

Applications of environmental DNA (eDNA) in ecology and conservation: opportunities, challenges and prospects

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1 **Applications of environmental DNA (eDNA) in ecology and conservation: opportunities,**
2 **challenges and prospects**

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16

17

18 **Abstract**

19 Conserving biodiversity in the face of ever-increasing human pressure is hampered by our lack
20 of basic information on species occurrence, distribution, abundance, habitat requirements, and
21 threats. Obtaining this information requires efficient and sensitive methods capable of detecting
22 and quantifying true occurrence and diversity, including rare, cryptic and elusive species.

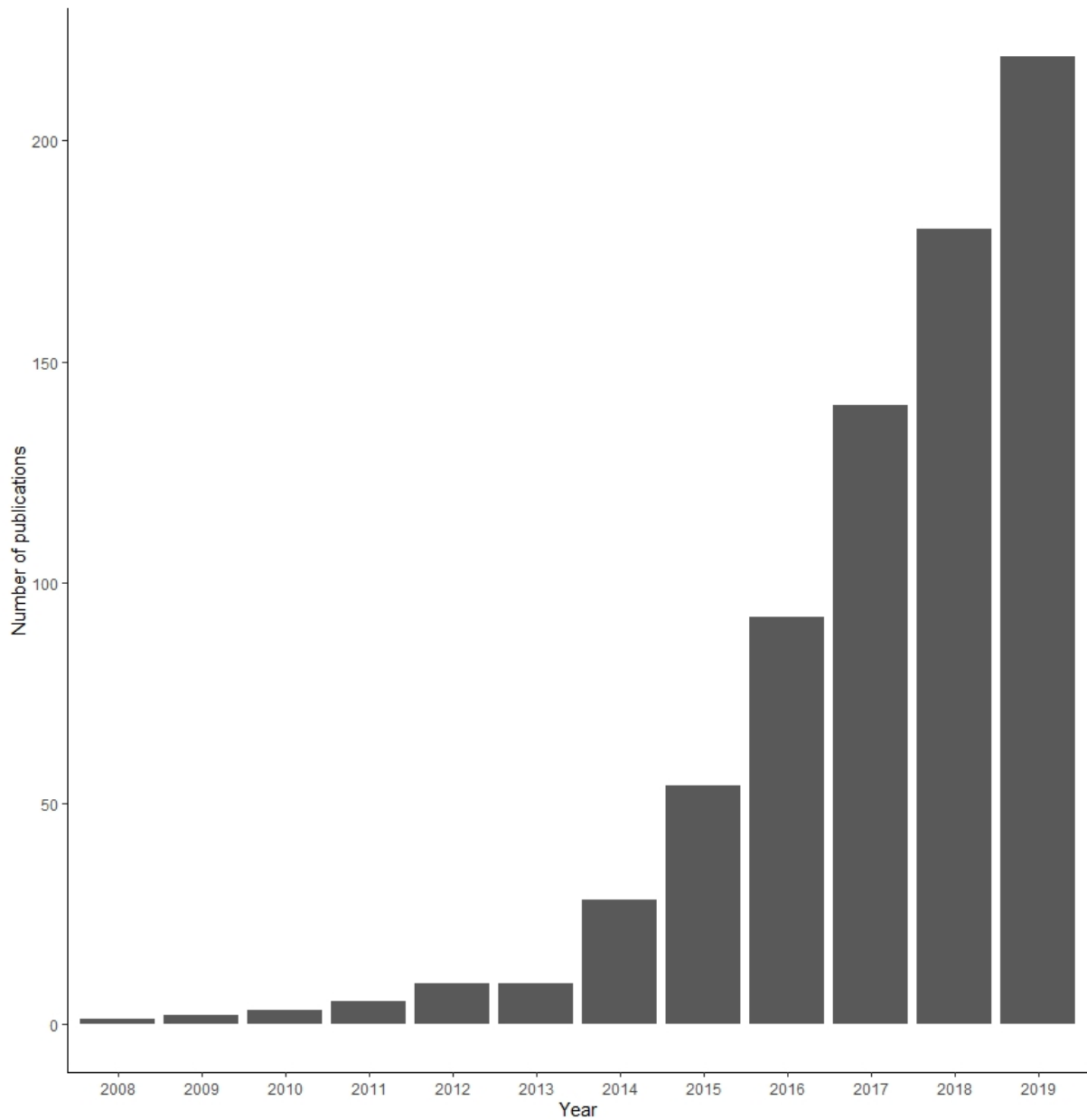
23 Environmental DNA (eDNA) is an emerging technique that can increase our ability to detect and
24 quantify biodiversity, by overcoming some of the challenges of labor-intensive traditional
25 surveys. The application of eDNA in ecology and conservation has grown enormously in recent
26 years, but without a concurrent growth in appreciation of its strengths and limitations. In many
27 situations, eDNA may either not work, or it may work but not provide the information needed.

28 Problems with (1) imperfect detection, (2) abundance quantification, (3) taxonomic assignment,
29 (4) eDNA spatial and temporal dynamics, (5) data analysis and interpretation, and (6) assessing
30 ecological status have all been significant. The technique has often been used without a careful
31 evaluation of the technical challenges and complexities involved, and a determination made that
32 eDNA is the appropriate method for the species or environment of interest. It is therefore
33 important to evaluate the scope and relevance of eDNA-based studies, and to identify critical
34 considerations that need to be taken into account before using the approach. We review and
35 synthesize eDNA studies published to date to highlight the opportunities and limitations of
36 utilizing eDNA in ecology and conservation. We identify potential ways of reducing limitations
37 in eDNA analysis, and demonstrate how eDNA and traditional surveys can complement each
38 other.

39 **Keywords:** Biodiversity monitoring, species detection, conservation tools, high-throughput
40 sequencing, traditional surveys, biological invasions

41 **Introduction**

42 Quantifying historical and contemporary biodiversity has traditionally relied on morphological
43 and behavioral data collected using direct observations, microscopes, binoculars, traps, and more
44 recently bioacoustics (Basset *et al.* 2012; Steenweg *et al.* 2017; Ovaskainen *et al.* 2018;
45 Burivalova *et al.* 2019; Khelifa 2019; Rajan *et al.* 2019; Outhwaite *et al.* 2020). These
46 techniques are often biased, invasive, destructive, and/or dependent on a declining pool of
47 taxonomic experts for identifying specimens. Traditional surveys are also generally labor
48 intensive and time consuming, and can be inefficient at detecting the true biodiversity present
49 (van der Heyde *et al.* ; Basset *et al.* 2012; Gómez-Zurita *et al.* 2016; Stoeckle *et al.* 2016; Evans
50 *et al.* 2017b; Rodriguez-Estrella *et al.* 2019; Zhang *et al.* 2020b). The advent of rapid and
51 relatively cheap DNA sequencing techniques has significantly enhanced biodiversity research by
52 overcoming some of the challenges of labor-intensive traditional surveys and offering the
53 opportunity to efficiently characterize biodiversity in time and space, using standardized methods
54 (Corlett 2017; Alexander *et al.* 2020; Cowart *et al.* 2020; Ji *et al.* 2020; Leempoel *et al.* 2020;
55 Sales *et al.* 2020; Yang & Zhang 2020). Among these techniques, environmental DNA (eDNA)
56 sampling has attracted worldwide attention, and interest in using this tool for biodiversity
57 assessment has grown rapidly in the past few years (Figure 1).



58

59 Figure 1 Number of studies using environmental DNA (eDNA) recovered from a literature search with the
 60 words 'environmental DNA' OR 'eDNA' for the period between 1 January 2008 and 31 December 2019.

61

62 Environmental DNA (eDNA) is genetic material originating from the hair, skin, urine, feces,

63 gametes, or carcasses of organisms that is present, in a more or less degraded form, in water, soil,

64 or sediment (TABERLET *et al.* 2012a; Thomsen & Willerslev 2015). DNA can persist in the
65 environment for periods from hours in temperate waters, to hundreds or thousands of years in
66 cold, dry permafrost, allowing direct isolation without any other obvious signs of an organism's
67 presence (Thomsen & Willerslev 2015). The utilization of eDNA has the potential to
68 revolutionize conservation science and practice in several ways. First, eDNA techniques are fast,
69 efficient and relatively cheap, thus providing the opportunity to monitor the dynamics of species,
70 populations and communities, and to map their geographic distribution over long time periods
71 and across large spatial scales (Ficetola *et al.* 2019; Itakura *et al.* 2019; Lecaudey *et al.* 2019;
72 Preissler *et al.* 2019; Reinhardt *et al.* 2019; Sutter & Kinziger 2019; Sales *et al.* 2020). Second,
73 eDNA sampling is simple, non-destructive, and non-invasive, causing no significant damage to
74 the target species or its habitats (Antognazza *et al.* 2019b; Mora *et al.* 2019; Leempoel *et al.*
75 2020). Third, eDNA can achieve high detection probabilities for rare, cryptic, and elusive species,
76 even at relatively low densities (Carvalho *et al.* 2019; Franklin *et al.* 2019; Shelton *et al.* 2019;
77 Takahara *et al.* 2020). Fourth, eDNA enables the early detection of biological invasions and their
78 timely eradication before full establishment (Lin *et al.* 2019; Nardi *et al.* 2019; Schumer *et al.*
79 2019; Tingley *et al.* 2019). Fifth, eDNA allows accurate identification of target organisms using
80 standardized, reproducible and auditable criteria that can be applied to different life stages and in
81 different environments (Preissler *et al.* 2019; Takeuchi *et al.* 2019a). Sixth, eDNA sampling
82 potentially offers a broad taxonomic breadth, allowing simultaneous biodiversity assessment for
83 a wide range of organisms (Sawaya *et al.* 2019; Thomsen & Sigsgaard 2019; Zhang *et al.* 2020b).

84 However, despite the ecological and conservation significance of the questions that can
85 potentially be addressed using eDNA, many challenges and limitations exist. eDNA does not
86 always work, and even when it does 'work', the results are not always what are needed. We

87 therefore review and synthesize eDNA studies published to date to highlight the opportunities
88 and limitations of utilizing eDNA in ecology and conservation. Additionally, we identify
89 potential routes to addressing fundamental assumptions and reducing the limitations of eDNA
90 (Table 1). We then propose a framework to discuss how eDNA can supplement traditional
91 biodiversity surveys. Lastly, we highlight new areas where eDNA studies are well positioned to
92 advance research in ecology, evolution and biodiversity.

93 **1. Literature search**

94 We searched for peer-reviewed journal papers in the Web of Science using the keywords
95 ‘environmental DNA’ and ‘eDNA’, and restricted the review to studies involving macro-
96 organisms. The final literature search was conducted on 16th January 2020 and covered the
97 period between 1 January 2008 and 31 December 2019 (2008 representing the year when eDNA
98 emerged as a survey tool in macro-ecology; (Ficetola *et al.* 2008)).

