen Claudia Junge Kara Layton Jann Martinsohn Iveta Matejusova Joana Robalo Naiara Rodriguez-Ezpeleta Goncalo Silva Ilona Strammer Anti Vasemägi Filip Volckaert
<b>Abstract:</b>	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

	<p>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.</p>
<p><b>Suggested Reviewers:</b></p>	<p>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.</p> <p>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)</p> <p>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.</p> <p>Linda Laikre, PhD  Professor, Stockholm University: Stockholms Universitet  linda.laikre@popgen.su.se</p>
<p><b>Response to Reviewers:</b></p>	

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.

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

John Gilbey<sup>a\*</sup>; Gary Carvalho<sup>b</sup>; Rita Castilho<sup>c</sup>; Ilaria Coscia<sup>d</sup>; Mark W. Coulson<sup>e</sup>; Geir Dahle<sup>f</sup>; Sofie Derycke<sup>g</sup>; Sara M. Francisco<sup>h</sup>; Sarah J. Helyar<sup>i</sup>; Torild Johansen<sup>j</sup>; Claudia Junge<sup>j</sup>; Kara K.S. Layton<sup>k</sup>; Jann Martinsohn<sup>l</sup>; Iveta Matejusova<sup>m</sup>; Joana I. Robalo<sup>h</sup>; Naiara Rodríguez-Ezpeleta<sup>n</sup>; Gonçalo Silva<sup>h</sup>; Ilona Strammer<sup>o</sup>; Anti Vasemägi<sup>p</sup>; Filip A.M. Volckaert<sup>q</sup>

<sup>a</sup> Marine Scotland, Freshwater Fisheries Laboratory, Faskally, Scotland, U.K.

<sup>b</sup> Molecular Ecology and Fisheries Genetics Laboratory, School of Biological Sciences, Bangor University, Deiniol Road, Bangor, UK

<sup>c</sup> Centre of Marine Sciences, CCMAR, University of Algarve, Campus de Gambelas, P-8005-139, Faro, Portugal

<sup>d</sup> School of Science, Engineering and Environment, Peel Building, University of Salford, Salford, UK

<sup>e</sup> Fisheries and Oceans Canada, 200 Kent Street, Ottawa, Ontario, Canada

<sup>f</sup> Institute of Marine Research, P.O. Box 1870, Nordnes, NO-5817 Bergen, Norway

<sup>g</sup> Aquatic Environment and Quality, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Ankerstraat 1, B-8400 Oostende, Belgium

<sup>h</sup> MARE—Marine and Environmental Sciences Centre, ISPA, Instituto Universitário, Rua Jardim do Tabaco 34, P-1149-041 Lisboa, Portugal

<sup>i</sup> Institute of Global Food Security, School of Biological Science, Queen's University Belfast, Northern Ireland, UK

<sup>j</sup> Institute of Marine Research, Tromsø, Troms, Norway

<sup>k</sup> School of Biological Sciences, University of Aberdeen, Aberdeen, U.K.

<sup>l</sup> Water and Marine Resources, E.C. Joint Research Centre, TP051 – Bldg. 5a, Via Enrico Fermi 2749, I-21027 Ispra, Italy

<sup>m</sup> Marine Scotland, Marine Laboratory, Victoria Road, Aberdeen, U.K.

<sup>n</sup> AZTI, Marine Research, Basque Research and Technology Alliance (BRTA). Txatxarramendi ugarteaz/g, E-48395 Sukarrieta - Bizkaia

<sup>o</sup> Université Catholique de Louvain, Place de l'Université 1, B-1348 Louvain-la-Neuve, Belgium

<sup>p</sup> Department of Aquatic Resources, Swedish University of Agricultural Sciences, SE-702 15 Drottningholm, Sweden

<sup>q</sup> Laboratory of Biodiversity and Evolutionary Genomics, KU Leuven, Charles Deberiotstraat 32 - box 2439, B-3000 Leuven, Belgium

\* Corresponding author: John.Gilbey@gov.scot

Marine Scotland, Freshwater Fisheries Laboratory, Faskally, Scotland, U.K.

**Declarations of interest: none**

### Acknowledgments

This review is the result of discussion within the ICES Working Group on the Application of Genetics in Fisheries and Aquaculture (ICES-WGAGFA) and the authors wish to thank members for a most stimulating scientific environment. NRE's contribution has been funded by the Spanish Ministry of Science, Innovation and Universities through the EDAMAME ("Environmental DNA based approaches for marine and aquatic monitoring and evaluation") project (code CTM2017-89500-R). FAMV acknowledges the Scientific Research Network "Eco-evolutionary dynamics in natural and anthropogenic communities" (grant W0.037.10 N of the Research Foundation - Flanders).

# 1 Life in a drop: sampling environmental DNA for marine fishery management 2 and ecosystem monitoring

## 3 Abstract

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

22  
23 **Keywords:** Environmental DNA, eDNA, management, ecosystem, fisheries

25

26 **1. Introduction**

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

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

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
50 Effective fishery and ecosystem management rely on the identification and quantification of the  
51 species living a certain environment, that is, characterising its biodiversity. There are two significant  
52 limitations in gathering such information using traditional techniques: how to representatively sample  
53 the biodiversity in an ecosystem and how to identify individuals to species level? Sampling requires  
54 complicated logistics, is costly, is biased in its sampling coverage, and is especially difficult for species  
55 with low abundance and/or elusive species. Identification also requires taxonomic expertise, which is  
56 often lacking and difficult to apply in some cryptic species. The requirement to overcome such  
57 impediments has stimulated the search for new tools and approaches to integrate the various  
58 environmental dimensions in decision making into an evidence-based policy approach [8]. One such  
59 approach is utilisation of DNA collected from the environment to identify and/or quantify the species  
60 present in the ecosystem.

26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
61 Environmental DNA (eDNA) stems from individual organisms which release DNA into the  
62 environment through waste products, skin/tissue, scales, gametes, mucus, blood and carcasses [9-  
63 12]. This extra-organismal DNA is termed environmental DNA (eDNA) [13]. In contrast to DNA  
64 extracted from tissue samples, or community DNA – where DNA is extracted from communities of  
65 whole organisms - eDNA does not require sampling the target organisms themselves, but instead the  
66 sampling of the environment they live in [14, 15]. The development of new ways of monitoring marine  
67 ecosystems and marine biodiversity using eDNA has advanced over recent years and has  
68 revolutionised the ability to track invasive species, monitor endangered species, assess the health of  
69 fish stocks, and explore the world of marine biodiversity [16]. The seeming simplicity and cost-  
70 effectiveness of eDNA-based approaches, together with the interest from wider stakeholder groups,  
71 has made such applications highly attractive [17].

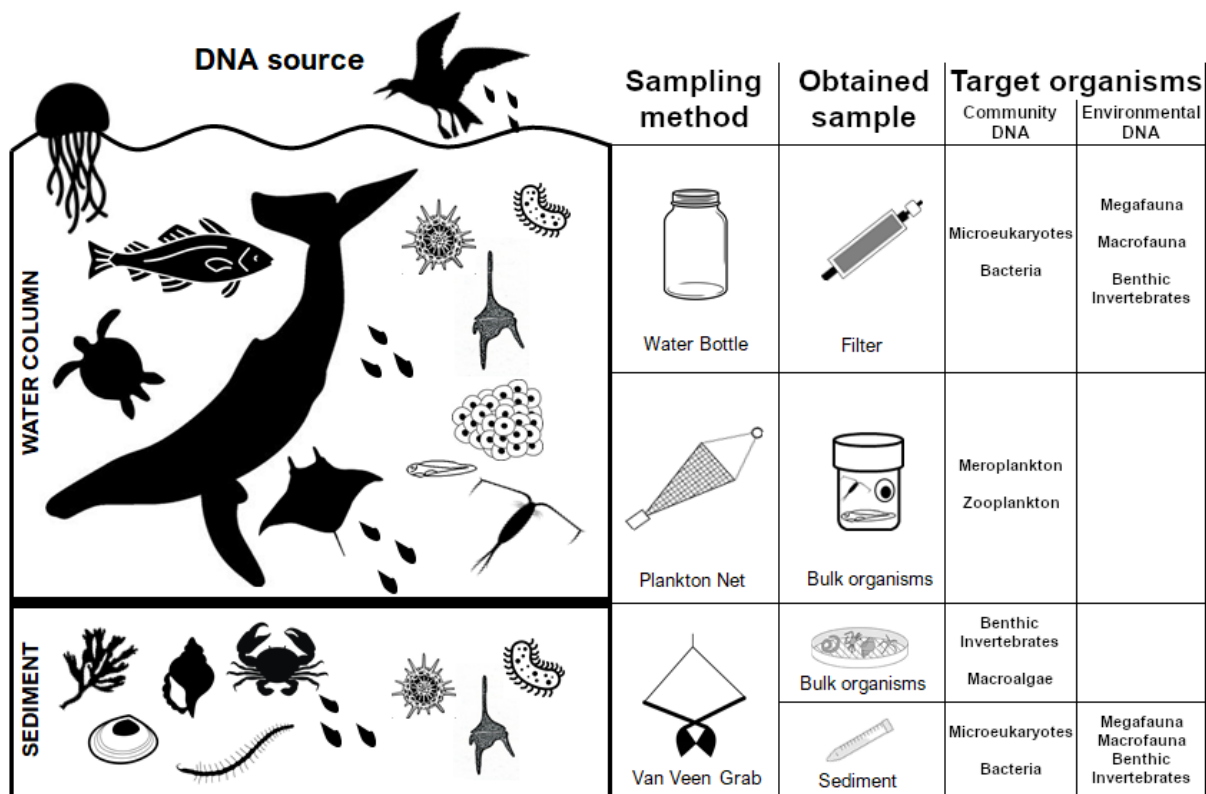
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65  
72 The development of genetic technologies to identify species and characterise whole  
73 communities through the collection and filtration of water and/or sediment sample is both a  
74 potentially invaluable tool for managers and an irresistible story for the popular press. Press articles  
75 focusing on such tools range from the very small, such as “New Nano Strategy Fights Superbugs” [18],

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
76 to the very large (and improbable) “Loch Ness Monster Hunters to Try DNA Search?” [19].  
77 Disentangling fact from fiction, and hyperbola from reality, is thus not a simple task for the manager  
78 striving to understand the field. As such this raises two opposing issues which could each negatively  
79 affect the ability to manage fisheries and monitor ecosystems using the most appropriate available  
80 scientific tools: the pre-emptive uptake of unproven approaches versus the failure to take advantage  
81 of robust new techniques. Stories in the press, together with questions from stakeholders, about new  
82 potential approaches that have been developed are often powerful incentives for major funding and  
83 uptake of these tools in practice [20]. Whilst in some cases this uptake may be justified, in others,  
84 especially in rapidly developing fields, such reliance may be potentially premature. However, each  
85 investment requires an accessible, robust and balanced evidence base as deriving management  
86 decisions on unproven and/or unreliable techniques brings obvious dangers and potential lack of trust  
87 in novel molecular technologies. Further, focusing effort and especially funding on such approaches  
88 means that other, perhaps more proven techniques with higher TRL (technology readiness levels) will  
89 be starved of resources. It is thus of particular importance that managers and policy makers can  
90 distinguish with confidence among approaches that although show promise, are at an early stage of  
91 validation.

37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
92 The converse of the dangers of using unproven tools is avoiding the utilisation of effective  
93 proven tools due to uncertainties about their efficacy. As scientific technologies develop it is often the  
94 case that some areas progress further and faster than others. Proven approaches emerge and begin  
95 to be utilised in limited applications. In order to take full advantage of such developments in a wider  
96 context, managers need a straightforward guideline explaining the potential of each molecular tool  
97 and its state of readiness for routine applications in order to navigate in the various information  
98 streams and stakeholder drivers they are exposed to.

54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65  
99 In order to bridge the information gap between the specialist and the manager, we provide here  
100 a non-technical synthesis of the evidence surrounding the use of eDNA based monitoring techniques  
101 for management of fisheries and ecosystems in the marine environment. It is not intended to be an

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



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

114  
 115 **2. Environmental DNA in a fisheries context**

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

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

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



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

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

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

168

169 3.1. Targeted species detection

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

181

182 **Table 1**

183 Selected applications of targeted species detection using marine eDNA.

184

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]

185

186 Applications are varied and are detailed with examples in Table 1. It can be observed from these  
187 examples that targeted species detection has shown its usefulness across many and varied situations  
188 of fishery management and ecosystem monitoring. Marine monitoring using traditional methods such  
189 as individual capture (with e.g. trawls, nets and traps) and visual surveys are time consuming, costly  
190 to carry out and in some cases simply impossible. Investigations using eDNA have shown that in  
191 numerous situations the approaches have the potential to add to the available information to inform  
192 a variety of management questions. Adding value to traditional programmes is, perhaps, the most  
193 cost-effective way to integrate eDNA screening into routine management and monitoring  
194 programmes (see below). However, in some specific situations the use of eDNA has the potential to  
195 replace traditional monitoring. For this to occur a number of technical and validation steps are  
196 required such as comparisons between eDNA and visual survey data in context, controls for type I  
197 (false-positive) and type II (false negative) errors, validation of experimental results in the laboratory,  
198 scaling up versus one-off sample collection, temporal and spatial replicates (see below). If such steps  
199 are successful, targeted species detection using eDNA has shown that it can fulfil the requirements of  
200 fishery and ecosystem monitoring programmes and can be used as an alternative approach to answer  
201 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.

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

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

222 3.2. Community characterisation

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

234 **Table 2**

235 Selected applications of community characterisation using marine eDNA.

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

1	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]
2			
3			
4	Connection of life stages	Linking distributions of adult and immature stages of South African marine fish species	[49]
5			
6			
7			
8	Clarification of feeding behaviour	Characterisation of prey species of invasive lionfish through gut content analysis in the Mexican Caribbean	[50]
9			
10			
11			
12	Ecosystem food-web structure and dynamics	Characterisation of community structure of Japanese coastal waters	[51]
13			
14			
15	The impact of aquaculture on benthic communities	Comparison of benthic Foraminifera communities at different distances from aquaculture sites	[52]
16			
17			
18			
19	Identification of non-indigenous species in ballast/harbour water	Detection of the transfer of North Sea molluscs across tropical waters in ballast water	[53]
20			
21			
22			
23	Monitoring of marine vertebrates	Distribution in space and water column of marine vertebrates in Monterey Bay	[54]
24			
25			
26	Habitat preference	Fine-scale geographic and temporal mapping of marine fish populations in the Hudson River estuary	[55]
27			
28			
29			
30	Characterisation of non-indigenous species	Detection of introduced and newly observed resident marine species around southern Britain	[27]
31			
32			
33			
34	Biodiversity assessment- marine sanctuaries	Characterisation of pelagic and benthic eukaryotic biodiversity in the Florida Keys National Marine Sanctuary	[56]
35			
36			
37			

237

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

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

249

**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<sup>2</sup> 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

273 Together with the identification of both individual and ecosystem-based biodiversity, eDNA can  
 1  
 2 274 be used to estimate either the relative abundance of multiple species using metabarcoding [62], or  
 3  
 4 275 the absolute abundance of individual species using qPCR [63]. At its simplest, such approaches involve  
 5  
 6  
 7 276 quantifying the amount of eDNA from a species represented in a sample and using that as a simple  
 8  
 9 277 proxy for abundance [64]. Such information may be used to estimate numbers of individuals and/or  
 10  
 11 278 biomass. The use of eDNA-based tools to quantify stocks of species of interest is of course of great  
 12  
 13  
 14 279 interest to fishery managers and policy makers, as population or stock assessment is a central  
 15  
 16 280 component of any management and/or conservation programme. Estimating absolute counts and/or  
 17  
 18  
 19 281 biomass, relies on the establishment of a robust correlation between DNA concentration and living  
 20  
 21 282 biomass whereas relative biomass estimates assume that the relative amounts of DNA measured in  
 22  
 23 283 the sample are representative of the relative abundance of the different species in the ecosystem.  
 24  
 25  
 26 284 While both approaches may seem to rely on fairly simple calculations and indeed are beginning to be  
 27  
 28 285 used (Table 3), in practice, there are many factors which interact to make the relationships upon which  
 29  
 30  
 31 286 the assumptions about the correlations are made very complex to disentangle and to obtain robust  
 32  
 33 287 estimates.

