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

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47 and ecosystem monitoring

48 **Abstract**

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

67

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

69

1. Introduction

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

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

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

106 Environmental DNA (eDNA) stems from individual organisms which release DNA into the
107 environment through waste products, skin/tissue, scales, gametes, mucus, blood and carcasses [9-
108 12]. This extra-organismal DNA is termed environmental DNA (eDNA) [13]. In contrast to DNA
109 extracted from tissue samples, or community DNA – where DNA is extracted from communities of
110 whole organisms - eDNA does not require sampling the target organisms themselves, but instead the
111 sampling of the environment they live in [14, 15]. The development of new ways of monitoring marine
112 ecosystems and marine biodiversity using eDNA has advanced over recent years and has
113 revolutionised the ability to track invasive species, monitor endangered species, assess the health of
114 fish stocks, and explore the world of marine biodiversity [16]. The seeming simplicity and cost-
115 effectiveness of eDNA-based approaches, together with the interest from wider stakeholder groups,
116 has made such applications highly attractive [17].

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

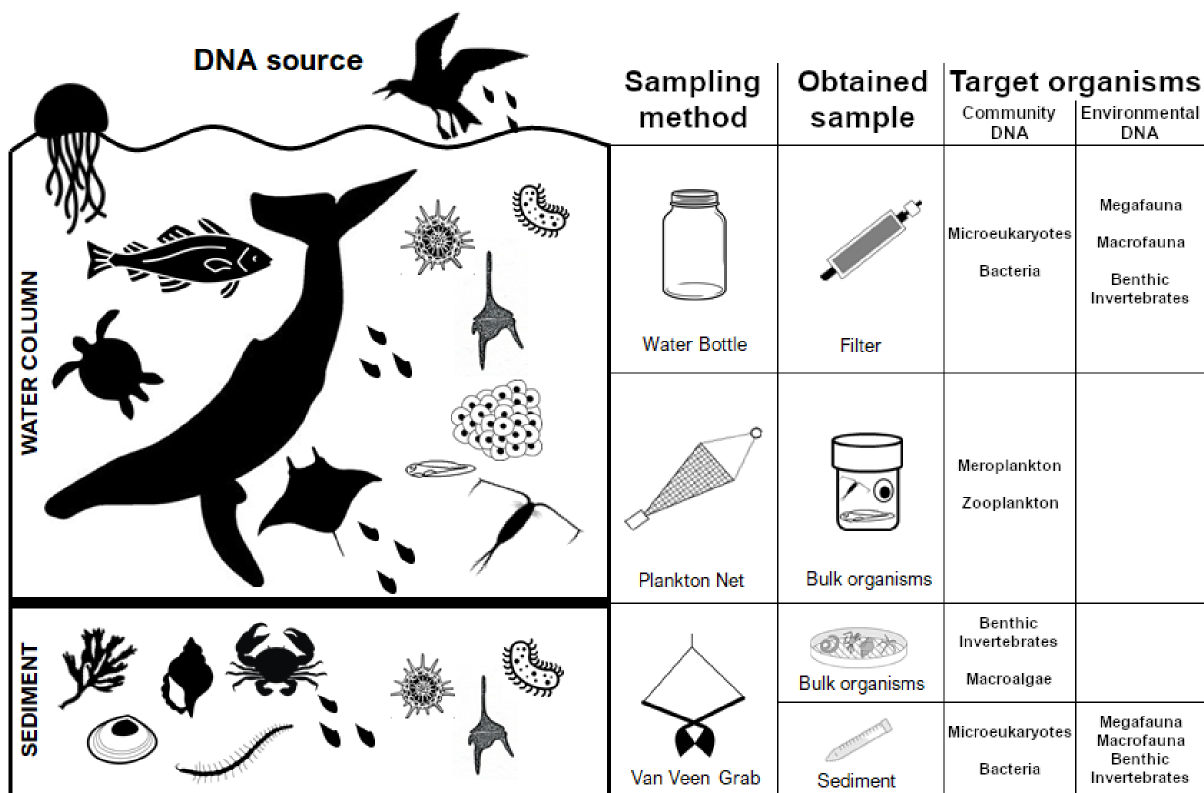
121 to the very large (and improbable) "Loch Ness Monster Hunters to Try DNA Search?" [19].
122 Disentangling fact from fiction, and hyperbola from reality, is thus not a simple task for the manager
123 striving to understand the field. As such this raises two opposing issues which could each negatively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