99 **2. Current ecological and conservation questions addressed using eDNA**

100 Two broad approaches that have received the most attention in eDNA-based studies are
101 barcoding and metabarcoding. The main difference between barcoding and metabarcoding is that
102 barcoding uses species-specific primers to detect the DNA fragments of a single species within
103 an environmental sample (Takahara *et al.* ; Franklin *et al.* 2019; Strickland & Roberts 2019;
104 Akamatsu *et al.* 2020; Harper *et al.* 2020; Kessler *et al.* 2020; Togaki *et al.* 2020) while
105 metabarcoding uses universal primers to simultaneously detect millions of DNA fragments from
106 the widest possible range of species from multiple trophic levels and domains of life (Alexander
107 *et al.* 2020; Cowart *et al.* 2020; Djurhuus *et al.* 2020; Yang & Zhang 2020; Zhang *et al.* 2020b).
108 For eDNA barcoding, conventional PCR (cPCR) is used to detect the presence of a species

109 (Jerde *et al.* 2011a; Dejean *et al.* 2012; Thomsen *et al.* 2012c; Mahon *et al.* 2013; Piaggio *et al.*
110 2014; Fukumoto *et al.* 2015) and quantitative PCR (qPCR) is used to quantifying the relative
111 abundance of DNA sequences (proxies for relative species abundance or biomass) or to improve
112 the sensitivity of species detection (Takahara *et al.* 2012; Goldberg *et al.* 2013; Pilliod *et al.*
113 2013; Doi *et al.* 2015; Klymus *et al.* 2015; Laramie *et al.* 2015; Balasingham *et al.* 2017). eDNA
114 barcoding has been particularly useful for detecting invasive, rare, and cryptic species, even in
115 difficult to access habitats, map their distributions, and design management strategies (Levi *et al.*
116 2019) (Levi *et al.* 2019; Qu & Stewart 2019; Reinhardt *et al.* 2019b). eDNA metabarcoding has
117 been successfully used to characterize past and present biodiversity patterns (Edwards *et al.* 2018;
118 Singer *et al.* 2018; Zinger *et al.* 2019), to understand trophic interactions and dietary preferences
119 (Galan *et al.* 2018; Harrer & Levi 2018; Mora *et al.* 2019; Thomsen & Sigsgaard 2019), to study
120 the spawning ecology of elusive species (Maruyama *et al.* 2018; Antognazza *et al.* 2019;
121 Bracken *et al.* 2019; Takeuchi *et al.* 2019b), and to monitor ecosystem health and dynamics
122 (Cordier *et al.* 2019; Evrard *et al.* 2019; Graham *et al.* 2019).

123 **Detecting rare, cryptic or endangered species**

124 Detection and monitoring of rare, cryptic, and endangered species using conventional techniques
125 is a difficult task that often involves huge amounts of time and effort (Qu & Stewart 2019b).
126 Repeated sampling (in space and time) with conventional surveys is expensive and can cause
127 irreparable damage to the target organism or its habitat. eDNA analysis offers a cost-efficient
128 approach to non-invasive monitoring of such species. Several studies have evaluated the
129 methodological efficiency of eDNA versus conventional surveys in detecting rare, cryptic, and
130 endangered species, and demonstrated that the probability of eDNA accurately detecting a target
131 species is relatively higher than or comparable to that of conventional surveys (Deiner *et al.*

132 2017). However, most eDNA-based studies have focused on aquatic taxa, especially fishes and
133 amphibians (Beauclerc *et al.* 2019; Deutschmann *et al.* 2019). Studies on other taxa and in
134 terrestrial environments are scarce.

135 **2.1 Estimating species distribution**

136 Although there is increasing global concern about declines in populations of wildlife (Jia *et al.*
137 2018; Saha *et al.* 2018; Sekercioglu *et al.* 2019), monitoring the population dynamics of some
138 species remains a challenge, partly due to large uncertainties in their geographic distributions,
139 limited understanding of their lifestyles, the complexity of their life histories, and
140 methodological constraints (Riggio *et al.* 2018; Srinivasan 2019; Wineland *et al.* 2019). eDNA
141 analyses have enhanced the monitoring of wildlife species distribution and abundance over large
142 spatial and temporal scales using efficient, sensitive and standardized methods (Matter *et al.*
143 2018; Hobbs *et al.* 2019; Itakura *et al.* 2019).

144 **2.2 Biomonitoring ecosystem health and dynamics**

145 Biological invasions, pests, and diseases constitute one of the most serious threats for global
146 biodiversity and cause adverse environmental, economic and public health impacts (Sengupta *et*
147 *al.* 2019; Tingley *et al.* 2019; Walsh *et al.* 2019). There is thus an urgent need to develop
148 effective monitoring and management strategies to contain the spread and establishment of these
149 harmful biological agents (Marshall & Stepien 2019; Orzechowski *et al.* 2019). However, such
150 efforts are constrained by our limited capacity to efficiently detect biological threats, especially
151 when these harmful agents are at low density (Manfrin *et al.* 2019). eDNA has proven to be a
152 very effective and sensitive sampling method, capable of monitoring the spread and
153 establishment of harmful biological agents through early detection, analysis of spread patterns,

154 and evaluation of population dynamics (Amberg *et al.* 2019; Ardura 2019; Fernanda Nardi *et al.*
155 2019; Gomes *et al.* 2019; Rudko *et al.* 2019).

156 **2.3 Diet and trophic interactions**

157 Understanding and quantifying biotic interactions, such as predator-prey and host-parasite
158 relationships, are key components of ecological research. However, these important biological
159 processes remain poorly investigated, primarily due to methodological challenges. eDNA is
160 increasingly being used in diet analysis to estimate diversity, composition and occurrence
161 frequency of prey items in predator feces (Galan *et al.* 2018; Jusino *et al.* 2019; Mata *et al.* 2019;
162 Mora *et al.* 2019). DNA deposited by pollinators on flowers, and by dispersers on seeds, also
163 offers an opportunity to investigate plant-animal interactions and the role of these interactions in
164 the maintenance of ecosystem functions and the provision of ecosystem services (Harrer & Levi
165 2018; Thomsen & Sigsgaard 2019).

166 **2.4 Spawning ecology**

167 Most aquatic animals, except for aquatic mammals and reptiles, reproduce through the process of
168 spawning. Identifying areas for spawning, as well as the spatial extent of spawning activities, is
169 vital for the effective management and conservation of these species. However, understanding
170 the natural reproductive ecology of these organisms have mostly relied on collections of eggs,
171 larvae and spawning-condition adults (Tsukamoto *et al.* 2011; Antognazza *et al.* 2019b). These
172 techniques are often biased, invasive, destructive, and/or strictly dependent on a declining pool
173 of taxonomic experts for identifying life history stages (Maruyama *et al.* 2018). Surveys of this
174 nature are also generally labor intensive and time consuming, and can be inefficient at detecting
175 certain life history stages (Antognazza *et al.* 2019b; Fritts *et al.* 2019). For instance, kick-

176 sampling for eggs is sometimes conducted in areas of relatively shallow waters or during the day
177 whereas the adults spawn in deep waters or at night (Antognazza *et al.* 2019a). eDNA enables
178 the detection of a species regardless of its life stage or gender, and is transforming our ability to
179 non-invasively quantify spawning activities, and identify the spatial extent of spawning, with
180 limited resources (Maruyama *et al.* 2018; Tillotson *et al.* 2018; Antognazza *et al.* 2019b;
181 Bracken *et al.* 2019; Fritts *et al.* 2019; Takeuchi *et al.* 2019b; Takeuchi *et al.* 2019c).

182 **2.5 Monitoring biodiversity**

183 Conserving biodiversity in the face of ever-increasing human pressure is hampered by our lack
184 of basic information on past and present species occurrences, distributions, abundances, habitat
185 requirements, and threats. Obtaining this information requires efficient and sensitive sampling
186 methods capable of detecting and quantifying true biodiversity, especially in megadiverse
187 regions with many cryptic and undescribed species (Kuzmina *et al.* 2018; Lacoursière-Roussel *et*
188 *al.* 2018). eDNA has increased our ability to monitor past and present biodiversity, by
189 overcoming some of the challenges of labor-intensive traditional surveys (Edwards *et al.* 2018;
190 Fraser *et al.* 2018; Montagna *et al.* 2018; Cilleros *et al.* 2019). It is now possible and cost-
191 efficient to assess the biodiversity of entire communities and infer diversity and assemblage
192 patterns for a wide range of taxonomic groups simultaneously (DiBattista *et al.* 2019; Zinger *et*
193 *al.* 2019).

194 **3. Challenges and limitations of eDNA**

195 The application of eDNA in ecology and conservation has grown enormously in recent years, but
196 without a concurrent growth in appreciation of its limitations. While there is evidence that eDNA
197 can increase the precision and resolution obtainable from traditional biodiversity surveys

198 (Thomsen & Willerslev 2015; Yamamoto *et al.* 2017), this is certainly not true in all
199 circumstances, even with standardized and highly sensitive assays (Hinlo *et al.* 2017; Ulibarri *et*
200 *al.* 2017). In cases where eDNA has been successful, it might not necessarily be the appropriate
201 tool if information is required on the abundance or biomass of species (although this may be
202 possible in some cases (Takahara *et al.* 2012; Pilliod *et al.* 2013; Doi *et al.* 2015; Baldigo *et al.*
203 2017)), its ecology (life-history, sex ratio, breeding status), or its conservation status (Evans *et al.*
204 2017b; Trebitz *et al.* 2017). Presence/absence information from eDNA is useful in conservation
205 for monitoring populations at large spatial scales and for identifying habitats that are of high
206 value to species of conservation concern (Voros *et al.* 2017; Weltz *et al.* 2017). eDNA can also
207 be used to detect the first occurrence of an invasive species or the continued presence of a native
208 species that was considered extinct, sometimes at relatively low densities (Stoeckle *et al.* 2017;
209 Trebitz *et al.* 2017). However, presence/absence can be misleading when eDNA is present in the
210 environment in the absence of living target organisms or when eDNA is not detected but the
211 target organism is present (Song *et al.* 2017). Abundance data provide far more information on
212 the status of a population than presence/absence data and thus potentially allow for more robust
213 assessments of the factors affecting populations.

214 To date, increased speed and reduced cost remain the key advantages of eDNA
215 (Sigsgaard *et al.* 2015b). Whether eDNA sampling is more sensitive and has higher resolution
216 than traditional surveys remain controversial. For some species or taxa, eDNA performs better
217 than traditional methods (Kraaijeveld *et al.* 2015; Deiner *et al.* 2016; Olds *et al.* 2016; Strickland
218 & Roberts 2019; Tingley *et al.* 2019), for others, eDNA is as good as traditional surveys
219 (Hanfling *et al.* 2016; Hopken *et al.* 2016; Yamamoto *et al.* 2017), while for some, eDNA
220 provide little additional benefit to surveillance (Rose *et al.* 2019; Walsh *et al.* 2019; Wood *et al.*

221 2019). However, studies in which eDNA has been unsuccessful are much less likely to be
222 published, so we inevitably know less about eDNA's failures than its successes. In addition to
223 the taxa- or species-specific differences in sensitivity between eDNA and traditional surveys, the
224 environment, time of the year, and biotic factors also play important roles (Dejean *et al.* 2011;
225 Pilliod *et al.* 2014; Barnes & Turner 2016; O'Donnell *et al.* 2017; Lacoursière-Roussel *et al.*
226 2018; Anglès d'Auriac *et al.* 2019; Takeuchi *et al.* 2019a). In aquatic ecosystems, for example,
227 eDNA can persist from a few hours to a month after release (Dejean *et al.* 2011; Pilliod *et al.*
228 2014). In addition, differences in eDNA persistence can occur even within the same environment,
229 for example, between the surface and bottom layers of a water body (O'Donnell *et al.* 2017;
230 Lacoursière-Roussel *et al.* 2018; Anglès d'Auriac *et al.* 2019).

231 Studies that have quantitatively assessed the cost-efficiency of eDNA relative to
232 traditional methods suggest that eDNA sampling is relatively cheaper than traditional surveys
233 (Biggs *et al.* 2015; Davy *et al.* 2015; Huver *et al.* 2015; Sigsgaard *et al.* 2015a; Qu & Stewart
234 2019a), although this can depend on the target taxa, site-specific detection rates, budgets, and
235 other considerations (Smart *et al.* 2016). For instance, Qu & Stewart (2019) found that the cost of
236 detecting and quantifying Yangtze finless porpoise (*Neophocaena asiaeorientalis asiaeorientalis*)
237 populations using visual surveys was 1.41-1.88 times (monthly cost) and 4.22-5.64 times
238 (seasonal cost) higher than using eDNA. Sigsgaard *et al.* (2015) found that using eDNA (\$4250)
239 to detect the European weather loach (*Misgurnus fossilis*) was 1.9 times cheaper than using a
240 combination of traditional methods (\$8100). Biggs *et al.* (2015) found that the cost of detecting
241 newts (*Triturus cristatus*) was 10.4 times cheaper using eDNA (€140 per site) compared to
242 traditional field sampling (€1450 per site). Davy *et al.* 2015 found that the cost of detecting nine
243 sympatric freshwater turtles using traditional surveys was 2–10 times higher than using eDNA.