34  
 35 288

37  
 38 **Table 3**

39  
 40 290 Selected applications of abundance estimation using marine eDNA.

41  
 42 291

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]

1 2 3 4 5	Invasive species abundance	Temporal abundance of invasive Codium seaweed in the Bay of Biscay	[67]
6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45	Stock assessment	Biomass estimation of Atlantic cod in oceanic waters around the Faroe Islands	[29]

---

292

293 Applications of using eDNA to assess abundance in the aquatic environment are at present most  
 294 advanced in freshwater [62]. Abundance estimation using traditional methods such as gillnet data and  
 295 trawling provides a relative index assumed to be directly proportional to density/absolute abundance  
 296 [29, 64, 68]. Such traditional non-genetic methods are the most common to estimate fish abundance  
 297 in lakes for fisheries management [69] and biodiversity characterisation [70], although they are often  
 298 expensive, time consuming and destructive. Initial results from experimental aquaria and ponds show  
 299 positive correlations between species abundance and eDNA concentration [71, 72]. However, even in  
 300 controlled tank situations, it has been found that “...quantification of eDNA samples can be highly  
 301 variable even when sampling from the same individual under controlled conditions” [72]. Approaches  
 302 have now moved from the experimental set-up to the field. The abundance of individual targeted  
 303 species has been characterised using eDNA in freshwater fish species including lake trout (*Salvelinus*  
 304 *namaycush*) [64], common carp (*Cyprinus carpio*) [73] and Atlantic salmon (*Salmo salar*) [74]. Similarity  
 305 between relative and absolute abundance has been reported in communities including both  
 306 amphibians [75] and fish [55, 76], including commercially important species such as Atlantic cod  
 307 (*Gadus morhua*) [29].

308

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

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



314 In order to determine the utility of eDNA for assessing commercial stocks a quantitative eDNA  
1  
2 315 survey of Atlantic cod was compared to results from a standardised demersal trawl survey. Important  
3  
4  
5 316 stock metrics such as regional cod biomass and Catch Per Unit Effort (CPUE) were determined using  
6  
7 317 traditional assessment analysis of trawl data. At 35 trawl stations water samples were also collected  
8  
9 318 4 m above the seafloor and eDNA analysed in the laboratory using cod-specific DNA probes.

11 319 There was an overall 80 % concordance between trawl and eDNA cod detection, with good  
12  
13  
14 320 spatial conformity between the two approaches. Nearly 70 % of all discrepancies in the detection of  
15  
16 321 Atlantic cod were at the sampling stations where actual or predicted Atlantic cod catch rates were  
17  
18  
19 322 very low ( $\leq 3$  fish  $h^{-1}$ ). Similarly, there were also significant positive correlations between the regional  
20  
21 323 integrals of cod biomass (kg) and eDNA quantities (copies) and between sampling effort-normalised  
22  
23 324 CPUE and eDNA concentrations.

26 325 This study shows that eDNA monitoring can provide valuable spatial and abundance  
27  
28 326 information which is comparable to traditional standardised trawl data but less costly and with less  
29  
30  
31 327 impact on the environment. The findings reinforce the opportunities for the incorporation of  
32  
33 328 approaches utilising eDNA into stock biomass assessments of commercially important fish stocks.  
34  
35

36 329  
37  
38 330 In the marine environment, abundance estimates using eDNA, while inherently more difficult  
39  
40 331 than a relatively enclosed freshwater ecosystem, are starting to be examined (Table 3). Approaches  
41  
42  
43 332 are developing rapidly and, while at present robust relationships between abundance quantification  
44  
45 333 using eDNA and more traditional methods are sometimes weak [62, 77, 78], in some cases the  
46  
47 334 approach seems to be comparable to that of other quantitative methods [29, 79]. The inherent  
48  
49  
50 335 uncertainty in the robustness of biomass quantification when utilising eDNA approaches is due to both  
51  
52 336 the assumptions on which the technique rests and the impact of extraneous factors on such  
53  
54 337 assumptions. eDNA abundance quantification relies on the assumption that local population numbers  
55  
56  
57 338 may be inferred by measuring the concentration of eDNA at a given locality and that this estimation  
58  
59 339 represents the quantitative relation between eDNA concentration and the underlying population size  
60  
61  
62  
63  
64  
65

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

340 [79, 80]. However, such a relationship may not be always true, or even present in most cases. The  
341 amount of eDNA at a location will vary depending on a number of biological, physical and  
342 environmental factors (see below). While these factors also have an impact on species detection, the  
343 impact of the fluctuations registered is higher if quantitative measurements are being attempted,  
344 rather than simple presence/absence results. Nevertheless, it may be possible to incorporate these  
345 impacts into modelling, to better predict how they can affect eDNA concentrations, therefore  
346 reducing the variance around such quantifications [79, 81-83]. However, due to the complexity of  
347 interacting factors, direct quantitative assessments remain highly challenging in marine ecosystems  
348 [17, 84].

21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

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

358

#### 359 **4. Considerations**

47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

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

366 drawbacks of this method, hence have been able to define the actions needed in order to limit errors  
1  
2 367 and uncertainties [85-87].  
3

4 368 An important consideration in any eDNA monitoring programme is the avoidance of  
5  
6  
7 369 contamination [88]. DNA molecules from many sources are everywhere around us, and if they enter  
8  
9 370 eDNA samples they have the potential to produce false positives. The use of sterile equipment, gloves,  
10  
11 371 and a dedicated eDNA laboratory (with strict protocols, controls and necessary separations of  
12  
13 372 processes handling high and low DNA templates) are necessary measurements to be taken in order to  
14  
15 373 reduce contaminations and resulting false positives [86]. It is possible to control for contamination, by  
16  
17 374 taking multiple replicates (usually three) of the same samples, and by using negative controls (i.e.  
18  
19 375 sterilised distilled water samples not containing any actual material) at every stage of the process  
20  
21 376 (field and laboratory blanks for DNA extraction and amplification) [88]. Any DNA that results from  
22  
23 377 these blanks (and there is likely to be some), is then 'subtracted' from the results of the actual samples.  
24  
25 378 Thus, like in any other monitoring approach, standardization is crucial, especially when it comes to  
26  
27 379 techniques of collection, essential negative control sample inclusion [89] and laboratory analysis [90],  
28  
29 380 as well as the interpretation of results [91].  
30  
31  
32  
33  
34

35 381 Another important consideration (which can be a significant drawback in certain situations) is  
36  
37 382 the availability of DNA reference sequences, or a reference database of taxonomically identified  
38  
39 383 species/groups [92]. Matching sequences obtained from actual eDNA samples against a reference  
40  
41 384 database is the final step in the workflow, one that will tell the user what species the sampled eDNA  
42  
43 385 belongs to. The reliability of such databases, together with the availability of high-quality reference  
44  
45 386 sequences of previously examined and taxonomically identified organisms is crucial for robust data  
46  
47 387 interpretation and to avoid false negatives and positives. There are a number of databases that can  
48  
49 388 be used, with the Barcode of Life Data System (iBOL) [93] being an important example. Yet, it is  
50  
51 389 advisable, when embarking on an eDNA project, to invest time assessing the reliability of the  
52  
53 390 databases for the geographic area and taxa investigated, and if required, build a project-specific  
54  
55 391 quality-controlled database.  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

392 Another pivotal consideration when interpreting results is that of eDNA transport. As  
1  
2 393 mentioned above, eDNA offers a snapshot of the species presence in a certain habitat in a given  
3  
4 394 timeframe. Environmental DNA sampled might indeed come from the organisms that live in the  
5  
6  
7 395 sampled area at that time, but it might also originate from degrading tissue, eggs and sperm and,  
8  
9 396 depending on environmental conditions, it might have simply been transported from elsewhere with  
10  
11 397 the currents or tides. Many researchers are now concentrating their efforts into understanding how  
12  
13  
14 398 long these molecules can persist in the environment and remain detectable [reviewed in 17].  
15

16 399

## 20 400 **5. Integration into existing management and monitoring programmes**

23 401 The development of new approaches to gather information of relevance to fisheries and  
24  
25 402 ecosystem monitoring through the use of eDNA sampling methods, and the associated novel insights  
26  
27  
28 403 such approaches generate, has the potential to revolutionise the information available to managers.  
29  
30 404 However, together with the requirement for the new methods to be able to provide robust results,  
31  
32 405 there is also a need to investigate the practicalities and cost-benefit of incorporating the new  
33  
34  
35 406 techniques into standardised monitoring surveys [94, 95]. In some situations, for example, the  
36  
37 407 requirement for targeted detection of specific species, it may be necessary to develop novel surveying  
38  
39  
40 408 programmes. However, by far the most preferred situation would be if the added value could be  
41  
42 409 embedded into existing survey programmes, through the addition of the collection of eDNA samples,  
43  
44 410 potentially requiring relatively little extra cost/effort on top of that already being invested. This is  
45  
46  
47 411 especially relevant as ship-based survey costs increase while genetic screening costs are decreasing.  
48  
49 412 Trawl surveys may be able to be supplemented by simultaneous eDNA collection from water samples,  
50  
51  
52 413 and benthic sediment monitoring by eDNA collection from grab samples. Indeed, in many if not most,  
53  
54 414 often costly, traditional fishery and ecosystem monitoring surveys there would seem to be an ideal  
55  
56 415 opportunity to collect such samples and add value in this way. It seems, therefore, that the design of  
57  
58  
59 416 future surveys, together with that of existing programmes, should be evaluated in the light of the  
60  
61  
62  
63  
64  
65

1  
2 417 developments in eDNA approaches outlined above and the added value that the integration of these  
3 418 approaches could bring.

4  
5 419

6  
7 420 **6. Conclusion**

8  
9 421 Rapid developments in the field of eDNA analysis have provided a range of new tools for  
10  
11 422 research scientists, and fishery and ecosystem managers. With such developments, it is not  
12  
13 423 straightforward for the manager to disentangle which tools can provide robust evidence to  
14  
15 424 incorporate into policy development discussions, and which are still in the developmental phase. In  
16  
17 425 tandem, reports about such advances in the mainstream media drive stakeholders to question  
18  
19 426 managers about the utility of the toolkits, including specific questions that might be difficult to answer  
20  
21 427 for a non-specialist. Here, we have attempted to provide a topic-based overview which goes some  
22  
23 428 way to address this problem, and thus can be of use to inform managers of the strengths and  
24  
25 429 weaknesses of the various approaches currently available.

26  
27 430 Environmental DNA-based tools have, for a number of years now, been providing reliable  
28  
29 431 evidence in areas such as single species detection, and the characterisation of ecosystem biodiversity.  
30  
31 432 As such, they represent a robust, cost-effective, and in an increasing number of cases a more sensible  
32  
33 433 option for managers and monitors for incorporation into their standard scientific toolkits. While  
34  
35 434 significant advances have been, and continue to be, made in the use of eDNA to quantify both relative  
36  
37 435 and absolute abundance, such analyses are less well developed and still suffer from uncertainties  
38  
39 436 associated with various environmental, biological and methodological challenges of these techniques  
40  
41 437 [17]. As these influences are studied and their impacts better understood such uncertainties will be  
42  
43 438 reduced. However, at present their application is likely to be more limited.

44  
45 439 Every scientific monitoring method has uncertainties and the field of eDNA research is no  
46  
47 440 exception. However, in many cases such uncertainty is well understood and as such, and considering  
48  
49 441 the potential significant benefits and potential cost-savings of the new tools available, managers and  
50  
51 442 monitors should consider the integration of these approaches in their management planning

443 discussions along with the more traditional techniques. The different approaches can work together  
1  
2 444 to provide complementary information. In the end they will allow enhanced scientific understanding,  
3  
4 445 resulting in improved science-based policy development in view of ecosystem-based management.  
5  
6

7 446

## 9 447 **7. Acknowledgments**

11 448 -

14 449

## 16 450 **References**

18  
19 451 [1] B. Worm, E.B. Barbier, N. Beaumont, J.E. Duffy, C. Folke, B.S. Halpern, J.B.C. Jackson, H.K. Lotze, F.  
20 452 Micheli, S.R. Palumbi, E. Sala, K.A. Selkoe, J.J. Stachowicz, R. Watson, Impacts of biodiversity loss on  
21 453 ocean ecosystem services, *Science* 314(5800) (2006) 787-790.

22 454 [2] E. Crist, C. Mora, R. Engelman, The interaction of human population, food production, and  
23 455 biodiversity protection, *Science* 356(6335) (2017) 260-264.

24 456 [3] United Nations, Transforming our World: The 2030 Agenda for Sustainable Development. Outcome  
25 457 Document for the UN Summit to Adopt the Post-2015 Development Agenda: Draft for Adoption, New  
26 458 York, 2015.

27 459 [4] Secretariat of the Convention on Biological Diversity, Sustaining life on Earth: how the Convention  
28 460 on Biological Diversity promotes nature and human well-being., Montreal, 2000.

29 461 [5] D. Hollis, T. Rosen, United Nations convention on law of the sea (UNCLOS), 1982, *The Encyclopedia*  
30 462 *of Earth* 22 (2010).

31 463 [6] OSPAR Commission, Convention for the protection of the marine environment of the North-East  
32 464 Atlantic, 1992.

33 465 [7] MSFD, Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008  
34 466 establishing a framework for community action in the field of marine environmental policy (Marine  
35 467 Strategy Framework Directive) L 164/19, Off. J. EU, 2008, p. 22.

36 468 [8] R. Cormier, C.R. Kelble, M.R. Anderson, J.I. Allen, A. Grehan, Ó. Gregersen, Moving from ecosystem-  
37 469 based policy objectives to operational implementation of ecosystem-based management measures,  
38 470 *ICES J Mar Sci* 74(1) (2016) 406-413.

39 471 [9] L. Livia, P. Antonella, L. Hovirag, N. Mauro, F. Panara, A nondestructive, rapid, reliable and  
40 472 inexpensive method to sample, store and extract high-quality DNA from fish body mucus and buccal  
41 473 cells, *Mol Ecol Notes* 6(1) (2006) 257-260.

42 474 [10] C.M. Merkes, S.G. McCalla, N.R. Jensen, M.P. Gaikowski, J.J. Amberg, Persistence of DNA in  
43 475 carcasses, slime and avian feces may affect interpretation of environmental DNA data, *PLoS One* 9(11)  
44 476 (2014) e113346.

45 477 [11] F. Pompanon, B.E. Deagle, W.O. Symondson, D.S. Brown, S.N. Jarman, P. Taberlet, Who is eating  
46 478 what: diet assessment using next generation sequencing, *Mol Ecol* 21(8) (2012) 1931-50.

47 479 [12] S. Alasaad, A. Sánchez, J.A. Marchal, A. Píriz, J.A. Garrido-García, F. Carro, I. Romero, R.C. Soriguer,  
48 480 Efficient identification of *Microtus cabreræ* excrements using noninvasive molecular analysis, *Conserv*  
49 481 *Genet Res* 3(1) (2011) 127-129.

50 482 [13] P.F. Thomsen, E. Willerslev, Environmental DNA – An emerging tool in conservation for  
51 483 monitoring past and present biodiversity, *Biol Conserv* 183 (2015) 4-18.