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

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

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

154



155

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

159

160 **2. Environmental DNA in a fisheries context**

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

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

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

187 temperature, organic matter, pH, UV radiation, and water currents, and by the type and amount of
188 material used during sampling [17]. Further, as eDNA is used as a proxy for species presence, any
189 biases in the transport and persistence of eDNA can result in its distribution being significantly
190 different from that of the actual ornagisms. Careful evaluation of these biases is needed for the correct
191 interpretation of eDNA results in the framework of fisheries management and conservation.
192

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

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

213

214 *3.1. Targeted species detection*

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

226

227 **Table 1**

228 Selected applications of targeted species detection using marine eDNA.

229

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]

230

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

247

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

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

258 In order to address these issues, species-specific genetic probes were developed and tested in
259 the laboratory by filtering and extracting eDNA from tank water containing eels. This showed that the
260 probes could identify the Japanese eel from a minute amount of eDNA. Samples were collected at
261 varying depths during an ocean survey on the southern West Mariana Ridge in the general spawning
262 area of the eel. eDNA positive signals were detected for *A. japonica* from 3 of the 108 samples.
263 This first attempt to detect Japanese eel eDNA suggests the approach has the potential to
264 provide information in near real-time about the spawning aggregations in a deep-water environment
265 which is very challenging to survey using traditional techniques.
266
267 *3.2. Community characterisation*
268 Community characterisation, often referred to as community metabarcoding, is a technique
269 used to characterise either the species composition or a selected subset of species, whose eDNA is
270 represented in a water sample [44, 45]. Using this approach, a region of DNA conserved within a
271 species and diverse across a wide range of taxa is specifically targeted and many targets are captured
272 simultaneously in a single reaction. Amplified products are sequenced, revealing unique species-
273 specific signatures (i.e. a barcode for that species) within a sample and sequences are compared to
274 reference sequences within a database. As such, each unique sequence match between the sample
275 and the reference database will identify DNA from a specific species in the sample [46]. Metabarcoding
276 has been utilized in a variety of settings, showing a broad potential application for biodiversity
277 monitoring (Table 2).
278
279 **Table 2**
280 Selected applications of community characterisation using marine eDNA.
281

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

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

282

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

292 effective characterisation of entire communities, and therefore it is especially useful in ecosystem
293 monitoring scenarios.
294

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

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

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

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

315
316

317 *3.3. Species Abundance Estimation*

318 Together with the identification of both individual and ecosystem-based biodiversity, eDNA can
319 be used to estimate either the relative abundance of multiple species using metabarcoding [62], or
320 the absolute abundance of individual species using qPCR [63]. At its simplest, such approaches involve
321 quantifying the amount of eDNA from a species represented in a sample and using that as a simple
322 proxy for abundance [64]. Such information may be used to estimate numbers of individuals and/or
323 biomass. The use of eDNA-based tools to quantify stocks of species of interest is of course of great
324 interest to fishery managers and policy makers, as population or stock assessment is a central
325 component of any management and/or conservation programme. Estimating absolute counts and/or
326 biomass, relies on the establishment of a robust correlation between DNA concentration and living
327 biomass whereas relative biomass estimates assume that the relative amounts of DNA measured in
328 the sample are representative of the relative abundance of the different species in the ecosystem.
329 While both approaches may seem to rely on fairly simple calculations and indeed are beginning to be
330 used (Table 3), in practice, there are many factors which interact to make the relationships upon which
331 the assumptions about the correlations are made very complex to disentangle and to obtain robust
332 estimates.
333
334 **Table 3**
335 Selected applications of abundance estimation using marine eDNA.
336