244 However, (Smart *et al.* 2016) evaluated the relative cost of eDNA and bottle-trapping for
245 detecting the European newt (*Lissotriton vulgaris vulgaris*) and found that eDNA sampling was
246 more cost-efficient than trapping under low setup costs but bottle-trapping was more
247 cost-efficient than eDNA under high setup costs.

248 Qualitatively novel applications with actual conservation outcomes are still largely
249 lacking, although researchers are now moving away from proof-of-concept research to studies
250 that quantify population dynamics across organisms and environments (Stewart *et al.* 2017;
251 Carraro *et al.* 2018). However, the ability of eDNA to detect the continuous presence of a species
252 not sighted in its habitat for many years also raises questions about the mechanisms and
253 processes by which eDNA is transported and the conservation implications of unexplained
254 variability in eDNA transport (Sigsgaard *et al.* 2015a; Jerde *et al.* 2016; Lim *et al.* 2016).
255 Although methods and models to handle imperfect detection are increasingly being improved
256 (Piggott 2016; Guillera-Arroita *et al.* 2017; Ji *et al.* 2019), it is not possible to simply ignore the
257 presence of eDNA in the absence of living target organisms and/or the absence of eDNA in the
258 presence of living target organisms without actual field surveys. Increased PCR replication can
259 maximize eDNA detection and minimize false positives and/or negatives (Piggott 2016) but this
260 cannot substitute for actual biological replicates and will increase cost (Ficetola *et al.* 2015;
261 Roussel *et al.* 2015; Evans *et al.* 2017b). Detection of species using eDNA relies on DNA
262 isolated from living and dead cells (characterized by low concentration and high degradation
263 (Deagle *et al.* 2006)), and on PCR amplification (subjected to high variability and stochasticity
264 (Kebschull & Zador 2015)), and is prone to imperfect detection (Pilliod *et al.* 2014; Ficetola *et al.*
265 2015). Increasing the number of DNA extracts per sample or the number of amplifications per
266 DNA extract does not necessarily increase the probability of detection but will require more

267 laboratory reagents, time, and effort. However, collecting biological samples from sites where
268 the target species is most likely to be detected—based on knowledge of the target species’
269 ecology—can enhance the detection probability (Ficetola *et al.* 2015; Akre *et al.* 2019; Wineland
270 *et al.* 2019; Wood *et al.* 2019; Bedwell & Goldberg 2020; Vimercati *et al.* 2020).

271 Degradation of eDNA in the environment limits the scope of eDNA studies, as often only
272 small segments of genetic material remain, particularly in warm, humid conditions (Strickler *et*
273 *al.* 2015; Collins *et al.* 2018; Goldberg *et al.* 2018; Harrison *et al.* 2019; Moushomi *et al.* 2019;
274 Murakami *et al.* 2019; Sirois & Buckley 2019). Additionally, the impacts of varying
275 environmental conditions on time to degradation and the potential of DNA to travel throughout
276 media such as water can affect inferences of fine-scale spatiotemporal trends in species and
277 communities (Coissac *et al.*, 2012; Taberlet *et al.*, 2012a; Eichmiller *et al.*, 2016; Goldberg *et al.*,
278 2016; Deiner *et al.*, 2017; Hering *et al.*, 2018)(Hering *et al.* 2018). However, eDNA workflows
279 have been improving continuously, including the optimization of protocols for improved sample
280 collection and preservation, library preparation, sequencing, and bioinformatics (Williams *et al.*
281 2016; Yamanaka *et al.* 2017; Ji *et al.* 2019; Jusino *et al.* 2019; Koziol *et al.* 2019; Muha *et al.*
282 2019; Singer *et al.* 2019; Thomas *et al.* 2019; Yamahara *et al.* 2019). For instance, Thomas *et al*
283 (2019) developed desiccating filter housings that can automatically preserve captured eDNA via
284 desiccation. These housings also reduce the amount of time (or steps) required to handle samples,
285 and do not require the addition of chemicals and/or cold storage, thus minimizing the risk of
286 contamination. Singer *et al* (2019) found that for the same eDNA sample, Illumina NovaSeq
287 detected 40% more metazoan families than MiSeq and attributed this difference to NovaSeq’s
288 advanced technology.

289 Despite the important role that eDNA already plays in biodiversity assessment, diet
290 analysis, and detection of rare or invasive species, we are concerned that it is being over-
291 promoted as a standalone technique for ecological and conservation initiatives that may not fully
292 benefit from it (Roussel *et al.* 2015). We emphasize, in particular, that it is challenging to
293 distinguish between detection of eDNA and detection of a species, or to quantify organismal
294 abundance and biomass using eDNA, without a clear understanding of the challenges and
295 limitations of the technique. Failure to address these problems may confound the interpretation
296 of eDNA data.

297 **3.1 Imperfect sampling of eDNA and false detection**

298 eDNA is prone to imperfect sampling and false detection, which can occur at various stages of
299 the project, including field collection, sample storage, molecular analysis, and bioinformatics
300 workflows (Ficetola *et al.* 2016; Deiner *et al.* 2017; Doi *et al.* 2019; Piñol *et al.* 2019). Cases
301 where eDNA is detected in the environment in the absence of target organisms (false positives,
302 (Ficetola *et al.* 2015; Ficetola *et al.* 2016; Lahoz-Monfort *et al.* 2016; Stoeckle *et al.* 2016;
303 Guillera-Arroita *et al.* 2017)) or where eDNA is not detected but the target organism is present
304 (false negatives, (Morin *et al.* 2001; Ficetola *et al.* 2008; Schmidt *et al.* 2013; Ficetola *et al.* 2015;
305 Willoughby *et al.* 2016; Doi *et al.* 2019)) are common. Although site occupancy models have
306 been proposed as a way to account for imperfect detection, they largely depend on the number of
307 replicate samples per site and on the number of replicate amplifications per DNA sample (PCR),
308 which vary considerably across taxa (Schmidt *et al.* 2013; Matter *et al.* 2018; Chen & Ficetola
309 2019; Doi *et al.* 2019; Strickland & Roberts 2019). Causes of false detections include

310 **i. Limited persistence of eDNA in the environment**

311 A key motivation for using eDNA is the fact that all organisms shed DNA into their environment,
312 allowing direct isolation without any obvious signs of the organism's presence (TABERLET *et*
313 *al.* 2012a). However, DNA released by aquatic or terrestrial organisms is not necessarily
314 concentrated at the site of its release, but is transported across space and degraded over time
315 (Deiner & Altermatt 2014a; Jane *et al.* 2015; Sansom & Sassoubre 2017; Rice *et al.* 2018;
316 Murakami *et al.* 2019). The eDNA release and decay rates depend on several biotic (e.g. life-
317 history traits, species interactions, microbes) and abiotic (e.g. UV radiation, temperature, salinity)
318 factors (Pilliod *et al.* 2014; Klymus *et al.* 2015; Lacoursière-Roussel *et al.* 2016; Stewart 2019).
319 Our current understanding of how eDNA persist under different environmental conditions for
320 different species is limited, but this information is critical for deciding on the most appropriate
321 time window to conduct eDNA surveys. Environmental conditions are constantly changing and
322 can be different in each location throughout the year. For example, Pilliod *et al.* 2014 detected
323 eDNA after 11 and 18 days in water samples that were stored in the dark but eDNA was no
324 longer detectable in samples that were exposed to full-sun after 8 days. Temperature directly
325 affects the metabolic rate of some organisms (e.g. amphibians, invertebrates, reptiles, and fish)
326 and consequently could strongly affect eDNA release rate (Clarke & Fraser 2004; Lacoursière-
327 Roussel *et al.* 2016). For instance, Lacoursière-Roussel *et al.* 2016 showed that fish released
328 more eDNA in warm water (14 °C) than in cold water (7 °C) and that the relationships between
329 eDNA concentration and fish abundance or biomass were stronger in warm water than in cold
330 water.

331 **ii. Primer biases**

332 The suite of molecular markers used in eDNA analysis is extremely important for the
333 identification of species in both single taxa and multi-species samples. However, successful

334 amplification of eDNA depends highly on primer specificity, sensitivity, and efficiency
335 (Stadhouders *et al.* 2010; Nichols *et al.* 2018). eDNA samples are characterized by highly
336 heterogeneous DNA from mixtures of many different taxa or haplotypes, making it difficult to
337 achieve full complementarity between primers and target sequences during PCR (Stadhouders *et*
338 *al.* 2010; Nichols *et al.* 2018; Wei *et al.* 2018). These primer-template mismatches can affect
339 both the stability of the primer-template duplex and the efficiency with which the polymerase
340 extends the primer, potentially leading to biased results or complete PCR failure (Stadhouders *et*
341 *al.* 2010). For instance, primer bias may lead to the preferential amplification of abundant
342 sequences compared to rare ones, or of shorter fragments compared to longer ones, or of non-
343 target organisms compared to target organisms (Nichols *et al.* 2018; Xia *et al.* 2018). Unlike
344 metabarcoding, primer bias is not a major issue for barcoding. However, targeted PCR-based
345 amplification of samples using species-specific primers, instead of universal primers, should be
346 strongly encouraged in eDNA barcoding (Wilcox *et al.* 2013; Davy *et al.* 2015; Cannon *et al.*
347 2016). Conventional PCR (cPCR) methods may cross-amplify and provide false positive results
348 but quantitative PCR (qPCR) methods are likely to be more sensitive (Wilcox *et al.* 2013).

349 **iii. Inhibition of DNA amplification**

350 eDNA analysis involves the collection of complex and heterogeneous mixtures from aquatic
351 ecosystems, soils, sediments, or feces (Koziol *et al.* 2019). The polymerase chain reaction (PCR)
352 is the standard method for detection and characterization of organisms and genetic markers in
353 these sample types. However, PCR is vulnerable to inhibitors, which are usually present in
354 eDNA samples and which may affect the sensitivity of the assay or even lead to false negative
355 results (Schrader *et al.* 2012; Nichols *et al.* 2018; Hunter *et al.* 2019). PCR inhibitors represent a
356 diverse group of substances including bile salts from feces, polysaccharides from plant materials,

357 collagen from tissues, heme from blood, humic acid from soil, urea from urine, and melanin and
358 eumelanin from hair and skin (Watson & Blackwell 2000; Radstrom *et al.* 2004; Schrader *et al.*
359 2012). Although PCR inhibitors have different properties and mechanisms of action, they
360 generally exert their effects through direct interaction with DNA or interference with
361 thermostable DNA polymerases (Schrader *et al.* 2012). Direct binding of inhibitors to DNA can
362 prevent amplification and facilitate co-purification of inhibitor and DNA (Schrader *et al.* 2012;
363 Jane *et al.* 2015). Inhibitors can also interact directly with a DNA polymerase to block enzyme
364 activity. Since some inhibitors are predominantly found in specific types of samples,
365 matrix-specific protocols for preparation of nucleic acids before PCR are essential (Schrader *et*
366 *al.* 2012; Hunter *et al.* 2019).