52 484 [14] P. Taberlet, E. Coissac, H. Mehrdad, L.H. Rieseberg, Environmental DNA, *Mol Ecol* 21 (2012) 1789-  
53 485 1793.

- 486 [15] K. Deiner, H.M. Bik, E. Mächler, M. Seymour, A. Lacoursière-Roussel, F. Altermatt, S. Creer, I. Bista,  
1 487 D.M. Lodge, N. de Vere, M.E. Pfrender, L. Bernatchez, Environmental DNA metabarcoding:  
2 488 Transforming how we survey animal and plant communities, *Mol Ecol* 26(21) (2017) 5872-5895.
- 3 489 [16] R.A. Weller, D.J. Baker, M.M. Glackin, S.J. Roberts, R.W. Schmitt, E.S. Twigg, D.J. Vimont, The  
4 490 challenge of sustaining ocean observations, *Frontiers in Marine Science* 6(105) (2019).
- 5 491 [17] B.K. Hansen, D. Bekkevold, L. Clausen, W., E.E. Nielsen, The sceptical optimist: challenges and  
6 492 perspectives for the application of environmental DNA in marine fisheries, *Fish Fish* 19(5) (2018) 751-  
7 493 768.
- 8 494 [18] ScienceDaily, New nano strategy fights superbugs, 2020.  
9 495 <https://www.sciencedaily.com/releases/2020/03/200312101030.htm>. Accessed 15/4/2020.
- 10 496 [19] National Geographic, Loch Ness Monster Hunters to Try DNA Search? Get the Facts., 2018.  
11 497 [https://www.nationalgeographic.com/news/2018/05/loch-ness-monster-scotland-environmental-](https://www.nationalgeographic.com/news/2018/05/loch-ness-monster-scotland-environmental-dna-science/)  
12 498 [dna-science/](https://www.nationalgeographic.com/news/2018/05/loch-ness-monster-scotland-environmental-dna-science/). Accessed 16/4/2020.
- 13 499 [20] M.S. Schäfer, Taking stock: A meta-analysis of studies on the media's coverage of science, *Public*  
14 500 *Understanding of Science* 21(6) (2010) 650-663.
- 15 501 [21] H.C. Rees, B.C. Maddison, D.J. Middleditch, J.R.M. Patmore, K.C. Gough, E. Crispo, REVIEW: The  
16 502 detection of aquatic animal species using environmental DNA - a review of eDNA as a survey tool in  
17 503 ecology, *Journal of Applied Ecology* 51(5) (2014) 1450-1459.
- 18 504 [22] M.A. Barnes, C.R. Turner, The ecology of environmental DNA and implications for conservation  
19 505 genetics, *Conserv Genet* 17(1) (2015) 1-17.
- 20 506 [23] E.E. Sigsgaard, M.R. Jensen, I.E. Winkelmann, P.R. Møller, M.M. Hansen, P.F. Thomsen,  
21 507 Population-level inferences from environmental DNA—Current status and future perspectives, *Evol*  
22 508 *Appl* 13(2) (2020) 245-262.
- 23 509 [24] W. Appeltans, Shane T. Ahyong, G. Anderson, Martin V. Angel, T. Artois, N. Bailly, R. Bamber, A.  
24 510 Barber, I. Bartsch, A. Berta, M. Błażewicz-Paszkowycz, P. Bock, G. Boxshall, Christopher B. Boyko,  
25 511 Simone N. Brandão, Rod A. Bray, Niel L. Bruce, Stephen D. Cairns, T.-Y. Chan, L. Cheng, Allen G. Collins,  
26 512 T. Cribb, M. Curini-Galletti, F. Dahdouh-Guebas, Peter J.F. Davie, Michael N. Dawson, O. De Clerck, W.  
27 513 Decock, S. De Grave, Nicole J. de Voogd, Daryl P. Domning, Christian C. Emig, C. Erséus, W. Eschmeyer,  
28 514 K. Fauchald, Daphne G. Fautin, Stephen W. Feist, Charles H.J.M. Franssen, H. Furuya, O. Garcia-Alvarez,  
29 515 S. Gerken, D. Gibson, A. Gittenberger, S. Gofas, L. Gómez-Daglio, Dennis P. Gordon, Michael D. Guiry,  
30 516 F. Hernandez, Bert W. Hoeksema, Russell R. Hopcroft, D. Jaume, P. Kirk, N. Koedam, S. Koenemann,  
31 517 Jürgen B. Kolb, Reinhardt M. Kristensen, A. Kroh, G. Lambert, David B. Lazarus, R. Lemaitre, M.  
32 518 Longshaw, J. Lowry, E. Macpherson, Laurence P. Madin, C. Mah, G. Mapstone, Patsy A. McLaughlin, J.  
33 519 Mees, K. Meland, Charles G. Messing, Claudia E. Mills, Tina N. Molodtsova, R. Mooi, B. Neuhaus,  
34 520 Peter K.L. Ng, C. Nielsen, J. Norenburg, Dennis M. Opresko, M. Osawa, G. Paulay, W. Perrin, John F.  
35 521 Pilger, Gary C.B. Poore, P. Pugh, Geoffrey B. Read, James D. Reimer, M. Rius, Rosana M. Rocha, José I.  
36 522 Saiz-Salinas, V. Scarabino, B. Schierwater, A. Schmidt-Rhaesa, Kareen E. Schnabel, M. Schotte, P.  
37 523 Schuchert, E. Schwabe, H. Segers, C. Self-Sullivan, N. Shenkar, V. Siegel, W. Sterrer, S. Stöhr, B. Swalla,  
38 524 Mark L. Tasker, Erik V. Thuesen, T. Timm, M.A. Todaro, X. Turon, S. Tyler, P. Uetz, J. van der Land, B.  
39 525 Vanhoorne, Leen P. van Ofwegen, Rob W.M. van Soest, J. Vanaverbeke, G. Walker-Smith, T.C. Walter,  
40 526 A. Warren, Gary C. Williams, Simon P. Wilson, Mark J. Costello, The magnitude of global marine  
41 527 species diversity, *Current Biology* 22(23) (2012) 2189-2202.
- 42 528 [25] E. Aylagas, Á. Borja, I. Muxika, N. Rodríguez-Ezpeleta, Adapting metabarcoding-based benthic  
43 529 biomonitoring into routine marine ecological status assessment networks, *Ecological Indicators* 95  
44 530 (2018) 194-202.
- 45 531 [26] J. Lobo, S. Shokralla, M.H. Costa, M. Hajibabaei, F.O. Costa, DNA metabarcoding for high-  
46 532 throughput monitoring of estuarine macrobenthic communities, *Sci Rep* 7(1) (2017) 15618.
- 47 533 [27] L.E. Holman, M. de Bruyn, S. Creer, G. Carvalho, J. Robidart, M. Rius, Detection of introduced and  
48 534 resident marine species using environmental DNA metabarcoding of sediment and water, *Sci Rep* 9(1)  
49 535 (2019) 11559.

536 [28] C.J. Closek, J.A. Santora, H.A. Starks, I.D. Schroeder, E.A. Andruszkiewicz, K.M. Sakuma, S.J.  
1 537 Bograd, E.L. Hazen, J.C. Field, A.B. Boehm, Marine vertebrate biodiversity and distribution within the  
2 538 central California current using environmental DNA (eDNA) metabarcoding and ecosystem surveys,  
3 539 *Frontiers in Marine Science* 6(732) (2019).

4 540 [29] I. Salter, M. Joensen, R. Kristiansen, P. Steingrund, P. Vestergaard, Environmental DNA  
5 541 concentrations are correlated with regional biomass of Atlantic cod in oceanic waters,  
6 542 *Communications Biology* 2(1) (2019) 461.

7 543 [30] K. Deiner, H.M. Bik, E. Machler, M. Seymour, A. Lacoursiere-Roussel, F. Altermatt, S. Creer, I. Bista,  
8 544 D.M. Lodge, N. de Vere, M.E. Pfrender, L. Bernatchez, Environmental DNA metabarcoding:  
9 545 Transforming how we survey animal and plant communities, *Mol Ecol* 26(21) (2017) 5872-5895.

10 546 [31] C.R. Turner, K.L. Uy, R.C. Everhart, Fish environmental DNA is more concentrated in aquatic  
11 547 sediments than surface water, *Biol Conserv* 183 (2015) 93-102.

12 548 [32] K. Deiner, J. Lopez, S. Bourne, L. Holman, M. Seymour, E.K. Grey, A. Lacoursière, Y. Li, M.A.  
13 549 Renshaw, M.E. Pfrender, M. Rius, L. Bernatchez, D.M. Lodge, Optimising the detection of marine  
14 550 taxonomic richness using environmental DNA metabarcoding: the effects of filter material, pore size  
15 551 and extraction method, *Metabarcoding and Metagenomics* 2 (2018) e28963.

16 552 [33] Y. Liu, G.H. Wikfors, J.M. Rose, R.S. McBride, L.M. Milke, R. Mercaldo-Allen, Application of  
17 553 Environmental DNA Metabarcoding to Spatiotemporal Finfish Community Assessment in a Temperate  
18 554 Embayment, *Frontiers in Marine Science* 6(674) (2019).

19 555 [34] N.K. Truelove, E.A. Andruszkiewicz, B.A. Block, A rapid environmental DNA method for detecting  
20 556 white sharks in the open ocean, *Methods Ecol Evol* 10(8) (2019) 1128-1135.

21 557 [35] F. Sanger, A.R. Coulson, A rapid method for determining sequences in DNA by primed synthesis  
22 558 with DNA polymerase, *Journal of Molecular Biology* 94(3) (1975) 441-448.

23 559 [36] K. Deiner, F. Altermatt, Transport Distance of Invertebrate Environmental DNA in a Natural River,  
24 560 *PLoS One* 9(2) (2014) e88786.

25 561 [37] J.L.A. Shaw, L. Weyrich, A. Cooper, Using environmental (e)DNA sequencing for aquatic  
26 562 biodiversity surveys: a beginner's guide, *Marine and Freshwater Research* 68(1) (2017) pp. 20-33-2017  
27 563 v.68 no.1.

28 564 [38] A.C. Thomas, S. Tank, P.L. Nguyen, J. Ponce, M. Sinnesael, C.S. Goldberg, A system for rapid eDNA  
29 565 detection of aquatic invasive species, *Environmental DNA* doi.org/10.1002/edn3.25 (2019).

30 566 [39] L. Miralles, M. Parrondo, A. Hernández de Rojas, E. Garcia-Vazquez, Y.J. Borrell, Development and  
31 567 validation of eDNA markers for the detection of *Crepidula fornicata* in environmental samples, *Marine*  
32 568 *Pollution Bulletin* 146 (2019) 827-830.

33 569 [40] G.M. Nester, M. De Brauwer, A. Koziol, K.M. West, J.D. DiBattista, N.E. White, M. Power, M.J.  
34 570 Heydenrych, E. Harvey, M. Bunce, Development and evaluation of fish eDNA metabarcoding assays  
35 571 facilitate the detection of cryptic seahorse taxa (family: Syngnathidae), *Environmental DNA*  
36 572 doi.org/10.1002/edn3.93 (2020).

37 573 [41] R.A. Collins, K.F. Armstrong, A.J. Holyoake, S. Keeling, Something in the water: biosecurity  
38 574 monitoring of ornamental fish imports using environmental DNA, *Biol Invasions* 15(6) (2012) 1209-  
39 575 1215.

40 576 [42] A. Takeuchi, S. Watanabe, S. Yamamoto, M.J. Miller, T. Fukuba, T. Miwa, T. Okino, T. Minamoto,  
41 577 K. Tsukamoto, First use of oceanic environmental DNA to study the spawning ecology of the Japanese  
42 578 eel *Anguilla japonica*, *Mar Ecol Prog Ser* 609 (2019) 187-196.

43 579 [43] M.V. Everett, L.K. Park, Exploring deep-water coral communities using environmental DNA, *Deep*  
44 580 *Sea Research Part II: Topical Studies in Oceanography* 150 (2018) 229-241.

45 581 [44] A. Valentini, P. Taberlet, C. Miaud, R. Civade, J. Herder, P.F. Thomsen, E. Bellemain, A. Besnard, E.  
46 582 Coissac, F. Boyer, C. Gaboriaud, P. Jean, N. Poulet, N. Roset, G.H. Copp, P. Geniez, D. Pont, C. Argillier,  
47 583 J.M. Baudoin, T. Peroux, A.J. Crivelli, A. Olivier, M. Acqueberge, M. Le Brun, P.R. Moller, E. Willerslev,  
48 584 T. Dejean, Next-generation monitoring of aquatic biodiversity using environmental DNA  
49 585 metabarcoding, *Mol Ecol* 25(4) (2016) 929-42.



- 586 [45] C.L. Jerde, E.A. Wilson, T.L. Dressler, Measuring global fish species richness with eDNA  
1 587 metabarcoding, *Mol Ecol Resour* 19(1) (2019) 19-22.
- 2 588 [46] P.D.N. Hebert, A. Cywinska, S.L. Ball, J.R. deWaard, Biological identifications through DNA  
3 589 barcodes, *Proc R Soc Lond B Biol Sci* 270(1512) (2003) 313-321.
- 4 590 [47] N. Fraija-Fernández, M.-C. Bouquieaux, A. Rey, I. Mendibil, U. Cotano, X. Irigoien, M. Santos, N.  
5 591 Rodríguez-Ezpeleta, Marine water environmental DNA metabarcoding provides a comprehensive fish  
6 592 diversity assessment and reveals spatial patterns in a large oceanic area, *Ecol Evol* 10(14) (2020) 7560-  
7 593 7584.
- 8 594 [48] B. Günther, T. Knebelsberger, H. Neumann, S. Laakmann, P. Martínez Arbizu, Metabarcoding of  
9 595 marine environmental DNA based on mitochondrial and nuclear genes, *Sci Rep* 8(1) (2018) 14822.
- 10 596 [49] D. Steinke, A.D. Connell, P.D.N. Hebert, Linking adults and immatures of South African marine  
11 597 fishes, *Genome* 59(11) (2016) 959-967.
- 12 598 [50] M. Valdez-Moreno, C. Quintal-Lizama, R. Gómez-Lozano, M.d.C. García-Rivas, Monitoring an alien  
13 599 invasion: DNA barcoding and the identification of lionfish and their prey on coral reefs of the Mexican  
14 600 Caribbean, *PLOS ONE* 7(6) (2012) e36636.
- 15 601 [51] S. Yamamoto, R. Masuda, Y. Sato, T. Sado, H. Araki, M. Kondoh, T. Minamoto, M. Miya,  
16 602 Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea, *Sci Rep*  
17 603 7 (2017) 40368.
- 18 604 [52] J. Pawlowski, P. Esling, F. Lejzerowicz, T. Cordier, J.A. Visco, C.I.M. Martins, A. Kvalvik, K. Staven,  
19 605 T. Cedhagen, Benthic monitoring of salmon farms in Norway using foraminiferal metabarcoding,  
20 606 *Aquaculture Environment Interactions* 8 (2016) 371-386.
- 21 607 [53] A. Ardura, A. Zaiko, J.L. Martinez, A. Samuiloviene, Y. Borrell, E. Garcia-Vazquez, Environmental  
22 608 DNA evidence of transfer of North Sea molluscs across tropical waters through ballast water, *Journal*  
23 609 *of Molluscan Studies* 81(4) (2015) 495-501.
- 24 610 [54] E.A. Andruszkiewicz, H.A. Starks, F.P. Chavez, L.M. Sassoubre, B.A. Block, A.B. Boehm,  
25 611 Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding, *PLOS ONE* 12(4)  
26 612 (2017) e0176343.
- 27 613 [55] M.Y. Stoeckle, L. Soboleva, Z. Charlop-Powers, Aquatic environmental DNA detects seasonal fish  
28 614 abundance and habitat preference in an urban estuary, *PLoS One* 12(4) (2017) e0175186.
- 29 615 [56] N.A. Sawaya, A. Djurhuus, C.J. Closek, M. Hepner, E. Olesin, L. Visser, C. Kelble, K. Hubbard, M.  
30 616 Breitbart, Assessing eukaryotic biodiversity in the Florida Keys National Marine Sanctuary through  
31 617 environmental DNA metabarcoding, *Ecol Evol* 9(3) (2019) 1029-1040.
- 32 618 [57] M. Seymour, F.K. Edwards, B.J. Cosby, M.G. Kelly, M. de Bruyn, G.R. Carvalho, S. Creer, Executing  
33 619 multi-taxa eDNA ecological assessment via traditional metrics and interactive networks, *Science of*  
34 620 *The Total Environment* 729 (2020) 138801.
- 35 621 [58] S. Fernandez, M.M. Sandin, P.G. Beaulieu, L. Clusa, J.L. Martinez, A. Ardura, E. Garcia-Vazquez,  
36 622 Environmental DNA for freshwater fish monitoring: insights for conservation within a protected area,  
37 623 *PeerJ* 6 (2018) e4486.
- 38 624 [59] A. Karahan, J. Douek, G. Paz, N. Stern, A.E. Kideys, L. Shaish, M. Goren, B. Rinkevich, Employing  
39 625 DNA barcoding as taxonomy and conservation tools for fish species censuses at the southeastern  
40 626 Mediterranean, a hot-spot area for biological invasion, *Journal for Nature Conservation* 36 (2017) 1-  
41 627 9.
- 42 628 [60] J. Bakker, O.S. Wangensteen, C. Baillie, D. Buddo, D.D. Chapman, A.J. Gallagher, T.L. Guttridge, H.  
43 629 Hertler, S. Mariani, Biodiversity assessment of tropical shelf eukaryotic communities via pelagic eDNA  
44 630 metabarcoding, *Ecol Evol* 9(24) (2019) 14341-14355.
- 45 631 [61] O. Laroche, O. Kersten, C.R. Smith, E. Goetze, From sea surface to seafloor: a benthic  
46 632 allochthonous eDNA survey for the abyssal ocean, [bioRxiv doi.org/10.1101/2020.05.07.082602](https://doi.org/10.1101/2020.05.07.082602)  
47 633 (2020).
- 48 634 [62] P.F. Thomsen, P.R. Moller, E.E. Sigsgaard, S.W. Knudsen, O.A. Jorgensen, E. Willerslev,  
49 635 Environmental DNA from seawater samples correlate with trawl catches of subarctic, deepwater  
50 636 fishes, *PLoS One* 11(11) (2016) e0165252.