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

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

337

338 Applications of using eDNA to assess abundance in the aquatic environment are at present most
339 advanced in freshwater [62]. Abundance estimation using traditional methods such as gillnet data and
340 trawling provides a relative index assumed to be directly proportional to density/absolute abundance
341 [29, 64, 68]. Such traditional non-genetic methods are the most common to estimate fish abundance
342 in lakes for fisheries management [69] and biodiversity characterisation [70], although they are often
343 expensive, time consuming and destructive. Initial results from experimental aquaria and ponds show
344 positive correlations between species abundance and eDNA concentration [71, 72]. However, even in
345 controlled tank situations, it has been found that "…quantification of eDNA samples can be highly
346 variable even when sampling from the same individual under controlled conditions" [72]. Approaches
347 have now moved from the experimental set-up to the field. The abundance of individual targeted
348 species has been characterised using eDNA in freshwater fish species including lake trout (*Salvelinus*
349 *namaycush*) [64], common carp (*Cyprinus carpio*) [73] and Atlantic salmon (*Salmo salar*) [74]. Similarity
350 between relative and absolute abundance has been reported in communities including both
351 amphibians [75] and fish [55, 76], including commercially important species such as Atlantic cod
352 (*Gadus morhua*) [29].

353

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

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

359 In order to determine the utility of eDNA for assessing commercial stocks a quantitative eDNA
360 survey of Atlantic cod was compared to results from a standardised demersal trawl survey. Important
361 stock metrics such as regional cod biomass and Catch Per Unit Effort (CPUE) were determined using
362 traditional assessment analysis of trawl data. At 35 trawl stations water samples were also collected
363 4 m above the seafloor and eDNA analysed in the laboratory using cod-specific DNA probes.

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

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

374

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

385 [79, 80]. However, such a relationship may not be always true, or even present in most cases. The
386 amount of eDNA at a location will vary depending on a number of biological, physical and
387 environmental factors (see below). While these factors also have an impact on species detection, the
388 impact of the fluctuations registered is higher if quantitative measurements are being attempted,
389 rather than simple presence/absence results. Nevertheless, it may be possible to incorporate these
390 impacts into modelling, to better predict how they can affect eDNA concentrations, therefore
391 reducing the variance around such quantifications [79, 81-83]. However, due to the complexity of
392 interacting factors, direct quantitative assessments remain highly challenging in marine ecosystems
393 [17, 84].

394 Abundance estimates in the marine environment can thus be summarised to be very much in
395 the developmental stage at the moment, notwithstanding some of the early applications being
396 examined. Significant questions still have to be addressed to allow the amount of eDNA collected to
397 be linked directly to either relative or absolute abundances. The three-dimensional nature of the
398 environment, together with the many physical, chemical and environmental factors whose impacts
399 have to be quantified means that the validity of abundance quantification using eDNA is still to be
400 determined in most if not all situations. Significant work is, however, being undertaken around the
401 world to determine if the method can be developed into a useful tool as, if so, it might in the future
402 provide a very cost-effective approach. At present, however, the jury is still out if this will be possible.
403

404 **4. Considerations**

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

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

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

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

437 Another pivotal consideration when interpreting results is that of eDNA transport. As
438 mentioned above, eDNA offers a snapshot of the species presence in a certain habitat in a given
439 timeframe. Environmental DNA sampled might indeed come from the organisms that live in the
440 sampled area at that time, but it might also originate from degrading tissue, eggs and sperm and,
441 depending on environmental conditions, it might have simply been transported from elsewhere with
442 the currents or tides. Many researchers are now concentrating their efforts into understanding how
443 long these molecules can persist in the environment and remain detectable [reviewed in 17].
444

445 **5. Integration into existing management and monitoring programmes**

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

462 developments in eDNA approaches outlined above and the added value that the integration of these
463 approaches could bring.
464
465 **6. Conclusion**

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

475 Environmental DNA-based tools have, for a number of years now, been providing reliable
476 evidence in areas such as single species detection, and the characterisation of ecosystem biodiversity.
477 As such, they represent a robust, cost-effective, and in an increasing number of cases a more sensible
478 option for managers and monitors for incorporation into their standard scientific toolkits. While
479 significant advances have been, and continue to be, made in the use of eDNA to quantify both relative
480 and absolute abundance, such analyses are less well developed and still suffer from uncertainties
481 associated with various environmental, biological and methodological challenges of these techniques
482 [17]. As these influences are studied and their impacts better understood such uncertainties will be
483 reduced. However, at present their application is likely to be more limited.

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

488 discussions along with the more traditional techniques. The different approaches can work together
489 to provide complementary information. In the end they will allow enhanced scientific understanding,
490 resulting in improved science-based policy development in view of ecosystem-based management.
491
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