367 **iv. Sample contamination**

368 Contamination occurs when DNA from an outside source (exogenous DNA) gets mixed with
369 DNA relevant to the research. For instance, if a frog is eaten at one pond, then the predator
370 defecates at another, this may introduce the frog's DNA to a pond where the frog is not present.
371 Because of the sensitivity of the technique, this is a serious issue in eDNA surveys and may
372 result in false positive detections and subsequent misinterpretation of results (Goldberg *et al.*
373 2016; Wilson *et al.* 2016). eDNA analysis requires multiple steps of sample handling and
374 manipulation in the field (collection, storage and transportation) and in the lab (storage, DNA
375 extraction, amplification, library preparation and sequencing), so contamination may occur at
376 various stages of the research (Goldberg *et al.* 2016; Doi *et al.* 2017). In the field, contamination
377 may occur when DNA from one or multiple samples is unintentionally transferred into another
378 sample, either from another site in the same study or from an unknown locality. This usually
379 occurs when the same field equipment (e.g. corers, filters, gloves) is used repeatedly for

380 sampling different sites without thorough treatment (e.g. sterilization). In the lab, contamination
381 may occur when remnant DNA from previous molecular experiments (e.g. DNA extraction,
382 amplification, library preparation and sequencing) spreads into new samples or when the same
383 lab equipment (e.g. tubes, pipettes, benchtops) is repeatedly used for conducting different
384 experiments without thorough decontamination. Instead of standard autoclaving (Unnithan *et al.*
385 2014) or the commonly used 10% bleach (sodium hypochlorite) solution (Prince & Andrus
386 1992), treatment of field and lab equipment with 50% bleach solution and thorough rinsing can
387 effectively destroy and remove unwanted DNA and PCR products (Kemp & Smith 2005;
388 Champlot *et al.* 2010; Goldberg *et al.* 2016; Wilcox *et al.* 2016).

389 **v. eDNA from dead individuals**

390 Both dead and live organisms release DNA into the environment and both contribute to the
391 eDNA pool. For most purposes, the researcher is only interested in the former – DNA from live
392 organisms – but distinguishing between them remains a challenge. Since DNA degrades with
393 time, the longer DNA fragments in a particular environment are likely to represent the most
394 recent DNA. (Jo *et al.* 2017) compared changes in copy numbers of long (719 bp) and short
395 (127 bp) eDNA fragments with time and suggested that the concentration of longer eDNA
396 fragments reflects fish biomass more accurately once the effects of decomposition and
397 contamination have been removed. However, removal of carcasses and avoidance of
398 contamination in natural settings is almost impossible, given that birth and mortality are key
399 processes in the dynamics of natural populations. The contribution of dead organisms to the
400 eDNA pool can vary considerably in different environments. For instance, in the tropics and sub-
401 tropics with relatively higher temperatures and faster degradation rates, carcasses do not persist

402 long. (Tsuji *et al.* 2017) found that ayu sweetfish (*Plecoglossus altivelis altivelis*) and common
403 carp (*Cyprinus carpio*) eDNA degradation rates increased with increasing water temperatures.

404 **vi. Ancient DNA (aDNA) resuspension**

405 Environmental DNA may occur as particle-bound or free-living dissolved molecules (Turner *et*
406 *al.* 2014a). Particles that bind DNA may settle over long periods and be resuspended through
407 natural phenomena like erosion, turbulence caused by fast-flow hydrological events, wind, and
408 wave action or bioturbation. In cases where the objective is to detect the continued presence of a
409 native species that was considered extinct, aDNA resuspension can lead to false positive results
410 and misinform management.

411 **3.2 Difficulties in quantifying abundance and biomass**

412 One of the most important issues limiting the application of eDNA in environmental monitoring
413 is the difficulty of quantifying species abundance and biomass. To date, results of most eDNA
414 studies have been interpreted as presence/absence (occurrence) information. However, some
415 studies have used mock communities with known and differing assemblage structures or
416 combined conventional surveys with eDNA in order to explore whether or not eDNA can
417 provide quantitative information (Piñol *et al.* 2019). The outcomes of these studies are still fairly
418 contentious, with strong, weak, and no quantitative estimates reported (Piñol *et al.* 2019). For
419 instance, (Pilliod *et al.* 2013) reported that eDNA concentrations of Rocky Mountain tailed frogs
420 (*Ascaphus montanus*) and Idaho giant salamanders (*Dicamptodon aterrimus*) were positively
421 associated with in-stream density, biomass, and proportion of area occupied by the two species.
422 (Takahara *et al.* 2012) showed that eDNA concentration in water samples correlated with the
423 biomass of common carp (*Cyprinus carpio*) in artificial ponds, and (Thomsen *et al.* 2012c)

424 showed that eDNA concentration was correlated with the density of common spadefoot toads
425 (*Pelobates fuscus*) and great crested newts (*Triturus cristatus*) in natural ponds. (Evans *et al.*
426 2016) found a modest, but positive relationship between species abundance and sequencing read
427 abundance for eight fish and one amphibian species in replicated mesocosms, while (Deagle *et al.*
428 2013) reported that the proportions of fish sequences recovered from 39 seal scats did not match
429 the proportions of the three fish species the seals consumed.

430 **3.2.1 Problems with interpreting relative abundance data generated from PCR-based** 431 **techniques and metabarcoding loci**

432 **i. Variability in eDNA deposition and preservation**

433 The production and stability of eDNA (origin, state, decay, transport, persistence (Barnes &
434 Turner 2016)) vary greatly among taxa, individuals, and even tissues within the same organism.
435 The concentration of DNA in the environment is influenced by several complex processes,
436 including movement and degradation, making it difficult to extract abundance information from
437 eDNA signals. Furthermore, an organism's size, age, condition, or biological activity can
438 influence the relationship between eDNA concentration and relative abundance (Spear *et al.*
439 2015; de Souza *et al.* 2016; Erickson *et al.* 2016; Stewart *et al.* 2017), interactions between a
440 target species and closely or distantly related species can influence the amount of eDNA released
441 (Sassoubre *et al.* 2016), and environmental conditions can influence eDNA release, persistence,
442 degradation, transport, location, and settlement (Laramie *et al.* 2015; Erickson *et al.* 2016;
443 Stewart *et al.* 2017). For instance, large-bodied, long-lived, year-round, and highly dispersed
444 species are more likely to be detected using eDNA than small-bodied, short-lived, seasonal, and

445 sedentary species (ANDERSEN *et al.* 2012a; Buxton *et al.* 2017; Dunn *et al.* 2017; Hemery *et al.*
446 2017; Rees *et al.* 2017; Nichols *et al.* 2018).

447 **ii. eDNA sampling and processing biases**

448 Key considerations in eDNA analysis are maximizing DNA capture in the field, minimizing
449 degradation during transport and storage, and successful isolation and amplification (Pilliod *et al.*
450 2013, 2014; Turner *et al.* 2014b; Renshaw *et al.* 2015a; Goldberg *et al.* 2016; Wood *et al.* 2019).
451 The choice of eDNA sampling and processing protocols can significantly influence DNA yield,
452 detection probability, and the resulting abundance and biodiversity estimates (Brannock &
453 Halanych 2015; Deiner *et al.* 2015b; Renshaw *et al.* 2015a; Djurhuus *et al.* 2017; Thomas *et al.*
454 2018). Specific protocols used in each study vary with sample type (water, feces, soil, sediment),
455 the ecosystem of interest (freshwater, marine, terrestrial), and the questions being investigated
456 (Renshaw *et al.* 2015a; Goldberg *et al.* 2016; Djurhuus *et al.* 2017). For diet analysis, individual
457 fecal samples are collected and dehydrated immediately using either alcohol or silica gel or a
458 combination of both (Deagle *et al.* 2009; Zeale *et al.* 2011; Galan *et al.* 2012; Pompanon *et al.*
459 2012; Clare *et al.* 2014; Mata *et al.* 2016). In terrestrial ecosystems, multiple soil cores are
460 collected and analyzed separately or are pooled together, homogenized and a representative
461 subsample is taken. DNA is extracted from the soil samples immediately after collection or
462 samples are stored at -20 °C or -80 °C for processing at a later date (van der Heyde *et al.* ;
463 Andersen *et al.* 2012b; Bienert *et al.* 2012; Epp *et al.* 2012; Taberlet *et al.* 2012b; Yoccoz *et al.*
464 2012). In aquatic ecosystems, different protocols are being used to collect water, capture eDNA
465 with filters, transport samples from the field, and to store water and/or filters prior to DNA
466 extraction and amplification (Goldberg *et al.* 2011; Pilliod *et al.* 2013; Biggs *et al.* 2015;
467 Renshaw *et al.* 2015a; Majaneva *et al.* 2018). Some studies filter, precipitate or centrifuge water

468 on-site, and preservation media (e.g. ice, sodium acetate, lysis buffers, and absolute ethanol) are
469 used to stabilize eDNA for enough time (up to 24 h) to safely transport it for storage and
470 processing (Ficetola *et al.* 2008; Goldberg *et al.* 2011; Pilliod *et al.* 2013; Biggs *et al.* 2015;
471 Valentini *et al.* 2016a). In other studies, water is transported in cold conditions and filtration or
472 precipitation is done in the laboratory (Jerde *et al.* 2011b; Thomsen *et al.* 2012c; Goldberg *et al.*
473 2013). Minimizing DNA degradation in these samples is challenging, especially in remote field
474 sites with little or no access to cooling and in situations where samples need to be transported for
475 several days (e.g. international flights with stop overs) before processing.

476 Various types of filters have been used to capture eDNA (Minamoto *et al.* 2012; Thomsen *et al.*
477 2012a; Goldberg *et al.* 2013; Jerde *et al.* 2013; Piaggio *et al.* 2014) and the efficiency of each
478 filter type depends on its pore size, the volume and chemical properties (e.g. pH, organic and
479 inorganic particles) of the water filtered, and the extraction method (Liang & Keeley 2013b;
480 Turner *et al.* 2014a; Renshaw *et al.* 2015a; Eichmiller *et al.* 2016; Djurhuus *et al.* 2017;
481 Majaneva *et al.* 2018). In general, filtration is relatively more efficient for eDNA capture than
482 precipitation and centrifugation methods (Deiner *et al.* 2015a; Renshaw *et al.* 2015b; Eichmiller
483 *et al.* 2016; Spens *et al.* 2017; Majaneva *et al.* 2018). Among filters, cellulose nitrate (CN) filters
484 capture relatively more eDNA than polyethene sulfone (PES), polyvinylidene fluoride (PVDF),
485 and polycarbonate (PC) filters, while glass microfiber (GMF) filters capture relatively more
486 eDNA than PC filters (Liang & Keeley 2013a; Eichmiller *et al.* 2016).

487 In some aquatic environments (e.g. muddy water), the pore size of a filter can influence filtration
488 rate, where larger pore size filters (e.g. 5 μm) or pre-filtration require less time than smaller pore
489 size filters (1 μm). However, larger pore size filters and pre-filtration are less efficient in DNA
490 recovery than smaller pore size filters (Liang & Keeley 2013a; Eichmiller *et al.* 2016).

491 iii. **PCR primer and sequencing biases**

492 eDNA species detection and quantification is usually accomplished using relatively short DNA
493 fragments. These increase detection probabilities with highly degraded eDNA, but they are prone
494 to high error rates and biases. Primers used to amplify these short DNA fragments may not
495 perfectly match the target organism's DNA, leading to primer–template mismatches and
496 differential amplification of target DNA (Leray *et al.* 2013; Elbrecht & Leese 2015; Bista *et al.*
497 2018). Primers can fail to detect low concentrations of eDNA, miss entire taxa or preferentially
498 amplify the eDNA of non-target organisms. For example, short DNA fragments are more likely
499 to represent ancient DNA (aDNA) that has persisted in the environment for very long periods,
500 bound to sediments, and represent historical biodiversity (Barnes *et al.* 2014; Barnes & Turner
501 2016). On the other hand, longer DNA fragments may represent more recent biological
502 information, but are present at lower concentrations in the environment, are less likely to persist
503 after release, and degrade (Lindahl 1993; Deagle *et al.* 2006; Hanfling *et al.* 2016; Bista *et al.*
504 2017). (Jo *et al.* 2017) showed that the decay rate of eDNA varied depending on the length of the
505 DNA fragment, while (Hanfling *et al.* 2016) found that smaller (~100 bp) fragments of 12S
506 rRNA persisted longer in lake water than longer (~460 bp) fragments of cytochrome b (CytB).
507 (Olson *et al.* 2012b) reported that primers targeting the mtDNA of the eastern hellbender
508 (*Cryptobranchus alleganiensis*) had six orders of magnitude higher sensitivity than primers
509 targeting the nuclear DNA. It has also been observed that polymerase choice can affect both
510 occurrence and relative abundance estimates and the main source of this bias can be attributed to
511 polymerase preference for sequences with specific GC contents (Fonseca 2018; Nichols *et al.*
512 2018). The addition of short indices to PCR primers can also introduce biases to the resulting
513 sequence counts, especially in mixed-template eDNA samples, presumably via differential

514 amplification efficiency among templates (O'Donnell *et al.* 2016; Leray & Knowlton 2017).
515 PCR amplification strategies also influence species detection and abundance estimation, with
516 quantitative PCR (qPCR) being relatively more effective for species detection and abundance
517 estimation than conventional PCR (cPCR) (Takahara *et al.* 2012; Turner *et al.* 2014b; Piggott
518 2016; Harper *et al.* 2018).