- 637 [63] T. Takahara, T. Minamoto, H. Yamanaka, H. Doi, Z. Kawabata, Estimation of fish biomass using  
1 638 environmental DNA, PLoS One 7(4) (2012) e35868.
- 2 639 [64] A. Lacoursière-Roussel, G. Côté, V. Leclerc, L. Bernatchez, Quantifying relative fish abundance  
3 640 with eDNA: a promising tool for fisheries management, Journal of Applied Ecology 53(4) (2016) 1148-  
4 641 1157.
- 5 642 [65] J.A. Port, J.L. O'Donnell, O.C. Romero-Maraccini, P.R. Leary, S.Y. Litvin, K.J. Nickols, K.M. Yamahara,  
6 643 R.P. Kelly, Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA, Mol  
7 644 Ecol 25(2) (2016) 527-541.
- 8 645 [66] L. Peters, S. Spatharis, M.A. Dario, T. Dwyer, I.J.T. Roca, A. Kintner, Ø. Kanstad-Hanssen, M.S.  
9 646 Llewellyn, K. Praebel, Environmental DNA: a new low-cost monitoring tool for pathogens in salmonid  
10 647 aquaculture, Frontiers in microbiology 9(3009) (2018).
- 11 648 [67] T.P. Muha, R. Skukan, Y.J. Borrell, J.M. Rico, C. Garcia de Leaniz, E. Garcia-Vazquez, S. Consuegra,  
12 649 Contrasting seasonal and spatial distribution of native and invasive *Codium* seaweed revealed by  
13 650 targeting species-specific eDNA, Ecol Evol 9(15) (2019) 8567-8579.
- 14 651 [68] W.A. Hubert, M.C. Fabrizio, Relative abundance and catch per unit effort, in: C.S. Guy, M.L. Brown  
15 652 (Eds.), Analysis and Interpretation of Freshwater Fisheries Data, American Fisheries Society, Bethesda,  
16 653 MA, 2007, pp. 279–325.
- 17 654 [69] S.A. Bonar, W.A. Hubert, D.W. Willis, Standard methods for sampling North American freshwater  
18 655 fishes, American Fisheries Society, Bethesda, MD, 2009.
- 19 656 [70] CEN, Water quality - Sampling of fish with multi-mesh gillnets. CEN EN 14757, 2005.
- 20 657 [71] P. Thomsen, Francis, Kielgast, J. O. S., L. Iversen, L., C. Wiuf, M. Rasmussen, M.T. Gilbert, P., L.  
21 658 Orlando, E. Willerslev, Monitoring endangered freshwater biodiversity using environmental DNA, Mol  
22 659 Ecol 21(11) (2012) 2565-2573.
- 23 660 [72] K.E. Klymus, C.A. Richter, D.C. Chapman, C. Paukert, Quantification of eDNA shedding rates from  
24 661 invasive bighead carp *Hypophthalmichthys nobilis* and silver carp *Hypophthalmichthys molitrix*, Biol  
25 662 Conserv 183 (2015) 77-84.
- 26 663 [73] H. Doi, T. Takahara, T. Minamoto, S. Matsushashi, K. Uchii, H. Yamanaka, Droplet digital  
27 664 polymerase chain reaction (PCR) outperforms real-time PCR in the detection of environmental DNA  
28 665 from an invasive fish species, Environmental Science & Technology 49(9) (2015) 5601-8.
- 29 666 [74] M.D. Tillotson, R.P. Kelly, J.J. Duda, M. Hoy, J. Kralj, T.P. Quinn, Concentrations of environmental  
30 667 DNA (eDNA) reflect spawning salmon abundance at fine spatial and temporal scales, Biol Conserv 220  
31 668 (2018) 1-11.
- 32 669 [75] D.S. Pilliod, C.S. Goldberg, R.S. Arkle, L.P. Waits, J. Richardson, Estimating occupancy and  
33 670 abundance of stream amphibians using environmental DNA from filtered water samples, Can J Fish  
34 671 Aquat Sci 70(8) (2013) 1123-1130.
- 35 672 [76] B. Hänfling, L. Lawson Handley, S. Read Daniel, C. Hahn, J. Li, P. Nichols, C. Blackman Rosetta, A.  
36 673 Oliver, J. Winfield Ian, Environmental DNA metabarcoding of lake fish communities reflects long-term  
37 674 data from established survey methods, Mol Ecol 25(13) (2016) 3101-3119.
- 38 675 [77] M.C. Schmelzle, A.P. Kinziger, Using occupancy modelling to compare environmental DNA to  
39 676 traditional field methods for regional-scale monitoring of an endangered aquatic species, Mol Ecol  
40 677 Resour 16(4) (2016) 895-908.
- 41 678 [78] S. Yamamoto, K. Minami, K. Fukaya, K. Takahashi, H. Sawada, H. Murakami, S. Tsuji, H. Hashizume,  
42 679 S. Kubonaga, T. Horiuchi, M. Hongo, J. Nishida, Y. Okugawa, A. Fujiwara, M. Fukuda, S. Hidaka, K.W.  
43 680 Suzuki, M. Miya, H. Araki, H. Yamanaka, A. Maruyama, K. Miyashita, R. Masuda, T. Minamoto, M.  
44 681 Kondoh, Environmental DNA as a 'Snapshot' of Fish Distribution: A Case Study of Japanese Jack  
45 682 Mackerel in Maizuru Bay, Sea of Japan, PLoS One 11(3) (2016) e0149786.
- 46 683 [79] K. Fukaya, H. Murakami, S. Yoon, K. Minami, Y. Osada, S. Yamamoto, R. Masuda, A. Kasai, K.  
47 684 Miyashita, T. Minamoto, M. Kondoh, Estimating fish population abundance by integrating quantitative  
48 685 data on environmental DNA and hydrodynamic modelling, bioRxiv doi.org/10.1101/482489 (2018).
- 49 686 [80] T. Chambert, D.S. Pilliod, C.S. Goldberg, H. Doi, T. Takahara, An analytical framework for  
50 687 estimating aquatic species density from environmental DNA, Ecol Evol 8(6) (2018) 3468-3477.

- 688 [81] L.M. Sassoubre, K.M. Yamahara, L.D. Gardner, B.A. Block, A.B. Boehm, Quantification of  
1 689 environmental DNA (eDNA) shedding and decay rates for three marine fish, *Environmental Science &*  
2 690 *Technology* 50(19) (2016) 10456-10464.
- 3 691 [82] E.A. Andruszkiewicz, L.M. Sassoubre, A.B. Boehm, Persistence of marine fish environmental DNA  
4 692 and the influence of sunlight, *PLoS One* 12(9) (2017) e0185043.
- 5 693 [83] T. Jo, M. Arimoto, H. Murakami, R. Masuda, T. Minamoto, Estimating shedding and decay rates  
6 694 of environmental nuclear DNA with relation to water temperature and biomass, *Environmental DNA*  
7 695 2(2) (2020) 140-151.
- 8 696 [84] L.L. Iversen, J. Kielgast, K. Sand-Jensen, Monitoring of animal abundance by environmental DNA  
9 697 — An increasingly obscure perspective: A reply to Klymus et al., 2015, *Biol Conserv* 192 (2015) 479-  
10 698 480.
- 11 699 [85] J.B. Harrison, J.M. Sunday, S.M. Rogers, Predicting the fate of eDNA in the environment and  
12 700 implications for studying biodiversity, *Proc R Soc Lond B Biol Sci* 286(1915) (2019) 20191409.
- 13 701 [86] G.F. Ficetola, P. Taberlet, E. Coissac, How to limit false positives in environmental DNA and  
14 702 metabarcoding?, *Mol Ecol Resour* 16(3) (2016) 604-7.
- 15 703 [87] C.L. Jerde, Can we manage fisheries with the inherent uncertainty from eDNA?, *J Fish Biol*  
16 704 doi:10.1111/jfb.14218 (2019).
- 17 705 [88] I.A. Dickie, S. Boyer, H.L. Buckley, R.P. Duncan, P.P. Gardner, I.D. Hogg, R.J. Holdaway, G. Lear, A.  
18 706 Makiola, S.E. Morales, J.R. Powell, L. Weaver, Towards robust and repeatable sampling methods in  
19 707 eDNA-based studies, *Mol Ecol Resour* 18(5) (2018) 940-952.
- 20 708 [89] K.M. Yamahara, C.M. Preston, J. Birch, K. Walz, R. Marin, S. Jensen, D. Pargett, B. Roman, W.  
21 709 Ussler, Y. Zhang, J. Ryan, B. Hobson, B. Kieft, B. Raanan, K.D. Goodwin, F.P. Chavez, C. Scholin, In situ  
22 710 autonomous acquisition and preservation of marine environmental DNA using an autonomous  
23 711 underwater vehicle, *Frontiers in Marine Science* 6(373) (2019).
- 24 712 [90] A. Djurhuus, J. Port, C.J. Closek, K.M. Yamahara, O. Romero-Maraccini, K.R. Walz, D.B. Goldsmith,  
25 713 R. Michisaki, M. Breitbart, A.B. Boehm, F.P. Chavez, Evaluation of filtration and DNA extraction  
26 714 methods for environmental DNA biodiversity assessments across multiple trophic levels, *Frontiers in*  
27 715 *Marine Science* 4 (2017) 314.
- 28 716 [91] R. Pinfield, E. Dillane, A.K.W. Runge, A. Evans, L. Mirimin, J. Niemann, T.E. Reed, D.G. Reid, E.  
29 717 Rogan, F.I.P. Samarra, E.E. Sigsgaard, A.D. Foote, False-negative detections from environmental DNA  
30 718 collected in the presence of large numbers of killer whales (*Orcinus orca*), *Environmental DNA* 1(4)  
31 719 (2019) 316-328.
- 32 720 [92] T. Schenekar, M. Schletterer, L.A. Lecaudey, S.J. Weiss, Reference databases, primer choice, and  
33 721 assay sensitivity for environmental metabarcoding: Lessons learnt from a re-evaluation of an eDNA  
34 722 fish assessment in the Volga headwaters, *River Research and Applications* doi.org/10.1002/rra.3610  
35 723 (2020).
- 36 724 [93] H. Weigand, A.J. Beermann, F. Čiampor, F.O. Costa, Z. Csabai, S. Duarte, M.F. Geiger, M.  
37 725 Grabowski, F. Rimet, B. Rulik, M. Strand, N. Szucsich, A.M. Weigand, E. Willassen, S.A. Wyler, A.  
38 726 Bouchez, A. Borja, Z. Čiamporová-Zaťovičová, S. Ferreira, K.-D.B. Dijkstra, U. Eisendle, J. Freyhof, P.  
39 727 Gadawski, W. Graf, A. Haegerbaeumer, B.B. van der Hoorn, B. Japoshvili, L. Keresztes, E. Keskin, F.  
40 728 Leese, J.N. Macher, T. Mamos, G. Paz, V. Pešić, D.M. Pfannkuchen, M.A. Pfannkuchen, B.W. Price, B.  
41 729 Rinkevich, M.A.L. Teixeira, G. Várbió, T. Ekrem, DNA barcode reference libraries for the monitoring of  
42 730 aquatic biota in Europe: Gap-analysis and recommendations for future work, *Science of The Total*  
43 731 *Environment* 678 (2019) 499-524.
- 44 732 [94] J. Pawlowski, M. Kelly-Quinn, F. Altermatt, L. Apothéoz-Perret-Gentil, P. Beja, A. Boggero, A.  
45 733 Borja, A. Bouchez, T. Cordier, I. Domaizon, M.J. Feio, A.F. Filipe, R. Fornaroli, W. Graf, J. Herder, B. van  
46 734 der Hoorn, J. Iwan Jones, M. Sagova-Mareckova, C. Moritz, J. Barquín, J.J. Piggott, M. Pinna, F. Rimet,  
47 735 B. Rinkevich, C. Sousa-Santos, V. Specchia, R. Trobajo, V. Vasselon, S. Vitecek, J. Zimmerman, A.  
48 736 Weigand, F. Leese, M. Kahlert, The future of biotic indices in the ecogenomic era: Integrating (e)DNA  
49 737 metabarcoding in biological assessment of aquatic ecosystems, *Science of The Total Environment* 637-  
50 738 638 (2018) 1295-1310.