519 **iv. Variation in DNA copy number of target loci**

520 Environmental DNA studies have mostly relied on mitochondrial (mt), chloroplast (cp), and
521 nuclear (n) DNA sequences, but the gene copy number of these target loci may vary between
522 taxa, individuals or tissues, even when the same number of cells is present in an environmental
523 sample (Moraes 2001; Morley & Nielsen 2016; Minamoto *et al.* 2017; Nichols *et al.* 2018). This
524 distorts the assumption that read abundance correlates with genic or taxon abundance, or that
525 there is a constant copy number to individual relationship. For instance, (Minamoto *et al.* 2017)
526 found that the copy numbers for nDNA of common carp (*Cyprinus carpio*) in environmental
527 samples were considerably higher for mtDNA, with the nDNA marker requiring much less
528 survey effort than the mtDNA marker, while (Piggott 2016) found that the 18S nDNA marker
529 required relatively higher survey effort to achieve a 0.95 detection probability for Macquarie
530 perch (*Macquaria australasica*) than two 12S mtDNA markers. These differences between
531 molecular markers can greatly influence species detection and abundance estimation, yet many
532 eDNA studies do not address this issue.

533 **v. Sequence filtering stringency**

534 Sequence filtering is a routine process in eDNA analysis and occurs at multiple steps of the
535 bioinformatics pipeline. For metabarcoding, raw sequence data are initially processed to filter

536 and correct (where possible) low-quality and erroneous reads (Valentini *et al.* 2016a; Evans *et al.*
537 2017a; Günther *et al.* 2018; Bakker *et al.* 2019; Rytönen *et al.* 2019; Cowart *et al.* 2020; Zhang
538 *et al.* 2020a). This quality control step removes any phiX reads (common in marker gene
539 sequencing) and chimeric sequences detected in the raw sequencing data. Other quality filtering
540 criteria include trimming off the first m bases of each sequence, and/or truncating each sequence
541 at position n (Bakker *et al.* 2019; Cowart *et al.* 2020). The appropriate number of bases to be
542 trimmed and the truncation length can be determined using read quality profiles. Filtering can
543 also be performed on an OTU-table or a species-by-site matrix to remove samples with a total
544 read frequency less than a given threshold and/or OTUs observed in less than a given number of
545 samples (Bakker *et al.* 2019; Rytönen *et al.* 2019; Cowart *et al.* 2020; Zhang *et al.* 2020a).
546 Filtering out OTUs that are detected in only one or a few samples is common, and this is based
547 on the suspicion that these low frequency OTUs are PCR or sequencing errors (Bakker *et al.*
548 2019; Rytönen *et al.* 2019; Cowart *et al.* 2020; Zhang *et al.* 2020a). Taxonomy-based filtering
549 is also being applied to retain target taxa and/or exclude non-target taxa from eDNA analysis
550 (Bakker *et al.* 2019; Cowart *et al.* 2020; Zhang *et al.* 2020a). Although there are accepted
551 thresholds, across studies, about which filtering criteria are suitable, differences in sequencing
552 depth, marker region, primer specificity, and taxonomic breadth makes it difficult to reach a
553 general consensus (Evans *et al.* 2017a). Sequence filtering stringency can affect species detection,
554 abundance and biomass quantification (Rivera *et al.* 2020). More stringent thresholds might filter
555 out true biological sequences from the dataset, whereas more flexible thresholds might treat
556 artefacts as true biological sequences (Laroche *et al.* 2017; Alberdi *et al.* 2018). (AMEND *et al.*
557 2010) reported a tradeoff between sequence quality stringency and quantification by showing

558 that read-quality based processing stringency profoundly affected the abundance estimate for one
559 fungal species.

560 **4.3 Incomplete reference databases and taxonomic assignment biases**

561 Environmental DNA of complex eukaryotic communities is increasingly being used to quantify
562 biodiversity in terrestrial, freshwater and marine ecosystems (Civade *et al.* 2016;
563 Andruszkiewicz *et al.* 2017; Gillet *et al.* 2018; Fujii *et al.* 2019; Thomsen & Sigsgaard 2019).
564 Assignment of OTUs to species or higher taxonomic levels is a fundamental step in such studies.
565 However, the incompleteness of reference sequence databases for most organisms is an
566 important limitation for biodiversity studies using eDNA (Thomsen & Sigsgaard 2019). The
567 taxonomic identification of taxa is as good as the reference database used (Thomsen & Sigsgaard
568 2019). Reference sequences for taxonomic assignment are only available for one or a few genes
569 for most species and the targeted marker regions (e.g. COI, 12S, 16S) cannot accurately resolve
570 most groups to species or higher taxonomic levels due to incompleteness of reference sequence
571 databases (Deagle *et al.* 2014; Liu *et al.* 2017; Thomsen & Sigsgaard 2019). Consequently,
572 eDNA studies are often interpreted using molecular operational taxonomic units (MOTUs) or
573 higher taxonomic ranks (genus, family, order) instead of binomial species names (Thomsen &
574 Sigsgaard 2019). This makes it difficult to associate eDNA data with existing biological and
575 ecological knowledge. Although user-friendly and cost-efficient methods that generate full-
576 length reference barcodes could improve future eDNA studies (Liu *et al.* 2017), unbalanced
577 barcoding efforts across regions of the world, taxonomic groups, and molecular markers
578 (Ratnasingham & Hebert 2007; Machida *et al.* 2017; Porter & Hajibabaei 2018) currently limit
579 the application of eDNA in ecology and conservation.

580 **4.4 Limited understanding of the ecology of eDNA**

581 We lack a clear understanding of the ecology of eDNA – its origin, state, transport, and fate.
582 This information is critical for deciding whether eDNA sampling is the appropriate technique to
583 make robust inferences about an organism’s presence, and to quantify abundance (Turner *et al.*
584 2014a; Barnes & Turner 2016; Stewart 2019). Environmental DNA originates as urine, feces,
585 epidermal tissues, secretions, reproductive cells or carcasses and this source material enters the
586 environment as particles of various sizes. These sources of eDNA may be rapidly transported
587 from the site of release, including leaching into the soil, downstream flow and dispersion by
588 water currents. Although particle size may be a major determinant of movement velocity, intact
589 genomic DNA within living cells may be transformed into extracellular fractions too small to be
590 detected (Barnes *et al.* 2014). (Murakami *et al.* 2019) found that eDNA of striped jack was
591 mostly detectable within 30 m of the source, (Jane *et al.* 2015) found that eDNA of brook trout
592 (*Salvelinus fontinalis*) could be detected 240 m downstream, (Deiner & Altermatt 2014a) found
593 that eDNA of daphnia (*Daphnia longispina*) could be detected 12.3 km downstream, and eDNA
594 of pelecypod (*Unio tumidus*) could be detected 9.1 km downstream. Despite the fact that eDNA
595 reflects the source within a range of distances (10–150 m; (O’Donnell *et al.* 2017; Yamamoto *et*
596 *al.* 2017; Murakami *et al.* 2019), the relationship between water current and eDNA transport is
597 not well known. Besides distance, many interacting factors can also influence eDNA detection
598 after leaving its source (Pilliod *et al.* 2014).

599 It is unlikely that all organisms release equal amounts of DNA into the environment and
600 that DNA from different sources degrades at the same rate, even under similar environmental
601 conditions. Therefore, the detection of a target species may be influenced by eDNA release and
602 degradation, which are in turn related to a species’ size, life history, biotic interactions, and
603 abiotic conditions (Barnes *et al.* 2014). For freshwater fish, eDNA degradation rates vary from

604 10.5%/h in common carp (*Cyprinus carpio*; (Barnes *et al.* 2014)) to 15.9%/h in bluegill sunfish
605 (*Lepomis macrochirus*; (Maruyama *et al.* 2014)), while for marine fish, eDNA degradation rates
606 vary from 1.5%/h in three-spined stickleback (*Gasterosteus aculeatus*; (Thomsen *et al.* 2012b)),
607 4.6%/h in European flounder (*Platichthys flesus*; (Thomsen *et al.* 2012b)) to >5.0%/h in northern
608 anchovy (*Engraulis mordax*), Pacific sardine (*Sardinops sagax*), and Pacific chub mackerel
609 (*Scomber japonicas*) (Sassoubre *et al.* 2016). These studies suggest that the degradation rate of
610 eDNA in aquatic fish, for instance, exhibit both species and environment effects.

611 DNA released into any environment is subjected to dynamic biological, physical, and
612 chemical processes that determine its fate (Levy-Booth *et al.* 2007). After release, DNA may be
613 bound to organic and inorganic particles that settle, and are later resuspended through natural
614 phenomena like erosion, turbulence caused by fast-flow hydrological events, wind and wave
615 action or bioturbation. However, whether eDNA is most abundant in the upper layer close to the
616 source (surface, (Moyer *et al.* 2014; Murakami *et al.* 2019)) or in the lower layer away from the
617 source (bottom, (Turner *et al.* 2015)) needs further investigation.

618 **4.5 Inconsistencies in data analysis and interpretation**

619 An important challenge in eDNA analysis is dealing with errors that occur during PCR
620 amplification and sequencing in a consistent way. Researchers have attempted to ameliorate this
621 issue using a variety of techniques including the deliberate and careful removal of erroneous
622 sequences.

623 **i. Minimum sequence threshold**

624 Setting a minimum sequence copy number below which sequences are discarded is the most
625 widely used strategy for eliminating erroneous sequences (Alberdi *et al.* 2018). However, this

626 minimum sequence threshold varies considerably across eDNA studies, with some researchers
627 only discarding singletons (i.e. a read with a sequence that is present only once (Andruszkiewicz
628 *et al.* 2017; Bista *et al.* 2017; Yamamoto *et al.* 2017)), while others only consider sequences
629 represented by ≥ 10 identical reads for downstream analyses (e.g. (Fujii *et al.* 2019)). In any case,
630 erroneous sequences must be removed with caution: more stringent thresholds might filter out
631 rare biological sequences from the dataset, whereas more flexible thresholds might treat artefacts
632 as true diversity (Laroche *et al.* 2017; Alberdi *et al.* 2018).

633 **ii. Chimeric sequence detection**

634 Chimeras are sequences formed when two or more biological sequences bind together during
635 PCR (Judo *et al.* 1998; Edgar *et al.* 2011). Chimera formation is common in eDNA analysis,
636 especially when DNA from closely related organisms is amplified (Edgar *et al.* 2011; Aas *et al.*
637 2017). Since chimeric sequences are very similar to their parent sequences (i.e. low divergence)
638 and sometimes have identical sequences to valid genes, it is very challenging to distinguish
639 chimeras from true biological sequences, even with dedicated software and complete reference
640 sequence databases (Edgar 2016; Aas *et al.* 2017; Alberdi *et al.* 2018). Detection and removal of
641 chimeras is of critical importance in eDNA studies because undetected chimeras can be
642 misinterpreted as real biological entities or novel taxa, causing inflated estimates of true diversity
643 and spurious inferences of differences in community composition (Edgar *et al.* 2011; Aas *et al.*
644 2017).