739 [95] T.E. Berry, B.J. Saunders, M.L. Coghlan, M. Stat, S. Jarman, A.J. Richardson, C.H. Davies, O. Berry,  
1 740 E.S. Harvey, M. Bunce, Marine environmental DNA biomonitoring reveals seasonal patterns in  
2 741 biodiversity and identifies ecosystem responses to anomalous climatic events, PLOS Genetics 15(2)  
3 742 (2019) e1007943.  
4  
5 743  
6  
7  
8 744  
9

10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

# 1 Life in a drop: sampling environmental DNA for marine fishery management 2 and ecosystem monitoring

## 3 Abstract

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

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

27

28

29 **1. Introduction**

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

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

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
52 ~~retrieval and analyses [8, 9]. Although there are often very good communication channels between~~  
53 ~~scientists and managers, science coverage in the mass media is the major channel that bridges the gap~~  
54 ~~between science and the general public [10]. Most people, including managers and policy makers,~~  
55 ~~acquire their information about scientific progress firstly, or for some stakeholder groups exclusively,~~  
56 ~~from the mass media. Accordingly, it is argued that media coverage contributes strongly to the image~~  
57 ~~and understanding of new developments and, importantly, influences their legitimization, public~~  
58 ~~support, and funding [10]. It is thus vital that the bidirectional information flow between managers~~  
59 ~~and scientists is well established and a mutual understanding assured [11]. This is often challenging,~~  
60 ~~particularly the communication of scientific approaches from specialists to managers and policy~~  
61 ~~makers in a rapidly developing and specialized field~~The integration of scientific advice into governance  
62 and policy development and implementation is often challenging, particularly the communication of  
63 scientific approaches from specialists to managers and policy makers in a rapidly developing and  
64 specialised field. This review seeks to address this issue with regards to new genetic based techniques  
65 in the fields of species identification and community characterisation and thus facilitate more effective  
66 development of marine fishery management and monitoring approaches.

35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65  
66  
67  
68  
69  
70  
71  
72  
73  
74  
75  
76  
77  
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 organismsbiodiversity 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

1 78 approach is utilisation of DNA collected from the environment to identify and/or quantify the species  
2 present in the ecosystem.  
3

4 80 Environmental DNA (eDNA) stems from individual organisms which release DNA into the  
5 environment through waste products, skin/tissue, scales, gametes, mucus, blood and carcasses [~~13-~~  
6 ~~169-12~~]. This extra-organismal DNA is termed environmental DNA (eDNA) [~~17~~].[13]. In contrast to DNA  
7 81 extracted from tissue samples, or community DNA – where DNA is extracted from the communities of  
8 whole organisms - eDNA does not require sampling the target organisms themselves, but instead the  
9 82 sampling of the environment they live in [~~18, 19~~14, 15]. The development of new ways of monitoring  
10 marine ecosystems and marine biodiversity using eDNA has advanced over recent years and has  
11 83 revolutionised the ability to track invasive species, monitor endangered species, assess the health of  
12 fish stocks, and explore the world of marine biodiversity [~~20~~].[16]. The seeming simplicity and cost-  
13 84 effectiveness of eDNA-based approaches, together with the interest from wider stakeholder groups,  
14 85 has made such applications highly attractive [~~21~~][17].  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30

31 91 The development of genetic technologies to identify species and characterise whole  
32 communities through the utilisation of a filtered collection and filtration of water and/or sediment  
33 92 sample is both a potentially invaluable tool for managers and an irresistible story for the popular press.  
34 Press articles focusing on such tools range from the very small, such as “New nano strategy fights  
35 93 superbugs Nano Strategy Fights Superbugs” [~~22~~][18], to the very large (and improbable) “Loch Ness  
36 94 Monster Hunters to Try DNA Search?” [~~23~~].[19]. Disentangling fact from fiction, and hyperbola from  
37 95 reality, is thus not a simple task for the manager striving to understand the field. As such this raises  
38 96 two opposing issues which could each negatively affect theirthe ability to manage fisheries and  
39 97 monitor ecosystems using the most appropriate available scientific tools: the pre-emptive uptake of  
40 98 unproven approaches versus the failure to take advantage of robust new techniques. Stories in the  
41 99 press, together with questions from stakeholders, about new potential approaches that have been  
42 100 developed are often powerful incentives for major funding and uptake of these tools in practice [~~10~~]-  
43 101 [~~1~~].[20]. Whilst in some cases this uptake may be justified, in others, especially in rapidly developing  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65



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

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

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

128

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26

DNA source

DNA source	Sampling method	Obtained sample	Target organisms	
			Community DNA	Environmental DNA
WATER COLUMN	Water Bottle	Filter	<i>Megafauna</i> Microeukaryotes Bacteria	
	Plankton Net	Bulk organisms	Meroplankton Zooplankton	
SEDIMENT	Van Veen Grab	Bulk organisms	Benthic Invertebrates Macroalgae	
		Sediment	<i>Megafauna</i> <i>Benthic Invertebrates</i> Microeukaryotes Bacteria	

129

27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55

DNA source

DNA source	Sampling method	Obtained sample	Target organisms	
			Community DNA	Environmental DNA
WATER COLUMN	Water Bottle	Filter	Microeukaryotes Bacteria	Megafauna Macrofauna Benthic Invertebrates
	Plankton Net	Bulk organisms	Meroplankton Zooplankton	
SEDIMENT	Van Veen Grab	Bulk organisms	Benthic Invertebrates Macroalgae	
		Sediment	Microeukaryotes Bacteria	Megafauna Macrofauna Benthic Invertebrates

130

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

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

## 136 2. Environmental DNA in a fisheries context

137 The marine environment harbours a huge diversity of species [2724], ranging from large and  
138 charismatic whales to tiny worms and unicellular plankton (Fig. 1). ~~Depending1). Compared to the~~  
139 ~~sampling of eDNA in freshwater it also poses its own set of, often difficult to address, issues when~~  
140 ~~trying to obtain unbiased samples, especially in relation to factors such, tides, currents, great depths~~  
141 ~~and rapid movements of individuals in three dimensions. Thus, depending~~ on the habitat and taxa of  
142 interest, ~~different~~various sampling methods are needed ~~to study, monitor, and provide information~~  
143 ~~of relevance to the management of marine communities (Fig. 1). These sampling methods aim to~~  
144 collect the full range of target species present at a given site so that, when possible, visual  
145 identification and quantification of the species is done ~~to study, monitor, and provide information of~~  
146 ~~relevance to the management of marine communities (Fig. 1).~~

147 Identification and characterization of these samples can be ~~either~~ accelerated ~~through~~using  
148 ~~genetic techniques. These will differ depending on the source of the DNA extraction of all obtained. In~~  
149 ~~the first case, community DNA can be collected. This refers to the collection of whole communities of~~  
150 organisms in the sample ~~(referred to as community DNA) with~~ from which DNA is extracted from the  
151 cells of the sampled individuals. Such analysis results in highly comparable results for monitoring and  
152 impact assessment, compared to traditional morphological analyses [28, 2925, 26] and at a fraction  
153 of the time and cost [28]. ~~Or the use of eDNA[25]. In the second case, organisms are not directly~~  
154 ~~sampled, rather extraorganismal DNA in the environment (eDNA) is collected and used to infer a~~  
155 ~~species presence. The use of eDNA in this way~~ may even further simplify sampling and increase  
156 throughput, decreasing the costs and allowing for large scale surveys of marine ecosystems.

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

159 [28](#)], and to determine their abundance [\[e.g. 32\]](#)[\[e.g. 29\]](#). Both community DNA and eDNA data are  
160 affected by technical (e.g. [DNA marker choice](#), [DNA primer choice](#), [laboratory assay choices](#), incomplete  
161 reference [databases](#)) and biological (e.g. size of the organisms) biases, which should be taken  
162 into account when interpreting the data for fisheries management and ecosystem monitoring [\[3330\]](#).  
163 ~~In addition,~~ [While the distribution of the entire organisms collected during community DNA surveys](#)  
164 [is, of course, affected by environmental parameters, extracellular eDNA is especially sensitive to such](#)  
165 [factors](#). eDNA data ~~are also~~ [thus](#) influenced by environmental factors such as water temperature,  
166 organic matter, pH, UV radiation, and water currents, and by the type and amount of material used  
167 during sampling [\[21\]](#)[\[17\]](#). [Further, as eDNA is used as a proxy for species presence, any biases in the](#)  
168 [transport and persistence of eDNA can result in its distribution being significantly different from that](#)  
169 [of the actual organisms](#). Careful evaluation of these biases is needed for the correct interpretation of  
170 eDNA results in the framework of fisheries management and conservation.

171

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

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

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

194 3.1. Targeted species detection

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

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

Application	Example <u>study outline</u>	Example
-------------	------------------------------	---------

1	Detection and mapping of the spread of	<del>[43]</del> <u>Invasive slipper shell on the European</u>	[39]
2	invasive or non-native species	<u>Atlantic coast</u>	
3	Identification and monitoring of	<del>[39]</del> <u>White sharks in the open ocean</u>	[34]
4	rare/endangered species		
5			
6	Detection of cryptic species	<del>[45]</del> <u>Cryptic seahorse species off western</u>	[40]
7		<u>Australia</u>	
8			
9	Biosecurity during import/export	<del>[40]</del> <u>Ornamental fish imports</u>	[41]
10			
11	Investigating spawning activity	<del>[47]</del> <u>Spawning ecology of the Japanese eel</u>	[42]
12			
13	<del>Monitoring of high diversity (multispecies) environments</del>	<del>[48]</del>	
14	Monitoring of hard to access	<del>[49]</del> <u>Deep-sea octocorals using remote</u>	[43]
15	environments	<u>submersibles</u>	
16			

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

18  
19 211  
20  
21  
22 212 Applications are varied and are detailed with examples in Table 1. It can be ~~seen~~observed from  
23  
24 213 these examples that targeted species detection has shown its usefulness across many and varied  
25  
26 214 situations of fishery management and ecosystem monitoring. Marine monitoring using traditional  
27  
28 215 methods such as individual capture (with e.g. trawls, nets, and traps etc) and visual surveys are time  
29  
30 216 consuming, costly to carry out and in some cases simply impossible. Investigations using eDNA have  
31  
32  
33 217 shown that in numerous situations ~~this tool~~the approaches have the potential to add to the available  
34  
35 218 information to inform a variety of management questions. Adding value to traditional programmes is,  
36  
37 219 perhaps, the most cost-effective way to integrate eDNA screening into routine management and  
38  
39 220 monitoring programmes (see below). However, in some specific situations the use of eDNA has the  
40  
41 221 potential to replace traditional monitoring. For this to occur a number of technical and validation steps  
42  
43 222 are required such as comparisons between eDNA and visual survey data in context, controls for type  
44  
45 223 I (false-positive) and type II (false negative) errors, validation of experimental results in the laboratory,  
46  
47 224 scaling up versus one-off sample collection, temporal and spatial replicates (see below). If such steps  
48  
49 225 are successful, targeted species detection using eDNA has shown that it can fulfil the requirements of  
50  
51 226 fishery and ecosystem monitoring programmes and can be used as an alternative approach to answer  
52  
53 227 relevant questions for managers.  
54  
55  
56  
57  
58  
59  
60 228  
61  
62  
63  
64  
65

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

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

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

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

247  
248 3.2. Community characterisation

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



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

259

260 **Table 2**

261 Selected applications of community characterisation using marine eDNA.

262

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

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

### 298 3.3. Species Abundance Estimation

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

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

### 313 3.3. Species Abundance Estimation

314 Together with the identification of both individual and ecosystem-based biodiversity, eDNA can  
 315 be used to estimate either the relative abundance of multiple species using metabarcoding [5062], or  
 316 the absolute abundance of individual species using qPCR [51].[63]. At its simplest, such approaches  
 317 involve quantifying the amount of eDNA from a species represented in a sample and using that as a  
 318 simple proxy for abundance [52]. ~~Such information may then~~[64]. ~~Such information may~~ be used to  
 319 estimate numbers of individuals and/or biomass. The use of eDNA-based tools to quantify stocks of  
 320 species of interest is of course of great interest to fishery managers and policy makers, as population  
 321 or stock assessment is a central component of any management and/or conservation programme.  
 322 Estimating absolute counts and/or biomass, relies on the establishment of a robust correlation  
 323 between DNA concentration and living biomass whereas relative biomass estimates assume that the  
 324 relative amounts of DNA measured in the sample are representative of the relative abundance of the  
 325 different species in the ecosystem. While both approaches may seem to rely on fairly simple  
 326 calculations and indeed are beginning to be used (Table 3), in practice, there are many factors which  
 327 interact to make the relationships upon which the assumptions about the correlations are made very  
 328 complex to disentangle and to obtain robust estimates.

330 **Table 3**

331 Selected applications of abundance estimation using marine eDNA.

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

	<u>continental slope off Southwest Greenland</u>	
Abundance of non-indigenous invasive species abundance	[84] <u>Temporal abundance of invasive <i>Codium</i> seaweed in the Bay of Biscay</u>	[67]
Stock assessment	[74] <u>Biomass estimation of Atlantic cod in oceanic waters around the Faroe Islands</u>	[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~~

354 between relative and absolute abundance has been reported in communities including both  
355 amphibians [75] and fish [64, 65, 55, 76] including commercially important species such as Atlantic cod  
356 (*Gadus morhua*) [56].  
357 , including commercially important species such as Atlantic cod (*Gadus morhua*) [29].

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

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

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

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

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

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

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

382  
383 In the marine environment, abundance estimates using eDNA, while inherently more difficult  
384 than a relatively enclosed freshwater ecosystem, are ~~increasingly reliable~~starting to be examined  
385 (Table 3). Approaches are developing rapidly and, while at present robust relationships between  
386 abundance quantification using eDNA and more traditional methods are sometimes weak [~~50, 66,~~  
387 ~~67~~62, 77, 78], in some cases the approach seems to be comparable to that of other quantitative  
388 methods [~~56, 68~~29, 79]. The inherent uncertainty in the robustness of biomass quantification when  
389 utilising eDNA approaches is due to both the assumptions on which the technique rests and the impact  
390 of extraneous factors on such assumptions. eDNA abundance quantification relies on the assumption  
391 that local population numbers may be inferred by measuring the concentration of eDNA at a given  
392 locality and that this estimation represents the quantitative relation between eDNA concentration  
393 and the underlying population size [~~68, 69~~79, 80]. However, such a relationship may not be always  
394 true, or even present in most cases. The amount of eDNA at a location will vary depending on a  
395 number of biological, physical and environmental factors (see below). While these factors also have  
396 an impact on species detection, the impact of the fluctuations registered is higher if quantitative  
397 measurements are being attempted, rather than simple presence/absence results. Nevertheless, it  
398 may be possible to incorporate these impacts into modelling, to better predict how they can affect  
399 eDNA concentrations, therefore reducing the variance around such quantifications [~~68, 70-72~~79, 81-  
400 83]. However, due to the complexity of interacting factors, direct quantitative assessments remain  
401 highly challenging in marine ecosystems [~~21, 73~~17, 84].

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

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

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

#### 411 412 **4. Considerations**

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

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

1  
2 432 ~~essential negative control sample inclusion~~[78] ~~and laboratory analysis~~ [79], as well as the  
3 433 ~~interpretation of results~~ [80].

4 434 An important consideration in any eDNA monitoring programme is the avoidance of  
5 contamination [88]. DNA molecules from many sources are everywhere around us, and if they enter  
6 435 eDNA samples they have the potential to produce false positives. The use of sterile equipment, gloves,  
7 436 and a dedicated eDNA laboratory (with strict protocols, controls and necessary separations of  
8 437 processes handling high and low DNA templates) are necessary measurements to be taken in order to  
9 438 reduce contaminations and resulting false positives [86]. It is possible to control for contamination, by  
10 439 taking multiple replicates (usually three) of the same samples, and by using negative controls (i.e.  
11 440 sterilised distilled water samples not containing any actual material) at every stage of the process  
12 441 (field and laboratory blanks for DNA extraction and amplification) [88]. Any DNA that results from  
13 442 these blanks (and there is likely to be some), is then 'subtracted' from the results of the actual samples.  
14 443 Thus, like in any other monitoring approach, standardization is crucial, especially when it comes to  
15 444 techniques of collection, essential negative control sample inclusion [89] and laboratory analysis [90],  
16 445 as well as the interpretation of results [91].

17 446  
18 447 Another important consideration (which can be a significant drawback in certain situations) is  
19 448 the availability of DNA reference sequences, or a reference database ~~[81].of taxonomically identified~~  
20 449 ~~species/groups~~ [92]. Matching sequences obtained from actual eDNA samples against a reference  
21 450 database is the final step in the workflow, one that will tell the user what species the sampled eDNA  
22 451 belongs to. The reliability of such databases ~~is thus, together with the availability of high-quality~~  
23 452 ~~reference sequences of previously examined and taxonomically identified organisms is~~ crucial for  
24 453 robust data interpretation and to avoid false negatives and positives. There are a number of databases  
25 454 that can be used, with the Barcode of Life Data System (iBOLD) ~~[8293]~~ being an important example.  
26 455 Yet, it is advisable, when embarking on an eDNA project, to invest time assessing the reliability of the  
27 456 databases for the geographic area and taxa investigated, and if required, build a project-specific  
28 457 quality-controlled database.



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

458 Another pivotal consideration when interpreting results is that of eDNA transport. As  
459 mentioned above, eDNA offers a snapshot of the species presence in a certain habitat in a given  
460 timeframe. Environmental DNA sampled might indeed come from the organisms that live in the  
461 sampled area at that time, but it might also originate from degrading tissue, eggs and sperm and,  
462 depending on environmental conditions, it might have simply been transported from elsewhere with  
463 the currents or tides. Many researchers are now concentrating their efforts into understanding how  
464 long these molecules can persist in the environment and remain detectable [reviewed in [2417](#)].

465

## 466 **5. Integration into existing management and monitoring programmes**

467 The development of new approaches to gather information of relevance to fisheries and  
468 ecosystem monitoring through the use of eDNA sampling methods, and the associated novel insights  
469 such approaches generate, has the potential to revolutionise the information available to managers.  
470 However, together with the requirement for the new methods to be able to provide robust results,  
471 there is also a need to investigate the practicalities and cost-benefit of incorporating the new  
472 techniques into standardised monitoring surveys [[83, 8494, 95](#)]. In some situations, for example, the  
473 requirement for targeted detection of specific species, it may be necessary to develop novel surveying  
474 programmes. However, by far the most preferred situation would be if the added value could be  
475 ~~brought to~~ embedded into existing survey programmes, through the addition of the collection of eDNA  
476 samples, potentially requiring relatively little extra cost/effort on top of that already being ~~employed.~~  
477 ~~Pelagic trawl~~ invested. This is especially relevant as ship-based survey costs increase while genetic  
478 screening costs are decreasing. Trawl surveys may be able to be supplemented by simultaneous eDNA  
479 collection from water samples, and benthic sediment monitoring by eDNA collection from grab  
480 samples. Indeed, in many if not most, often costly, traditional fishery and ecosystem monitoring  
481 surveys there would seem to be an ideal opportunity to collect such samples and add value in this  
482 way. It ~~would seem~~ seems, therefore, that the design of future surveys, together with that of existing

1 483 programmes, should be evaluated in the light of the developments in eDNA approaches outlined  
2 484 above and the added value that the integration of these approaches could bring.

3  
4 485

## 5 6 7 486 **6. Conclusion**

8  
9 487 Rapid developments in the field of eDNA analysis have provided a range of new tools for  
10  
11 488 research scientists, and fishery and ecosystem managers. With such developments, it is not  
12  
13  
14 489 straightforward for the manager to disentangle which tools can provide robust evidence ~~for them~~ to  
15  
16 490 incorporate into policy development discussions, and which are still in the developmental phase. In  
17  
18  
19 491 tandem, reports about such advances in the mainstream media drive stakeholders to question  
20  
21 492 managers about the utility of the toolkits, ~~such~~ including specific questions ~~again being that might be~~  
22  
23 493 difficult to answer for a non-specialist ~~to answer~~. Here, we have attempted to provide a topic-based  
24  
25  
26 494 overview which goes some way to address this problem, and thus can be of use to inform managers  
27  
28 495 of the strengths and weaknesses of the various approaches currently available.

29  
30 496 Environmental DNA-based tools have, for a number of years now, been providing reliable  
31  
32  
33 497 evidence in areas such as single species detection, and the characterisation of ecosystem biodiversity.  
34  
35 498 As such, they represent a robust, cost-effective, and in an increasing number of cases a more sensible  
36  
37  
38 499 option for managers and monitors for incorporation into their standard scientific toolkits. While  
39  
40 500 significant advances have been, and continue to be, made in the use of eDNA to quantify both relative  
41  
42 501 and absolute abundance, such analyses are less well developed and still suffer from uncertainties  
43  
44  
45 502 associated with various environmental, biological and methodological challenges of these techniques  
46  
47 503 ~~[21],[17]~~. As these influences are studied and their impacts better understood such uncertainties will  
48  
49  
50 504 be reduced. However, at present their application is likely to be more limited.

51  
52 505 Every scientific monitoring method has uncertainties and the field of eDNA research is no  
53  
54 506 exception. However, in many cases such uncertainty is well understood and as such, and considering  
55  
56  
57 507 the potential significant benefits and potential cost-savings of the new tools available, managers and  
58  
59 508 monitors should consider the integration of these approaches in their management planning

509 discussions along with the more traditional techniques. The different approaches can work together  
1  
2 510 to provide complementary information—to. In the end they will allow enhanced scientific  
3  
4  
5 511 understanding, resulting in improved science-based policy development in view of ecosystem-based  
6  
7 512 management.

8  
9 513

## 11 514 **7. Acknowledgments**

13  
14 515 -

15  
16 516

## 18 517 **References**

- 20  
21 518 [1] B. Worm, E.B. Barbier, N. Beaumont, J.E. Duffy, C. Folke, B.S. Halpern, J.B.C. Jackson, H.K. Lotze, F.  
22 519 Micheli, S.R. Palumbi, E. Sala, K.A. Selkoe, J.J. Stachowicz, R. Watson, Impacts of Biodiversity Loss on  
23 520 Ocean Ecosystem Servicesbiodiversity loss on ocean ecosystem services, Science 314(5800) (2006)  
24 521 787-790.  
25  
26 522 [2] E. Crist, C. Mora, R. Engelman, The interaction of human population, food production, and  
27 523 biodiversity protection, Science 356(6335) (2017) 260-264.  
28 524 [3] United Nations, Transforming our World: The 2030 Agenda for Sustainable Development. Outcome  
29 525 Document for the UN Summit to Adopt the Post-2015 Development Agenda: Draft for Adoption, New  
30 526 York, 2015.  
31 527 [4] Secretariat of the Convention on Biological Diversity, Sustaining life on Earth: how the Convention  
32 528 on Biological Diversity promotes nature and human well-being., Montreal, 2000.  
33  
34 529 [5] D. Hollis, T. Rosen, United Nations convention on law of the sea (UNCLOS), 1982, The Encyclopedia  
35 530 of Earth 22 (2010).  
36 531 [6] OSPAR Commission, Convention for the protection of the marine environment of the North-East  
37 532 Atlantic, 1992.  
38  
39 533 [7] MSFD, Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008  
40 534 establishing a framework for community action in the field of marine environmental policy (Marine  
41 535 Strategy Framework Directive) L 164/19, Off. J. EU, 2008, p. 22.  
42 536 [8] R. Landry, N. Amara, M. Lamari, Climbing the Ladder of Research Utilization: Evidence from Social  
43 537 Science Research, Science Communication 22(4) (2001) 396-422.  
44  
45 538 [9] S.S. Soomai, The science-policy interface in fisheries management: Insights about the influence of  
46 539 organizational structure and culture on information pathways, Marine Policy 81 (2017) 53-63.  
47 540 [10] M.S. Schäfer, Taking stock: A meta-analysis of studies on the media's coverage of science, Public  
48 541 Understanding of Science 21(6) (2010) 650-663.  
49 542 [11] M.F. Weigold, Communicating Science: A Review of the Literature, Science Communication 23(2)  
50 543 (2001) 164-193.  
51 544 [12] R. Cormier, C.R. Kelble, M.R. Anderson, J.I. Allen, A. Grehan, Ó. Gregersen, Moving from  
52 545 ecosystem-based policy objectives to operational implementation of ecosystem-based management  
53 546 measures, ICES J Mar Sci 74(1) (2016) 406-413.  
54  
55 547 [13] L. Livia, P. Antonella, L. Hovirag, N. Mauro, F. Panara, A nondestructive, rapid, reliable and  
56 548 inexpensive method to sample, store and extract high-quality DNA from fish body mucus and buccal  
57 549 cells, Mol Ecol Notes 6(1) (2006) 257-260.

550 [4410] C.M. Merkes, S.G. McCalla, N.R. Jensen, M.P. Gaikowski, J.J. Amberg, Persistence of DNA in  
1 551 carcasses, slime and avian feces may affect interpretation of environmental DNA data, PLoS One 9(11)  
2 552 (2014) e113346.

3 553 [4511] F. Pompanon, B.E. Deagle, W.O. Symondson, D.S. Brown, S.N. Jarman, P. Taberlet, Who is eating  
4 554 what: diet assessment using next generation sequencing, Mol Ecol 21(8) (2012) 1931-50.

5 555 [4612] S. Alasaad, A. Sánchez, J.A. Marchal, A. Píriz, J.A. Garrido-García, F. Carro, I. Romero, R.C.  
6 556 Soriguer, Efficient identification of *Microtus cabreræ* excrements using noninvasive molecular  
7 557 analysis, Conserv Genet Res 3(1) (2011) 127-129.

8 558 [4713] P.F. Thomsen, E. Willerslev, Environmental DNA – An emerging tool in conservation for  
9 559 monitoring past and present biodiversity, Biol Conserv 183 (2015) 4-18.

10 560 [4814] P. Taberlet, E. Coissac, H. Mehrdad, L.H. Rieseberg, Environmental DNA, Mol Ecol 21 (2012)  
11 561 1789-1793.

12 562 [4915] K. Deiner, H.M. Bik, E. Mächler, M. Seymour, A. Lacoursière-Roussel, F. Altermatt, S. Creer, I.  
13 563 Bista, D.M. Lodge, N. de Vere, M.E. Pfrender, L. Bernatchez, Environmental DNA metabarcoding:  
14 564 Transforming how we survey animal and plant communities, Mol Ecol 26(21) (2017) 5872-5895.

15 565 [2016] R.A. Weller, D.J. Baker, M.M. Glackin, S.J. Roberts, R.W. Schmitt, E.S. Twigg, D.J. Vimont, The  
16 566 ~~Challenge~~challenge of ~~Sustaining Ocean Observations~~sustaining ocean observations, Frontiers in  
17 567 Marine Science 6(105) (2019).

18 568 [2417] B.K. Hansen, D. Bekkevold, L. Clausen, W., E.E. Nielsen, The sceptical optimist: challenges and  
19 569 perspectives for the application of environmental DNA in marine fisheries, Fish Fish 19(5) (2018) 751-  
20 570 768.

21 571 [2218] ScienceDaily, New nano strategy fights superbugs, 2020.  
22 572 <https://www.sciencedaily.com/releases/2020/03/200312101030.htm>. {Accessed 15/4/2020.

23 573 [2319] National Geographic, Loch Ness Monster Hunters to Try DNA Search? Get the Facts., 2018.  
24 574 [https://www.nationalgeographic.com/news/2018/05/loch-ness-monster-scotland-environmental-](https://www.nationalgeographic.com/news/2018/05/loch-ness-monster-scotland-environmental-dna-science/)  
25 575 [dna-science/](https://www.nationalgeographic.com/news/2018/05/loch-ness-monster-scotland-environmental-dna-science/). {Accessed 16/4/2020.

26 576 [2420] M.S. Schäfer, Taking stock: A meta-analysis of studies on the media's coverage of science,  
27 577 Public Understanding of Science 21(6) (2010) 650-663.

28 578 [21] H.C. Rees, B.C. Maddison, D.J. Middleditch, J.R.M. Patmore, K.C. Gough, E. Crispo, REVIEW: The  
29 579 detection of aquatic animal species using environmental DNA - a review of eDNA as a survey tool in  
30 580 ecology, Journal of Applied Ecology 51(5) (2014) 1450-1459.

31 581 [2522] M.A. Barnes, C.R. Turner, The ecology of environmental DNA and implications for conservation  
32 582 genetics, Conserv Genet 17(1) (2015) 1-17.

33 583 [2623] E.E. Sigsgaard, M.R. Jensen, I.E. Winkelmann, P.R. Møller, M.M. Hansen, P.F. Thomsen,  
34 584 Population-level inferences from environmental DNA—Current status and future perspectives, Evol  
35 585 Appl 13(2) (2020) 245-262.

36 586 [2724] W. Appeltans, Shane T. Ahyong, G. Anderson, Martin V. Angel, T. Artois, N. Bailly, R. Bamber,  
37 587 A. Barber, I. Bartsch, A. Berta, M. Błażewicz-Paszkowycz, P. Bock, G. Boxshall, Christopher B. Boyko,  
38 588 Simone N. Brandão, Rod A. Bray, Niel L. Bruce, Stephen D. Cairns, T.-Y. Chan, L. Cheng, Allen G. Collins,  
39 589 T. Cribb, M. Curini-Galletti, F. Dahdouh-Guebas, Peter J.F. Davie, Michael N. Dawson, O. De Clerck, W.  
40 590 Decock, S. De Grave, Nicole J. de Voogd, Daryl P. Domning, Christian C. Emig, C. Erséus, W. Eschmeyer,  
41 591 K. Fauchald, Daphne G. Fautin, Stephen W. Feist, Charles H.J.M. Franssen, H. Furuya, O. Garcia-Alvarez,  
42 592 S. Gerken, D. Gibson, A. Gittenberger, S. Gofas, L. Gómez-Daglio, Dennis P. Gordon, Michael D. Guiry,  
43 593 F. Hernandez, Bert W. Hoeksema, Russell R. Hopcroft, D. Jaume, P. Kirk, N. Koedam, S. Koenemann,  
44 594 Jürgen B. Kolb, Reinhardt M. Kristensen, A. Kroh, G. Lambert, David B. Lazarus, R. Lemaitre, M.  
45 595 Longshaw, J. Lowry, E. Macpherson, Laurence P. Madin, C. Mah, G. Mapstone, Patsy A. McLaughlin, J.  
46 596 Mees, K. Meland, Charles G. Messing, Claudia E. Mills, Tina N. Molodtsova, R. Mooi, B. Neuhaus,  
47 597 Peter K.L. Ng, C. Nielsen, J. Norenburg, Dennis M. Opresko, M. Osawa, G. Paulay, W. Perrin, John F.  
48 598 Pilger, Gary C.B. Poore, P. Pugh, Geoffrey B. Read, James D. Reimer, M. Rius, Rosana M. Rocha, José I.  
49 599 Saiz-Salinas, V. Scarabino, B. Schierwater, A. Schmidt-Rhaesa, Kareen E. Schnabel, M. Schotte, P.  
50 600 Schuchert, E. Schwabe, H. Segers, C. Self-Sullivan, N. Shenkar, V. Siegel, W. Sterrer, S. Stöhr, B. Swalla,