645 **iii. Clustering strategy and percent identity cutoff for OTU assignment**

646 eDNA metabarcoding typically clusters amplicon sequences into operational taxonomic units
647 (OTUs) as an initial step in data processing. Many quality assurance and quality control

648 approaches, such as denoising, also require sequence clustering prior to further analyses,
649 including abundance and diversity estimation. Clustering groups sequences into OTUs based on
650 percent identity thresholds that represent intraspecific differences and approximate species
651 boundaries (Alberdi *et al.* 2018). The choice of clustering strategy for OTUs is crucial for
652 estimating the true diversity of biological communities, so choosing the wrong strategy may
653 result in either inflated or underestimated species richness and affect final conclusions (Alberdi
654 *et al.* 2018; Xiong & Zhan 2018; Rytönen *et al.* 2019). While OTUs are typically constructed
655 using a percent identity cutoff of 97% (Bista *et al.* 2017; Bista *et al.* 2018), lower and higher
656 thresholds (Fujii *et al.* 2019; Rytönen *et al.* 2019) have also been used. Moreover, lineages
657 evolve at variable rates, so no single cut-off value can accommodate the entire tree of life.
658 Developers of other programs, such as Swarm, argue that a single global clustering threshold will
659 inevitably be too relaxed for slow-evolving lineages and too stringent for rapidly evolving ones
660 (Mahe *et al.* 2014, 2015; Andruszkiewicz *et al.* 2017; Sawaya *et al.* 2019).

661 **iv. Taxonomic assignment threshold**

662 Taxonomic assignment is performed using a wide variety of methods and programs, but in
663 general, a search of reference sequence databases is conducted and query sequences (OTUs)
664 within a predefined percent similarity to the reference sequence are assigned to the lowest
665 possible taxonomic level. Taxonomic assignments may be considered valid if the percent
666 similarity is above the predefined threshold, but some studies use different similarity thresholds
667 to make assignments at different taxonomic levels, while some programs generate taxonomic
668 predictions with confidence estimates specified by bootstrapping (Andruszkiewicz *et al.* 2017;
669 Alberdi *et al.* 2018; Bista *et al.* 2018). Sometimes, OTUs are discarded because they do not

670 match any sequence in the reference database (Laroche *et al.* 2017). This is problematic for
671 accurate abundance and diversity estimation.

672 **4.6 Lack of ecological information**

673 eDNA analyses mostly report presence/absence and/or recent occupancy. Information on the
674 ecological status of target organisms, including the life history stages (adults, eggs, larvae)
675 present, the sex ratio, body condition (sick or healthy), and activity (e.g. breeding or non-
676 breeding) cannot be obtained, but may be crucial for making informed management and
677 conservation decisions. For instance, amphibians have complex life cycles and live both on land
678 and in water, as eggs, tadpoles or adults. Many amphibians are highly threatened and each threat
679 operates on different, sometimes multiple, life history stages (Klein *et al.* 2017). Thus,
680 knowledge of an organism's life history stages and their respective threats is critical for effective
681 management of their population (Klein *et al.* 2017). Moreover, life history traits that cannot be
682 assessed using eDNA can be key considerations for designing a successful eDNA-based study.
683 For instance, a species' life history can influence how well (when, where, and how) it can be
684 detected via eDNA surveys (Olson *et al.* 2012a; Barnes & Turner 2016; Bylemans *et al.* 2017;
685 Eiler *et al.* 2018; Takeuchi *et al.* 2019a; Wineland *et al.* 2019).

686 **5. Potential ways of reducing limitations in eDNA analysis**

687 Researchers have long been focusing on the comparisons between the detection probability of
688 eDNA and traditional survey methods (Ficetola *et al.* 2008; Jerde *et al.* 2011b). But only recently
689 have they begun to explore the origin, state, transport, and fate of eDNA and how these attributes
690 influence species detection and quantification, data analysis, and result interpretation (Deiner &
691 Altermatt 2014b; Barnes & Turner 2016; Jerde *et al.* 2016; Collins *et al.* 2018; Lugg *et al.* 2018;

692 Seymour *et al.* 2018; Seymour 2019). Most of the current limitations in eDNA analysis are
 693 directly or indirectly linked to technical aspects of the tool (Table 1). Developing improved
 694 techniques, optimizing current ones or combining eDNA with traditional surveys could
 695 overcome many of these limitations (Table 1).

696 Table 1 Potential ways of reducing limitations in environmental DNA (eDNA) analysis

Challenge and limitation	Causes	Potential solution(s)	Reference (s)
Imperfect sampling of eDNA and false detection	Limited persistence of eDNA	Use multiple field and PCR replicates Estimate detection rates using occupancy or other models	(Roussel <i>et al.</i> 2015; Valentini <i>et al.</i> 2016b; Willoughby <i>et al.</i> 2016; Alberdi <i>et al.</i> 2018)
	PCR primer biases	Use multiple markers and primers, even when targeting the same taxonomic group	(Alberdi <i>et al.</i> 2018; Collins <i>et al.</i> 2019)
	Inhibition of DNA amplification	Use inhibition-reducing assays	(Jane <i>et al.</i> 2015)
	Sample contamination	Use negative and positive controls Use particle size-based selective capture/enrichment of target eDNA	(Turner <i>et al.</i> 2014a; Bista <i>et al.</i> 2017)
	eDNA from dead organisms	Co-extract extracellular and intracellular DNA Co-extract DNA and RNA Amplify both longer and shorter DNA fragments	(Bista <i>et al.</i> 2017; Laroche <i>et al.</i> 2017)
	Ancient DNA (aDNA) resuspension	Confirm the organism's presence with traditional surveys	(Wu <i>et al.</i> 2018)
Difficulties in quantifying abundance and biomass	Variability in eDNA deposition and preservation	Quantify the relationship between eDNA release and biotic, and abiotic factors	(Laramie <i>et al.</i> 2015; Sassoubre <i>et al.</i> 2016)
	Choice of eDNA sampling and processing protocols	Use fully integrated environmental DNA sampling systems	(Thomas <i>et al.</i> 2018)
	PCR primer and sequencing biases	Use PCR-free and capture-based approaches	(Zhou <i>et al.</i> 2013; Wilcox <i>et al.</i> 2018; Ji <i>et al.</i> 2019)
	Variation in DNA copy number of target loci	Use multiple DNA markers	(Ma <i>et al.</i> 2016; Bylemans <i>et al.</i> 2018)
	Sequence filtering stringency	Adapt workflows based on sequencing technology and library	(Divoll <i>et al.</i> 2018)

Taxonomic assignment biases	Incomplete reference databases	Increase barcode efforts	(Young <i>et al.</i>)
Limited understanding of the ecology of eDNA	eDNA origin, state, transport, and fate	Use experimental validation in laboratory and natural settings	(Barnes & Turner 2016; Maruyama <i>et al.</i> 2019; Murakami <i>et al.</i> 2019)
Inconsistencies in data analysis and interpretation	Minimum sequence threshold	Use relative thresholds (e.g. 0.01% of total reads) rather than absolute copy number thresholds	(Bista <i>et al.</i> 2017; Alberdi <i>et al.</i> 2018)
	Chimeric sequence detection and removal	Predict <i>in silico</i> and remove using <i>de novo</i> delimitation approaches	(Bista <i>et al.</i> 2017; Alberdi <i>et al.</i> 2018)
	Percent identity for OTU clustering	Use existing knowledge of intraspecific diversity for target taxa	(Bista <i>et al.</i> 2017)
	Percent similarity for taxonomic assignment	Evaluate the completeness and accuracy of reference database used	(Bista <i>et al.</i> 2017)
Lack of ecological information	Target organisms not sighted	Conduct eDNA and traditional surveys simultaneously	(Biggs <i>et al.</i> 2015)

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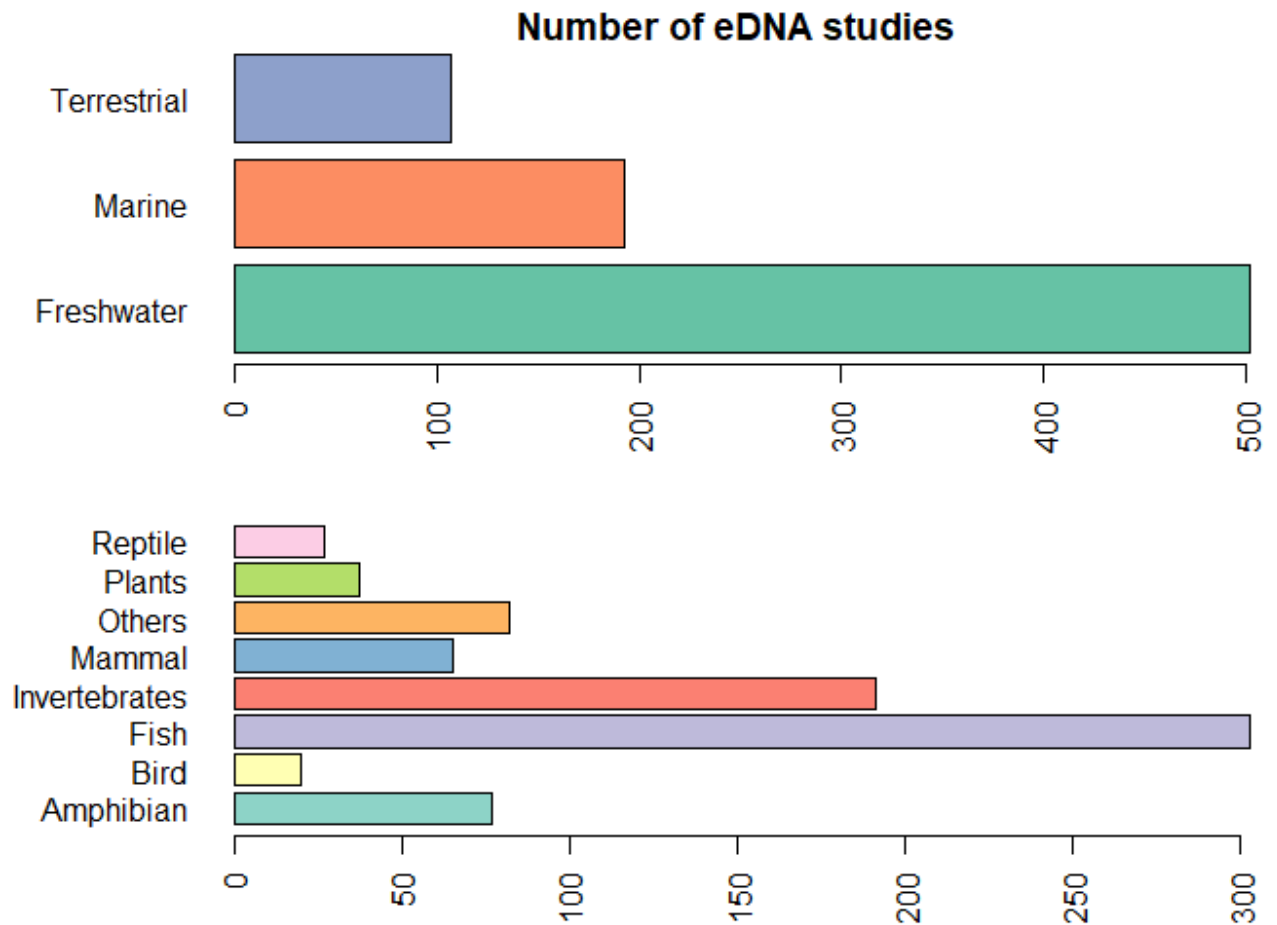
698

699 **6. How eDNA and traditional surveys can complement each other**

700 eDNA and traditional survey methods should not usually be considered as alternative methods
701 for assessing and monitoring biodiversity, since they can give such different information
702 (Ulibarri *et al.* 2017; Bailey *et al.* 2019; Rose *et al.* 2019; Leempoel *et al.* 2020; Takahara *et al.*
703 2020). Researchers must consider which of the two methods—or the use of both—is most
704 appropriate for addressing the questions they want to investigate (Qu & Stewart 2019b).
705 Information from eDNA often needs to be followed up with traditional surveys, but eDNA can
706 help guide these surveys in the right direction (Rose *et al.* 2019; Ji *et al.* 2020; Sales *et al.* 2020).
707 For example, Ji *et al.* 2020 found that leech-derived eDNA provides valuable information on the
708 spatial distributions of vertebrate species and on the environmental and anthropogenic correlates
709 of those distributions, making it a useful tool to efficiently measure the effectiveness of protected
710 areas and to help optimize the deployment of management resources within reserves. The way in
711 which eDNA and traditional surveys are implemented will largely be determined by the research
712 questions, but will also be influenced by practical considerations, such as the availability of
713 resources (including funding, time and the knowledge and skills of the persons undertaking the
714 research), and sound methodology. Knowing when to employ eDNA techniques rather than—or
715 in addition to—traditional sampling would enable practitioners to make more informed choices
716 concerning data collection (Franklin *et al.* 2019; Qu & Stewart 2019b). Based on the proportion
717 of eDNA studies published (between 1 January 2008 and 31 December 2019, Figure 2), eDNA
718 might be the first choice for hard-to-collect aquatic species (e.g. marine macroinvertebrates) and
719 would probably always be a useful supplement for fish and other cryptic aquatic species
720 (Wineland *et al.* 2019). The complex nature of some projects can sometimes make it difficult for
721 all aspects of a research question to be answered by a single method. In such cases, more than

722 one method can be used to collect and analyze data, integrate the findings, and draw inferences
723 (Harper *et al.* 2019; Jeunen *et al.* 2019; Knudsen *et al.* 2019; Wineland *et al.* 2019). eDNA can
724 be an exceptionally useful ecological and conservation tool when used in combination with
725 historical and other sources of data (e.g. citizen science) (Tingley *et al.* 2019). However, if
726 conditions permit, traditional biodiversity surveys will usually still be the first choice, because of
727 the additional types of information they can provide.

728



729

730 Figure 2 Number of studies using environmental DNA (eDNA) recovered from a literature search with the
 731 words 'environmental DNA' OR 'eDNA' for the period between 1 January 2008 and 31 December 2019
 732 that utilized a different organismal group and ecosystem.

733 **References**

734 Aas, A.B., Davey, M.L. & Kauserud, H. (2017). ITS all right mama: investigating the formation of chimeric
 735 sequences in the ITS2 region by DNA metabarcoding analyses of fungal mock communities of
 736 different complexities. *Mol Ecol Resour*, 17, 730-741.

737 Akamatsu, Y., Kume, G., Gotou, M., Kono, T., Fujii, T., Inui, R. *et al.* (2020). Using environmental DNA
 738 analyses to assess the occurrence and abundance of the endangered amphidromous fish
 739 *Plecoglossus altivelis ryukyuensis*. *Biodiversity Data Journal*, 8.

740 Akre, T.S., Parker, L.D., Ruther, E., Maldonado, J.E., Lemmon, L. & McInerney, N.R. (2019). Concurrent
 741 visual encounter sampling validates eDNA selectivity and sensitivity for the endangered wood
 742 turtle (*Glyptemys insculpta*). *Plos One*, 14.

743 Alberdi, A., Aizpurua, O., Gilbert, M.T.P. & Bohmann, K. (2018). Scrutinizing key steps for reliable
744 metabarcoding of environmental samples. *Methods Ecol Evol*, 9, 134-147.

745 Alexander, J.B., Bunce, M., White, N., Wilkinson, S.P., Adam, A.A.S., Berry, T. *et al.* (2020). Development
746 of a multi-assay approach for monitoring coral diversity using eDNA metabarcoding. *Coral Reefs*,
747 39, 159-171.

748 Amberg, J.J., Merkes, C.M., Stott, W., Rees, C.B. & Erickson, R.A. (2019). Environmental DNA as a tool to
749 help inform zebra mussel, *Dreissena polymorpha*, management in inland lakes. *Manag Biol*
750 *Invasion*, 10, 96-110.

751 AMEND, A.S., SEIFERT, K.A. & BRUNS, T.D. (2010). Quantifying microbial communities with 454
752 pyrosequencing: does read abundance count? *Mol Ecol*, 19, 5555-5565.

753 ANDERSEN, K., BIRD, K.L., RASMUSSEN, M., HAILE, J., BREUNING-MADSEN, H., KJÆR, K.H. *et al.* (2012a).
754 Meta-barcoding of 'dirt' DNA from soil reflects vertebrate biodiversity. *Mol Ecol*, 21, 1966-1979.

755 Andersen, K., Bird, K.L., Rasmussen, M., Haile, J., Breuning-Madsen, H., Kjaer, K.H. *et al.* (2012b). Meta-
756 barcoding of 'dirt' DNA from soil reflects vertebrate biodiversity. *Mol Ecol*, 21, 1966-1979.

757 Andruszkiewicz, E.A., Starks, H.A., Chavez, F.P., Sassoubre, L.M., Block, B.A. & Boehm, A.B. (2017).
758 Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding. *PloS one*, 12,
759 e0176343-e0176343.

760 Anglès d'Auriac, M.B., Strand, D.A., Mjelde, M., Demars, B.O.L. & Thaulow, J. (2019). Detection of an
761 invasive aquatic plant in natural water bodies using environmental DNA. *Plos One*, 14, e0219700.

762 Antognazza, C.M., Britton, J.R., Potter, C., Franklin, E., Hardouin, E.A., Gutmann Roberts, C. *et al.* (2019a).
763 Environmental DNA as a non-invasive sampling tool to detect the spawning distribution of
764 European anadromous shads (*Alosa* spp.). *Aquatic Conservation: Marine and Freshwater*
765 *Ecosystems*, 29, 148-152.

766 Antognazza, C.M., Britton, J.R., Potter, C., Franklin, E., Hardouin, E.A., Roberts, C.G. *et al.* (2019b).
767 Environmental DNA as a non-invasive sampling tool to detect the spawning distribution of
768 European anadromous shads (*Alosa* spp.). *Aquat Conserv*, 29, 148-152.

769 Ardura, A. (2019). Species-specific markers for early detection of marine invertebrate invaders through
770 eDNA methods: Gaps and priorities in GenBank as database example. *J Nat Conserv*, 47, 51-57.

771 Bailey, L.L., Jones, P., Thompson, K.G., Foutz, H.P., Logan, J.M., Wright, F.B. *et al.* (2019). Determining
772 Presence of Rare Amphibian Species: Testing and Combining Novel Survey Methods. *J Herpetol*,
773 53, 115-124.

774 Bakker, J., Wangenstein, O.S., Baillie, C., Buddo, D., Chapman, D.D., Gallagher, A.J. *et al.* (2019).
775 Biodiversity assessment of tropical shelf eukaryotic communities via pelagic eDNA
776 metabarcoding. *Ecol Evol*, 9, 14341-14355.

777 Balasingham, K.D., Walter, R.P. & Heath, D.D. (2017). Residual eDNA detection sensitivity assessed by
778 quantitative real-time PCR in a river ecosystem. *Mol Ecol Resour*, 17, 523-532.

779 Baldigo, B.P., Sporn, L.A., George, S.D. & Ball, J.A. (2017). Efficacy of Environmental DNA to Detect and
780 Quantify Brook Trout Populations in Headwater Streams of the Adirondack Mountains, New
781 York. *T Am Fish Soc*, 146, 99-111.

782 Barnes, M.A. & Turner, C.R. (2016). The ecology of environmental DNA and implications for conservation
783 genetics. *Conservation Genetics*, 17, 1-17.

784 Barnes, M.A., Turner, C.R., Jerde, C.L., Renshaw, M.A., Chadderton, W.L. & Lodge, D.M. (2014).
785 Environmental Conditions Influence eDNA Persistence in Aquatic Systems. *Environ Sci Technol*,
786 48, 1819-1827.

787 Basset, Y., Cizek, L., Cuénoud, P., Didham, R.K., Guilhaumon, F., Missa, O. *et al.* (2012). Arthropod
788 Diversity in a Tropical Forest. *Science*, 338, 1481-1484.

789 Beauclerc, K., Wozney, K., Smith, C. & Wilson, C. (2019). Development of quantitative PCR primers and
790 probes for environmental DNA detection of amphibians in Ontario. *Conserv Genet Resour*, 11,
791 43-46.

792 Bedwell, M.E. & Goldberg, C.S. (2020). Spatial and temporal patterns of environmental DNA detection to
793 inform sampling protocols in lentic and lotic systems. *Ecol Evol*.

794 Bienert, F., De Danieli, S., Miquel, C., Coissac, E., Poillot, C., Brun, J.J. *et al.* (2012). Tracking earthworm
795 communities from soil DNA. *Mol Ecol*, 21, 2017-2030.

796 Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Dejean, T., Griffiths, R.A. *et al.* (2015). Using eDNA to
797 develop a national citizen science-based monitoring programme for the great crested newt
798 (*Triturus cristatus*). *Biol Conserv*, 183, 19-28.

799 Bista, I., Carvalho, G.R., Tang, M., Walsh, K., Zhou, X., Hajibabaei, M. *et al.* (2018). Performance of
800 amplicon and shotgun sequencing for accurate biomass estimation in invertebrate community
801 samples. *Mol Ecol Resour*, 18, 1020-1034.

802 Bista, I., Carvalho, G.R., Walsh, K., Seymour, M., Hajibabaei, M., Lallias, D. *et al.* (2017). Annual time-
803 series analysis of aqueous eDNA reveals ecologically relevant dynamics of lake ecosystem
804 biodiversity. *Nat Commun*, 8, 14087.

805 Bracken, F.S.A., Rooney, S.M., Kelly-Quinn, M., King, J.J. & Carlsson, J. (2019). Identifying spawning sites
806 and other critical habitat in lotic systems using eDNA "snapshots": A case study using the sea
807 lamprey *Petromyzon marinus* L. *Ecol Evol*, 9, 553-567.

808 Brannock, P.M. & Halanych, K.M. (2015). Meiofaunal community analysis by high-throughput
809 sequencing: Comparison of extraction, quality filtering, and clustering methods. *Mar Genom*, 23,
810 67-75.

811 Burivalova, Z., Game, E.T. & Butler, R.A. (2019). The sound of a tropical forest. *Science*, 363, 28-29.

812 Buxton, A.S., Groombridge, J.J., Zakaria, N.B. & Griffiths, R.A. (2017). Seasonal variation in environmental
813 DNA in relation to population size and environmental factors. *Scientific Reports*, 7.

814 Bylemans, J., Furlan, E.M., Gleeson, D.M., Hardy, C.M. & Duncan, R.P. (2018). Does Size Matter? An
815 Experimental Evaluation of the Relative Abundance and Decay Rates of Aquatic Environmental
816 DNA. *Environ Sci Technol*, 52, 6408-6416.

817 Bylemans, J., Furlan, E.M., Hardy, C.M., McGuffie, P., Lintermans, M. & Gleeson, D.M. (2017). An
818 environmental DNA-based method for monitoring spawning activity: a case study, using the
819 endangered Macquarie perch (*Macquaria australasica*). *Methods Ecol Evol*, 8, 646-655.

820 Cannon, M.V., Hester, J., Shalkhauser, A., Chan, E.R., Logue, K., Small, S.T. *et al.* (2016). In silico
821 assessment of primers for eDNA studies using PrimerTree and application to characterize the
822 biodiversity surrounding the Cuyahoga River. *Scientific Reports*, 6, 22908.

823 Carraro, L., Hartikainen, H., Jokela, J., Bertuzzo, E. & Rinaldo, A. (2018). Estimating species distribution
824 and abundance in river networks using environmental DNA. *Proceedings of the National
825 Academy of Sciences*, 115, 11724-11729.

826 Carvalho, S., Aylagas, E., Villalobos, R., Kattan, Y., Berumen, M. & Pearman, J.K. (2019). Beyond the visual:
827 using metabarcoding to characterize the hidden reef cryptobiome. *P Roy Soc B-Biol Sci*, 286.

828 Champlot, S., Berthelot, C., Pruvost, M., Bennett, E.A., Grange, T. & Geigl, E.M. (2010). An Efficient
829 Multistrategy DNA Decontamination Procedure of PCR Reagents for Hypersensitive PCR
830 Applications. *Plos One*, 5.