601 Mark L. Tasker, Erik V. Thuesen, T. Timm, M.A. Todaro, X. Turon, S. Tyler, P. Uetz, J. van der Land, B.  
602 Vanhoorne, Leen P. van Ofwegen, Rob W.M. van Soest, J. Vanaverbeke, G. Walker-Smith, T.C. Walter,  
603 A. Warren, Gary C. Williams, Simon P. Wilson, Mark J. Costello, The ~~Magnitudemagnitude~~ of ~~Global~~  
604 ~~Marine Species Diversity~~ [global marine species diversity](#), *Current Biology* 22(23) (2012) 2189-2202.  
605 ~~[2825]~~ E. Aylagas, Á. Borja, I. Muxika, N. Rodríguez-Ezpeleta, Adapting metabarcoding-based benthic  
606 biomonitoring into routine marine ecological status assessment networks, *Ecological Indicators* 95  
607 (2018) 194-202.  
608 ~~[2926]~~ J. Lobo, S. Shokralla, M.H. Costa, M. Hajibabaei, F.O. Costa, DNA metabarcoding for high-  
609 throughput monitoring of estuarine macrobenthic communities, *Sci Rep* 7(1) (2017) 15618.  
610 ~~[30]~~~~[27]~~ [L.E. Holman, M. de Bruyn, S. Creer, G.F. Ficetola, C. Miaud, F. Pompanon, P. Taberlet, Species](#)  
611 ~~detection~~ [Carvalho, J. Robidart, M. Rius, Detection of introduced and resident marine species using](#)  
612 [environmental DNA from metabarcoding of sediment and water samples, \*Biology Letters\* 4\(4\) \(2008\)](#)  
613 [423-425, \*Sci Rep\* 9\(1\) \(2019\) 11559.](#)  
614 ~~[28]~~ [C.J. Closek, J.A. Santora, H.A. Starks, I.D. Schroeder, E.A. Andruszkiewicz, K.M. Sakuma, S.J.](#)  
615 [Bograd, E.L. Hazen, J.C. Field, A.B. Boehm, Marine vertebrate biodiversity and distribution within the](#)  
616 [central California current using environmental DNA \(eDNA\) metabarcoding and ecosystem surveys,](#)  
617 [Frontiers in Marine Science 6\(732\) \(2019\).](#)  
618 ~~[29]~~ [I. Salter, M. Joensen, R. Kristiansen, P. Steingrund, P. Vestergaard, Environmental DNA](#)  
619 [concentrations are correlated with regional biomass of Atlantic cod in oceanic waters,](#)  
620 [Communications Biology 2\(1\) \(2019\) 461.](#)  
621 ~~[31]~~ [R.A. Erickson, C.B. Rees, A.A. Coulter, C.M. Merkes, S.G. McCalla, K.F. Touzinsky, L. Walleiser, R.R.](#)  
622 [Goforth, J.J. Amberg, Detecting the movement and spawning activity of bigheaded carps with](#)  
623 [environmental DNA, \*Mol Ecol Resour\* 16\(4\) \(2016\) 957-65.](#)  
624 ~~[32]~~ [H. Doi, R. Inui, Y. Akamatsu, K. Kanno, H. Yamanaka, T. Takahara, T. Minamoto, Environmental](#)  
625 [DNA analysis for estimating the abundance and biomass of stream fish, \*Freshwater Biol\* 62\(1\) \(2017\)](#)  
626 [30-39.](#)  
627 ~~[33]~~~~[30]~~ K. Deiner, H.M. Bik, E. Machler, M. Seymour, A. Lacoursiere-Roussel, F. Altermatt, S. Creer, I.  
628 Bista, D.M. Lodge, N. de Vere, M.E. Pfrender, L. Bernatchez, Environmental DNA metabarcoding:  
629 Transforming how we survey animal and plant communities, *Mol Ecol* 26(21) (2017) 5872-5895.  
630 ~~[3431]~~ C.R. Turner, K.L. Uy, R.C. Everhart, Fish environmental DNA is more concentrated in aquatic  
631 sediments than surface water, *Biol Conserv* 183 (2015) 93-102.  
632 ~~[3532]~~ K. Deiner, J. Lopez, S. Bourne, L. Holman, M. Seymour, E.K. Grey, A. Lacoursière, Y. Li, M.A.  
633 Renshaw, M.E. Pfrender, M. Rius, L. Bernatchez, D.M. Lodge, Optimising the detection of marine  
634 taxonomic richness using environmental DNA metabarcoding: the effects of filter material, pore size  
635 and extraction method, *Metabarcoding and Metagenomics* 2 (2018) e28963.  
636 ~~[3633]~~ Y. Liu, G.H. Wikfors, J.M. Rose, R.S. McBride, L.M. Milke, R. Mercaldo-Allen, Application of  
637 Environmental DNA Metabarcoding to Spatiotemporal Finfish Community Assessment in a Temperate  
638 Embayment, *Frontiers in Marine Science* 6(674) (2019).  
639 ~~[3734]~~ N.K. Truelove, E.A. Andruszkiewicz, B.A. Block, A rapid environmental DNA method for  
640 detecting white sharks in the open ocean, *Methods Ecol Evol* 10(8) (2019) 1128-1135.  
641 ~~[38]~~~~[35]~~ [F. Sanger, A.R. Coulson, A rapid method for determining sequences in DNA by primed synthesis](#)  
642 [with DNA polymerase, \*Journal of Molecular Biology\* 94\(3\) \(1975\) 441-448.](#)  
643 ~~[36]~~ [K. Deiner, F. Altermatt, Transport Distance of Invertebrate Environmental DNA in a Natural River,](#)  
644 [PLoS One 9\(2\) \(2014\) e88786.](#)  
645 ~~[37]~~ J.L.A. Shaw, L. Weyrich, A. Cooper, Using environmental (e)DNA sequencing for aquatic  
646 biodiversity surveys: a beginner's guide, *Marine and Freshwater Research* 68(1) (2017) pp. 20-33-2017  
647 v.68 no.1.  
648 ~~[3938]~~ A.C. Thomas, S. Tank, P.L. Nguyen, J. Ponce, M. Sinnesael, C.S. Goldberg, A system for rapid  
649 eDNA detection of aquatic invasive species, *Environmental DNA* doi.org/10.1002/edn3.25 (2019).



650 ~~[40]~~[39] L. Miralles, M. Parrondo, A. Hernández de Rojas, E. Garcia-Vazquez, Y.J. Borrell, Development  
651 and validation of eDNA markers for the detection of *Crepidula fornicata* in environmental samples,  
652 *Marine Pollution Bulletin* 146 (2019) 827-830.

653 [40] G.M. Nester, M. De Brauwer, A. Koziol, K.M. West, J.D. DiBattista, N.E. White, M. Power, M.J.  
654 Heydenrych, E. Harvey, M. Bunce, Development and evaluation of fish eDNA metabarcoding assays  
655 facilitate the detection of cryptic seahorse taxa (family: Syngnathidae), *Environmental DNA*  
656 [doi.org/10.1002/edn3.93](https://doi.org/10.1002/edn3.93) (2020).

657 [41] R.A. Collins, K.F. Armstrong, A.J. Holyoake, S. Keeling, Something in the water: biosecurity  
658 monitoring of ornamental fish imports using environmental DNA, *Biol Invasions* 15(6) (2012) 1209-  
659 1215.

660 ~~[41]~~[42] A. Takeuchi, S. Watanabe, S. Yamamoto, M.J. Miller, T. Fukuba, T. Miwa, T. Okino, T.  
661 Minamoto, K. Tsukamoto, First use of oceanic environmental DNA to study the spawning ecology of  
662 the Japanese eel *Anguilla japonica*, *Mar Ecol Prog Ser* 609 (2019) 187-196.

663 [43] M.V. Everett, L.K. Park, Exploring deep-water coral communities using environmental DNA, *Deep*  
664 *Sea Research Part II: Topical Studies in Oceanography* 150 (2018) 229-241.

665 [44] A. Valentini, P. Taberlet, C. Miaud, R. Civade, J. Herder, P.F. Thomsen, E. Bellemain, A. Besnard, E.  
666 Coissac, F. Boyer, C. Gaboriaud, P. Jean, N. Poulet, N. Roset, G.H. Copp, P. Geniez, D. Pont, C. Argillier,  
667 J.M. Baudoin, T. Peroux, A.J. Crivelli, A. Olivier, M. Acqueberge, M. Le Brun, P.R. Moller, E. Willerslev,  
668 T. Dejean, Next-generation monitoring of aquatic biodiversity using environmental DNA  
669 metabarcoding, *Mol Ecol* 25(4) (2016) 929-42.

670 [42,45] C.L. Jerde, E.A. Wilson, T.L. Dressler, Measuring global fish species richness with eDNA  
671 metabarcoding, *Mol Ecol Resour* 19(1) (2019) 19-22.

672 [43,46] P.D.N. Hebert, A. Cywinska, S.L. Ball, J.R. deWaard, Biological identifications through DNA  
673 barcodes, *Proc R Soc Lond B Biol Sci* 270(1512) (2003) 313-321.

674 [47] N. Fraija-Fernández, M.-C. Bouquieaux, A. Rey, I. Mendibil, U. Cotano, X. Irigoien, M. Santos, N.  
675 Rodríguez-Ezpeleta, Marine water environmental DNA metabarcoding provides a comprehensive fish  
676 diversity assessment and reveals spatial patterns in a large oceanic area, *Ecol Evol* 44] A. Rey, K.J.  
677 Carney, L.E. Quinones, K.M. Pagenkopp Lohan, G.M. Ruiz, O.C. Basurko, N. Rodríguez-Ezpeleta,  
678 *Environmental DNA* 10(14) (2020) 7560-7584.

679 [48] B. Günther, T. Knebelsberger, H. Neumann, S. Laakmann, P. Martínez Arbizu, Metabarcoding: of  
680 marine environmental DNA based on mitochondrial and nuclear genes, *Sci Rep* 8(1) (2018) 14822.

681 [49] D. Steinke, A Promising Tool for Ballast Water. D. Connell, P.D.N. Hebert, Linking adults and  
682 immatures of South African marine fishes, *Genome* 59(11) (2016) 959-967.

683 [50] M. Valdez-Moreno, C. Quintal-Lizama, R. Gómez-Lozano, M.d.C. García-Rivas, Monitoring an alien  
684 invasion: DNA barcoding and the identification of lionfish and their prey on coral reefs of the Mexican  
685 Caribbean, *PLOS ONE* 7(6) (2012) e36636.

686 [51] S. Yamamoto, R. Masuda, Y. Sato, T. Sado, H. Araki, M. Kondoh, T. Minamoto, M. Miya,  
687 Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea, *Sci Rep*  
688 7 (2017) 40368.

689 [52] J. Pawlowski, P. Esling, F. Lejzerowicz, T. Cordier, J.A. Visco, C.I.M. Martins, A. Kvalvik, K. Staven,  
690 T. Cedhagen, Benthic monitoring of salmon farms in Norway using foraminiferal metabarcoding,  
691 *Aquaculture Environment Interactions* 8 (2016) 371-386.

692 [53] A. Ardura, A. Zaiko, J.L. Martinez, A. Samuiloviene, Y. Borrell, E. Garcia-Vazquez, Environmental  
693 DNA evidence of transfer of North Sea molluscs across tropical waters through ballast water, *Journal*  
694 *of Molluscan Studies* 81(4) (2015) 495-501.

695 [54] E.A. Andruszkiewicz, H.A. Starks, F.P. Chavez, L.M. Sassoubre, B.A. Block, A.B. Boehm,  
696 Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding, *PLOS ONE* 12(4)  
697 (2017) e0176343.

698 [55] M.Y. Stoeckle, L. Soboleva, Z. Charlop-Powers, Aquatic environmental DNA detects seasonal fish  
699 abundance and habitat preference in an urban estuary, *PLoS One* 12(4) (2017) e0175186.

700 [Science & Technology 53\(20\)\[56\] N.A. Sawaya, A. Djurhuus, C.J. Closek, M. Hepner, E. Olesin, L. Visser,](#)  
701 [C. Kelble, K. Hubbard, M. Breitbart, Assessing eukaryotic biodiversity in the Florida Keys National](#)  
702 [Marine Sanctuary through environmental DNA metabarcoding, Ecol Evol 9\(3\) \(2019\) 11849-](#)  
703 [118591029-1040.](#)  
704 [4557] M. Seymour, F.K. Edwards, B.J. Cosby, M.G. Kelly, M. de Bruyn, G.R. Carvalho, S. Creer,  
705 Executing multi-taxa eDNA ecological assessment via traditional metrics and interactive networks,  
706 Science of The Total Environment 729 (2020) 138801.  
707 [4658] S. Fernandez, M.M. Sandin, P.G. Beaulieu, L. Clusa, J.L. Martinez, A. Ardura, E. Garcia-Vazquez,  
708 Environmental DNA for freshwater fish monitoring: insights for conservation within a protected area,  
709 PeerJ 6 (2018) e4486.  
710 [4759] A. Karahan, J. Douek, G. Paz, N. Stern, A.E. Kideys, L. Shaish, M. Goren, B. Rinkevich, Employing  
711 DNA barcoding as taxonomy and conservation tools for fish species censuses at the southeastern  
712 Mediterranean, a hot-spot area for biological invasion, Journal for Nature Conservation 36 (2017) 1-  
713 9.  
714 [4860] J. Bakker, O.S. Wangensteen, C. Baillie, D. Buddo, D.D. Chapman, A.J. Gallagher, T.L. Guttridge,  
715 H. Hertler, S. Mariani, Biodiversity assessment of tropical shelf eukaryotic communities via pelagic  
716 eDNA metabarcoding, Ecol Evol 9(24) (2019) 14341-14355.  
717 [4961] O. Laroche, O. Kersten, C.R. Smith, E. Goetze, From sea surface to seafloor: a benthic  
718 allochthonous eDNA survey for the abyssal ocean, bioRxiv doi.org/10.1101/2020.05.07.082602  
719 (2020).  
720 [5062] P.F. Thomsen, P.R. Moller, E.E. Sigsgaard, S.W. Knudsen, O.A. Jorgensen, E. Willerslev,  
721 Environmental DNA from seawater samples correlate with trawl catches of subarctic, deepwater  
722 fishes, PLoS One 11(11) (2016) e0165252.  
723 [5163] T. Takahara, T. Minamoto, H. Yamanaka, H. Doi, Z. Kawabata, Estimation of fish biomass using  
724 environmental DNA, PLoS One 7(4) (2012) e35868.  
725 [5264] A. Lacoursière-Roussel, G. Côté, V. Leclerc, L. Bernatchez, Quantifying relative fish abundance  
726 with eDNA: a promising tool for fisheries management, Journal of Applied Ecology 53(4) (2016) 1148-  
727 1157.  
728 [53[65] J.A. Port, J.L. O'Donnell, O.C. Romero-Maraccini, P.R. Leary, S.Y. Litvin, K.J. Nickols, K.M.  
729 Yamahara, R.P. Kelly, Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental  
730 DNA, Mol Ecol 25(2) (2016) 527-541.  
731 [66] L. Peters, S. Spatharis, M.A. Dario, T. Dwyer, I.J.T. Roca, A. Kintner, Ø. Kanstad-Hanssen, M.S.  
732 Llewellyn, K. Praebel, Environmental DNA: a new low-cost monitoring tool for pathogens in salmonid  
733 aquaculture, Frontiers in microbiology 9(3009) (2018).  
734 [67] T.P. Muha, R. Skukan, Y.J. Borrell, J.M. Rico, C. Garcia de Leaniz, E. Garcia-Vazquez, S. Consuegra,  
735 Contrasting seasonal and spatial distribution of native and invasive *Codium* seaweed revealed by  
736 targeting species-specific eDNA, Ecol Evol 9(15) (2019) 8567-8579.  
737 [68] ~~N. Fraija-Fernández, M. C. Bouquicaux, A. Rey, I. Mendibil, U. Cotano, X. Irigoien, M. Santos, N.~~  
738 ~~Rodríguez-Ezpeleta, Marine water environmental DNA metabarcoding provides a comprehensive fish~~  
739 ~~diversity assessment and reveals spatial patterns in a large oceanic area, Ecol Evol~~  
740 ~~doi.org/10.1002/ece3.6482 (2020).~~  
741 [54] ~~A. Takeuchi, S. Watanabe, S. Yamamoto, M.J. Miller, T. Fukuba, T. Miwa, T. Okino, T. Minamoto,~~  
742 ~~K. Tsukamoto, First use of oceanic environmental DNA to study the spawning ecology of the Japanese~~  
743 ~~eel *Anguilla japonica*, Mar Ecol Prog Ser 609 (2019) 187-196.~~  
744 [55] W.A. Hubert, M.C. Fabrizio, Relative abundance and catch per unit effort, in: C.S. Guy, M.L. Brown  
745 (Eds.), Analysis and Interpretation of Freshwater Fisheries Data, American Fisheries Society, Bethesda,  
746 MA, 2007, pp. 279–325.  
747 [56[69] ~~I. Salter, M. Joensen, R. Kristiansen, P. Steingrund, P. Vestergaard, Environmental DNA~~  
748 ~~concentrations are correlated with regional biomass of Atlantic cod in oceanic waters,~~  
749 ~~Communications Biology 2(1) (2019) 461.~~

750 [57] S.A. Bonar, W.A. Hubert, D.W. Willis, Standard methods for sampling North American freshwater  
 1 751 fishes, American Fisheries Society, Bethesda, MD, 2009.

2 752 [5870] CEN, Water quality - Sampling of fish with multi-mesh gillnets. CEN EN 14757, 2005.

3 753 [5971] P. Thomsen, Francis, Kielgast, J. O. S., L. Iversen, L., C. Wiuf, M. Rasmussen, M.T. Gilbert, P., L.  
 4 754 Orlando, E. Willerslev, Monitoring endangered freshwater biodiversity using environmental DNA, Mol  
 5 755 Ecol 21(11) (2012) 2565-2573.

6 756 [6072] K.E. Klymus, C.A. Richter, D.C. Chapman, C. Paukert, Quantification of eDNA shedding rates  
 7 757 from invasive bighead carp *Hypophthalmichthys nobilis* and silver carp *Hypophthalmichthys molitrix*,  
 8 758 Biol Conserv 183 (2015) 77-84.

9 759 [6173] H. Doi, T. Takahara, T. Minamoto, S. Matsushashi, K. Uchii, H. Yamanaka, Droplet digital  
 10 760 polymerase chain reaction (PCR) outperforms real-time PCR in the detection of environmental DNA  
 11 761 from an invasive fish species, Environmental Science & Technology 49(9) (2015) 5601-8.

12 762 [6274] M.D. Tillotson, R.P. Kelly, J.J. Duda, M. Hoy, J. Kralj, T.P. Quinn, Concentrations of environmental  
 13 763 DNA (eDNA) reflect spawning salmon abundance at fine spatial and temporal scales, Biol Conserv 220  
 14 764 (2018) 1-11.

15 765 [6375] D.S. Pilliod, C.S. Goldberg, R.S. Arkle, L.P. Waits, J. Richardson, Estimating occupancy and  
 16 766 abundance of stream amphibians using environmental DNA from filtered water samples, Can J Fish  
 17 767 Aquat Sci 70(8) (2013) 1123-1130.

18 768 [6476] B. Hänfling, L. Lawson Handley, S. Read Daniel, C. Hahn, J. Li, P. Nichols, C. Blackman Rosetta,  
 19 769 A. Oliver, J. Winfield Ian, Environmental DNA metabarcoding of lake fish communities reflects long-  
 20 770 term data from established survey methods, Mol Ecol 25(13) (2016) 3101-3119.

21 771 [6577] M.Y. Stoeckle, L. Soboleva, Z. Charlop Powers, Aquatic environmental DNA detects seasonal  
 22 772 fish abundance and habitat preference in an urban estuary, PLoS One 12(4) (2017) e0175186.

23 773 [66] M.C. Schmelzle, A.P. Kinziger, Using occupancy modelling to compare environmental DNA to  
 24 774 traditional field methods for regional-scale monitoring of an endangered aquatic species, Mol Ecol  
 25 775 Resour 16(4) (2016) 895-908.

26 776 [6778] S. Yamamoto, K. Minami, K. Fukaya, K. Takahashi, H. Sawada, H. Murakami, S. Tsuji, H.  
 27 777 Hashizume, S. Kubonaga, T. Horiuchi, M. Hongo, J. Nishida, Y. Okugawa, A. Fujiwara, M. Fukuda, S.  
 28 778 Hidaka, K.W. Suzuki, M. Miya, H. Araki, H. Yamanaka, A. Maruyama, K. Miyashita, R. Masuda, T.  
 29 779 Minamoto, M. Kondoh, Environmental DNA as a 'Snapshot' of Fish Distribution: A Case Study of  
 30 780 Japanese Jack Mackerel in Maizuru Bay, Sea of Japan, PLoS One 11(3) (2016) e0149786.

31 781 [6879] K. Fukaya, H. Murakami, S. Yoon, K. Minami, Y. Osada, S. Yamamoto, R. Masuda, A. Kasai, K.  
 32 782 Miyashita, T. Minamoto, M. Kondoh, Estimating fish population abundance by integrating quantitative  
 33 783 data on environmental DNA and hydrodynamic modelling, bioRxiv doi.org/10.1101/482489 (2018).

34 784 [6980] T. Chambert, D.S. Pilliod, C.S. Goldberg, H. Doi, T. Takahara, An analytical framework for  
 35 785 estimating aquatic species density from environmental DNA, Ecol Evol 8(6) (2018) 3468-3477.

36 786 [7081] L.M. Sassoubre, K.M. Yamahara, L.D. Gardner, B.A. Block, A.B. Boehm, Quantification of  
 37 787 Environmental environmental DNA (eDNA) Sheddingshedding and Decay Ratesdecay rates for Three  
 38 788 Marine Fishthree marine fish, Environmental Science & Technology 50(19) (2016) 10456-10464.

39 789 [7182] E.A. Andruszkiewicz, L.M. Sassoubre, A.B. Boehm, Persistence of marine fish environmental  
 40 790 DNA and the influence of sunlight, PLoS One 12(9) (2017) e0185043.

41 791 [7283] T. Jo, M. Arimoto, H. Murakami, R. Masuda, T. Minamoto, Estimating shedding and decay rates  
 42 792 of environmental nuclear DNA with relation to water temperature and biomass, Environmental DNA  
 43 793 2(2) (2020) 140-151.

44 794 [7384] L.L. Iversen, J. Kielgast, K. Sand-Jensen, Monitoring of animal abundance by environmental DNA  
 45 795 — An increasingly obscure perspective: A reply to Klymus et al., 2015, Biol Conserv 192 (2015) 479-  
 46 796 480.

47 797 [7485] J.B. Harrison, J.M. Sunday, S.M. Rogers, Predicting the fate of eDNA in the environment and  
 48 798 implications for studying biodiversity, Proc R Soc Lond B Biol Sci 286(1915) (2019) 20191409.

49 799 [7586] G.F. Ficetola, P. Taberlet, E. Coissac, How to limit false positives in environmental DNA and  
 50 800 metabarcoding?, Mol Ecol Resour 16(3) (2016) 604-7.



801 [7687] C.L. Jerde, Can we manage fisheries with the inherent uncertainty from eDNA?, J Fish Biol  
802 doi:10.1111/jfb.14218 (2019).

803 [7788] I.A. Dickie, S. Boyer, H.L. Buckley, R.P. Duncan, P.P. Gardner, I.D. Hogg, R.J. Holdaway, G. Lear,  
804 A. Makiola, S.E. Morales, J.R. Powell, L. Weaver, Towards robust and repeatable sampling methods in  
805 eDNA-based studies, Mol Ecol Resour 18(5) (2018) 940-952.

806 [7889] K.M. Yamahara, C.M. Preston, J. Birch, K. Walz, R. Marin, S. Jensen, D. Pargett, B. Roman, W.  
807 Ussler, Y. Zhang, J. Ryan, B. Hobson, B. Kieft, B. Raanan, K.D. Goodwin, F.P. Chavez, C. Scholin, In situ  
808 ~~Autonomous—Acquisition~~autonomous acquisition and ~~Preservation~~preservation of ~~Marine~~  
809 ~~Environmental~~marine environmental DNA ~~Using~~using an ~~Autonomous—Underwater~~  
810 ~~Vehicle~~autonomous underwater vehicle, Frontiers in Marine Science 6(373) (2019).

811 [7990] A. Djurhuus, J. Port, C.J. Closek, K.M. Yamahara, O. Romero-Maraccini, K.R. Walz, D.B.  
812 Goldsmith, R. Michisaki, M. Breitbart, A.B. Boehm, F.P. Chavez, Evaluation of ~~Filtration~~filtration and  
813 DNA ~~Extraction—Method~~extraction methods for ~~Environmental~~environmental DNA ~~Biodiversity~~  
814 ~~Assessments~~biodiversity assessments across ~~Multiple Trophic Levels~~multiple trophic levels, Frontiers  
815 in Marine Science 4 (2017) 314.

816 [8091] R. Pinfield, E. Dillane, A.K.W. Runge, A. Evans, L. Mirimin, J. Niemann, T.E. Reed, D.G. Reid, E.  
817 Rogan, F.I.P. Samarra, E.E. Sigsgaard, A.D. Foote, False-negative detections from environmental DNA  
818 collected in the presence of large numbers of killer whales (*Orcinus orca*), Environmental DNA 1(4)  
819 (2019) 316-328.

820 [8192] T. Schenekar, M. Schletterer, L.A. Lecaudey, S.J. Weiss, Reference databases, primer choice,  
821 and assay sensitivity for environmental metabarcoding: Lessons learnt from a re-evaluation of an  
822 eDNA fish assessment in the Volga headwaters, River Research and Applications  
823 doi.org/10.1002/rra.3610 (2020).

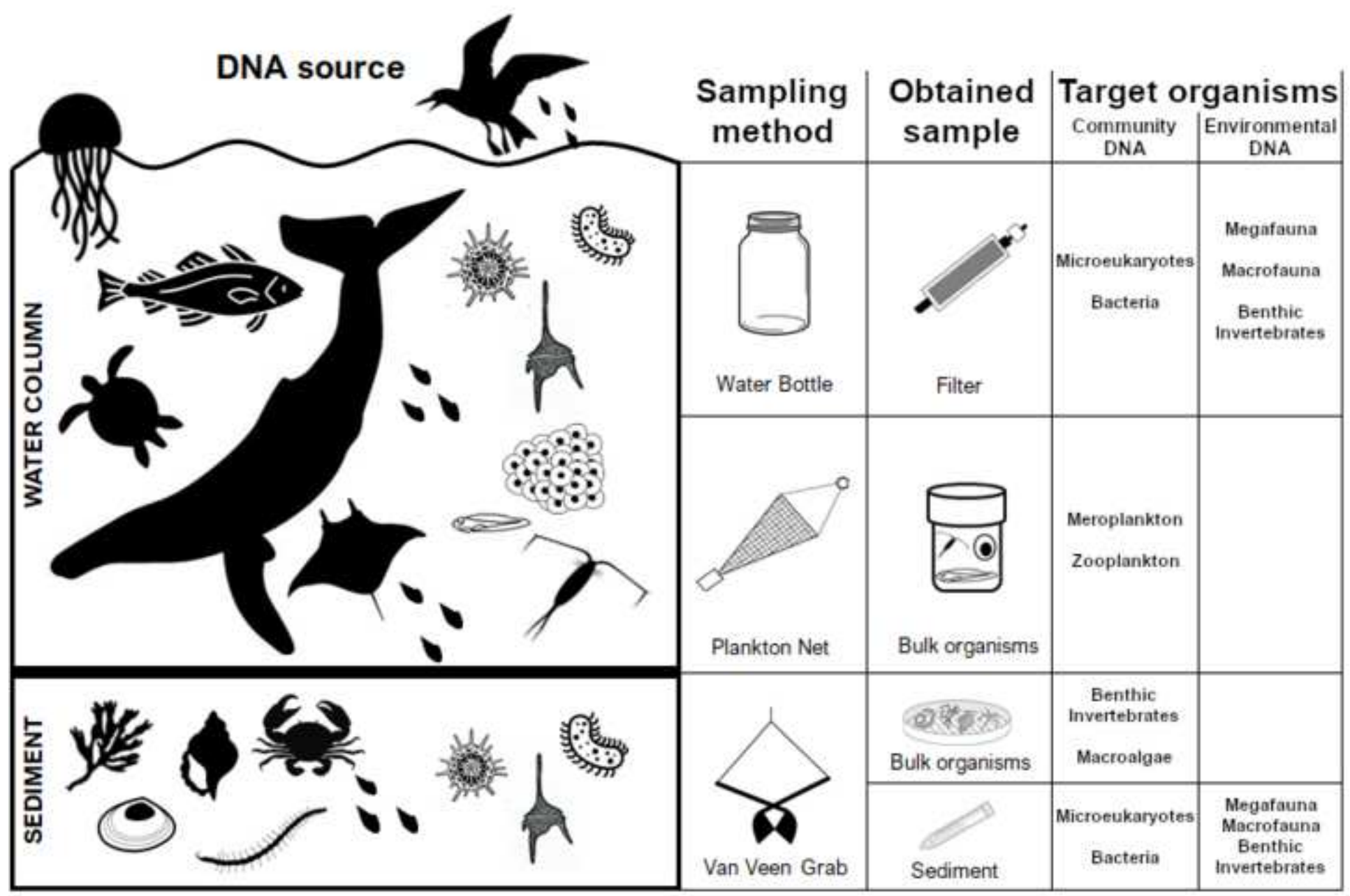
824 [8293] H. Weigand, A.J. Beermann, F. Čiampor, F.O. Costa, Z. Csabai, S. Duarte, M.F. Geiger, M.  
825 Grabowski, F. Rimet, B. Rulik, M. Strand, N. Szucsich, A.M. Weigand, E. Willassen, S.A. Wyler, A.  
826 Bouchez, A. Borja, Z. Čiamporová-Zaťovičová, S. Ferreira, K.-D.B. Dijkstra, U. Eisendle, J. Freyhof, P.  
827 Gadawski, W. Graf, A. Haegerbaeumer, B.B. van der Hoorn, B. Japoshvili, L. Keresztes, E. Keskin, F.  
828 Leese, J.N. Macher, T. Mamos, G. Paz, V. Pešić, D.M. Pfannkuchen, M.A. Pfannkuchen, B.W. Price, B.  
829 Rinkevich, M.A.L. Teixeira, G. Várбірó, T. Ekrem, DNA barcode reference libraries for the monitoring of  
830 aquatic biota in Europe: Gap-analysis and recommendations for future work, Science of The Total  
831 Environment 678 (2019) 499-524.

832 [8394] J. Pawlowski, M. Kelly-Quinn, F. Altermatt, L. Apothéloz-Perret-Gentil, P. Beja, A. Boggero, A.  
833 Borja, A. Bouchez, T. Cordier, I. Domaizon, M.J. Feio, A.F. Filipe, R. Fornaroli, W. Graf, J. Herder, B. van  
834 der Hoorn, J. Iwan Jones, M. Sagova-Mareckova, C. Moritz, J. Barquín, J.J. Piggott, M. Pinna, F. Rimet,  
835 B. Rinkevich, C. Sousa-Santos, V. Specchia, R. Trobajo, V. Vasselon, S. Vitecek, J. Zimmerman, A.  
836 Weigand, F. Leese, M. Kahlert, The future of biotic indices in the ecogenomic era: Integrating (e)DNA  
837 metabarcoding in biological assessment of aquatic ecosystems, Science of The Total Environment 637-  
838 638 (2018) 1295-1310.

839 [8495] T.E. Berry, B.J. Saunders, M.L. Coghlan, M. Stat, S. Jarman, A.J. Richardson, C.H. Davies, O. Berry,  
840 E.S. Harvey, M. Bunce, Marine environmental DNA biomonitoring reveals seasonal patterns in  
841 biodiversity and identifies ecosystem responses to anomalous climatic events, PLOS Genetics 15(2)  
842 (2019) e1007943.

843

844



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.