831 Chen, W.T. & Ficetola, G.F. (2019). Conditionally autoregressive models improve occupancy analyses of
832 autocorrelated data: An example with environmental DNA. *Mol Ecol Resour*, 19, 163-175.

833 Cilleros, K., Valentini, A., Allard, L., Dejean, T., Etienne, R., Grenouillet, G. *et al.* (2019). Unlocking
834 biodiversity and conservation studies in high-diversity environments using environmental DNA
835 (eDNA): A test with Guianese freshwater fishes. *Mol Ecol Resour*, 19, 27-46.

836 Civade, R., Dejean, T., Valentini, A., Roset, N., Raymond, J.-C., Bonin, A. *et al.* (2016). Spatial
837 Representativeness of Environmental DNA Metabarcoding Signal for Fish Biodiversity
838 Assessment in a Natural Freshwater System. *PloS one*, 11, e0157366-e0157366.

839 Clare, E.L., Symondson, W.O.C., Broders, H., Fabianek, F., Fraser, E.E., MacKenzie, A. *et al.* (2014). The
840 diet of *Myotis lucifugus* across Canada: assessing foraging quality and diet variability. *Mol Ecol*,
841 23, 3618-3632.

842 Clarke, A. & Fraser, K.P.P. (2004). Why does metabolism scale with temperature? *Functional Ecology*, 18,
843 243-251.

844 Collins, R.A., Bakker, J., Wangensteen, O.S., Soto, A.Z., Corrigan, L., Sims, D.W. *et al.* (2019). Non-specific
845 amplification compromises environmental DNA metabarcoding with COI. *Methods Ecol Evol*.

846 Collins, R.A., Wangensteen, O.S., O’Gorman, E.J., Mariani, S., Sims, D.W. & Genner, M.J. (2018).
847 Persistence of environmental DNA in marine systems. *Communications Biology*, 1, 185.

848 Cordier, T., Frontalini, F., Cermakova, K., Apothéoz-Perret-Gentil, L., Treglia, M., Scantamburlo, E. *et al.*
849 (2019). Multi-marker eDNA metabarcoding survey to assess the environmental impact of three
850 offshore gas platforms in the North Adriatic Sea (Italy). *Marine Environmental Research*, 146, 24-
851 34.

852 Corlett, R.T. (2017). A Bigger Toolbox: Biotechnology in Biodiversity Conservation. *Trends Biotechnol*, 35,
853 55-65.

854 Cowart, D.A., Matabos, M., Brandt, M.I., Marticorena, J. & Sarrazin, J. (2020). Exploring Environmental
855 DNA (eDNA) to Assess Biodiversity of Hard Substratum Faunal Communities on the Lucky Strike
856 Vent Field (Mid-Atlantic Ridge) and Investigate Recolonization Dynamics After an Induced
857 Disturbance. *Front Mar Sci*, 6.

858 Davy, C.M., Kidd, A.G. & Wilson, C.C. (2015). Development and Validation of Environmental DNA (eDNA)
859 Markers for Detection of Freshwater Turtles. *Plos One*, 10, e0130965-e0130965.

860 de Souza, L.S., Godwin, J.C., Renshaw, M.A. & Larson, E. (2016). Environmental DNA (eDNA) Detection
861 Probability Is Influenced by Seasonal Activity of Organisms. *Plos One*, 11.

862 Deagle, B.E., Eveson, J.P. & Jarman, S.N. (2006). Quantification of damage in DNA recovered from highly
863 degraded samples--a case study on DNA in faeces. *Front Zool*, 3, 11.

864 Deagle, B.E., Jarman, S.N., Coissac, E., Pompanon, F. & Taberlet, P. (2014). DNA metabarcoding and the
865 cytochrome c oxidase subunit I marker: not a perfect match. *Biology letters*, 10, 20140562.

866 Deagle, B.E., Kirkwood, R. & Jarman, S.N. (2009). Analysis of Australian fur seal diet by pyrosequencing
867 prey DNA in faeces. *Mol Ecol*, 18, 2022-2038.

868 Deagle, B.E., Thomas, A.C., Shaffer, A.K., Trites, A.W. & Jarman, S.N. (2013). Quantifying sequence
869 proportions in a DNA-based diet study using Ion Torrent amplicon sequencing: which counts
870 count? *Mol Ecol Resour*, 13, 620-633.

871 Deiner, K. & Altermatt, F. (2014a). Transport distance of invertebrate environmental DNA in a natural
872 river. *PloS one*, 9, e88786-e88786.

873 Deiner, K. & Altermatt, F. (2014b). Transport Distance of Invertebrate Environmental DNA in a Natural
874 River. *PLOS ONE*, 9, e88786.

875 Deiner, K., Bik, H.M., Machler, E., Seymour, M., Lacoursiere-Roussel, A., Altermatt, F. *et al.* (2017).
876 Environmental DNA metabarcoding: Transforming how we survey animal and plant communities.
877 *Mol Ecol*, 26, 5872-5895.

878 Deiner, K., Fronhofer, E.A., Mächler, E., Walser, J.-C. & Altermatt, F. (2016). Environmental DNA reveals
879 that rivers are conveyor belts of biodiversity information. *Nat Commun*, 7, 12544.

880 Deiner, K., Walser, J.-C., Mächler, E. & Altermatt, F. (2015a). Choice of capture and extraction methods
881 affect detection of freshwater biodiversity from environmental DNA. *Biological Conservation*,
882 183, 53-63.

883 Deiner, K., Walser, J.C., Machler, E. & Altermatt, F. (2015b). Choice of capture and extraction methods
884 affect detection of freshwater biodiversity from environmental DNA. *Biol Conserv*, 183, 53-63.

885 Dejean, T., Valentini, A., Duparc, A., Pellier-Cuit, S., Pompanon, F., Taberlet, P. *et al.* (2011). Persistence
886 of Environmental DNA in Freshwater Ecosystems. *Plos One*, 6.

887 Dejean, T., Valentini, A., Miquel, C., Taberlet, P., Bellemain, E. & Miaud, C. (2012). Improved detection of
888 an alien invasive species through environmental DNA barcoding: the example of the American
889 bullfrog *Lithobates catesbeianus*. *J Appl Ecol*, 49, 953-959.

890 Deutschmann, B., Mueller, A.-K., Hollert, H. & Brinkmann, M. (2019). Assessing the fate of brown trout
891 (*Salmo trutta*) environmental DNA in a natural stream using a sensitive and specific dual-labelled
892 probe. *Sci Total Environ*, 655, 321-327.

893 DiBattista, J.D., Reimer, J.D., Stat, M., Masucci, G.D., Biondi, P., De Brauwer, M. *et al.* (2019). Digging for
894 DNA at depth: rapid universal metabarcoding surveys (RUMS) as a tool to detect coral reef
895 biodiversity across a depth gradient. *Peerj*, 7.

896 Divoll, T.J., Brown, V.A., Kinne, J., McCracken, G.F. & O'Keefe, J.M. (2018). Disparities in second-
897 generation DNA metabarcoding results exposed with accessible and repeatable workflows. *Mol*
898 *Ecol Resour*, 18, 590-601.

899 Djurhuus, A., Closek, C.J., Kelly, R.P., Pitz, K.J., Michisaki, R.P., Starks, H.A. *et al.* (2020). Environmental
900 DNA reveals seasonal shifts and potential interactions in a marine community. *Nature*
901 *Communications*, 11, 254.

902 Djurhuus, A., Port, J., Closek, C.J., Yamahara, K.M., Romero-Maraccini, O., Walz, K.R. *et al.* (2017).
903 Evaluation of Filtration and DNA Extraction Methods for Environmental DNA Biodiversity
904 Assessments across Multiple Trophic Levels. *Frontiers in Marine Science*, 4.

905 Doi, H., Akamatsu, Y., Watanabe, Y., Goto, M., Inui, R., Katano, I. *et al.* (2017). Water sampling for
906 environmental DNA surveys by using an unmanned aerial vehicle. *Limnol Oceanogr-Meth*, 15,
907 939-944.

908 Doi, H., Fukaya, K., Oka, S., Sato, K., Kondoh, M. & Miya, M. (2019). Evaluation of detection probabilities
909 at the water-filtering and initial PCR steps in environmental DNA metabarcoding using a
910 multispecies site occupancy model. *Scientific Reports*, 9.

911 Doi, H., Uchii, K., Takahara, T., Matsushashi, S., Yamanaka, H. & Minamoto, T. (2015). Use of Droplet
912 Digital PCR for Estimation of Fish Abundance and Biomass in Environmental DNA Surveys. *Plos*
913 *One*, 10.

914 Dunn, N., Priestley, V., Herraiz, A., Arnold, R. & Savolainen, V. (2017). Behavior and season affect crayfish
915 detection and density inference using environmental DNA. *Ecol Evol*, 7, 7777-7785.

916 Edgar, R.C. (2016). UCHIME2: improved chimera prediction for amplicon sequencing. *bioRxiv*, 074252.

917 Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C. & Knight, R. (2011). UCHIME improves sensitivity and
918 speed of chimera detection. *Bioinformatics*, 27, 2194-2200.

919 Edwards, M.E., Alsos, I.G., Yoccoz, N., Coissac, E., Goslar, T., Gielly, L. *et al.* (2018). Metabarcoding of
920 modern soil DNA gives a highly local vegetation signal in Svalbard tundra. *Holocene*, 28, 2006-
921 2016.

922 Eichmiller, J.J., Miller, L.M. & Sorensen, P.W. (2016). Optimizing techniques to capture and extract
923 environmental DNA for detection and quantification of fish. *Molecular Ecology Resources*, 16,
924 56-68.

925 Eiler, A., Löfgren, A., Hjerne, O., Nordén, S. & Saetre, P. (2018). Environmental DNA (eDNA) detects the
926 pool frog (*Pelophylax lessonae*) at times when traditional monitoring methods are insensitive.
927 *Scientific Reports*, 8, 5452.

- 928 Elbrecht, V. & Leese, F. (2015). Can DNA-Based Ecosystem Assessments Quantify Species Abundance?
 929 Testing Primer Bias and Biomass—Sequence Relationships with an Innovative Metabarcoding
 930 Protocol. *PLOS ONE*, 10, e0130324.
- 931 Epp, L.S., Boessenkool, S., Bellemain, E.P., Haile, J., Esposito, A., Riaz, T. *et al.* (2012). New environmental
 932 metabarcodes for analysing soil DNA: potential for studying past and present ecosystems. *Mol*
 933 *Ecol*, 21, 1821-1833.
- 934 Erickson, R.A., Rees, C.B., Coulter, A.A., Merkes, C.M., Mccalla, S.G., Touzinsky, K.F. *et al.* (2016).
 935 Detecting the movement and spawning activity of bigheaded carps with environmental DNA.
 936 *Mol Ecol Resour*, 16, 957-965.
- 937 Evans, N.T., Li, Y.Y., Renshaw, M.A., Olds, B.P., Deiner, K., Turner, C.R. *et al.* (2017a). Fish community
 938 assessment with eDNA metabarcoding: effects of sampling design and bioinformatic filtering.
 939 *Can J Fish Aquat Sci*, 74, 1362-1374.
- 940 Evans, N.T., Olds, B.P., Renshaw, M.A., Turner, C.R., Li, Y., Jerde, C.L. *et al.* (2016). Quantification of
 941 mesocosm fish and amphibian species diversity via environmental DNA metabarcoding. *Mol Ecol*
 942 *Resour*, 16, 29-41.
- 943 Evans, N.T., Shirey, P.D., Wieringa, J.G., Mahon, A.R. & Lamberti, G.A. (2017b). Comparative Cost and
 944 Effort of Fish Distribution Detection via Environmental DNA Analysis and Electrofishing. *Fisheries*,
 945 42, 90-99.
- 946 Evrard, O., Lacey, J.P., Ficetola, G.F., Gielly, L., Huon, S., Lefèvre, I. *et al.* (2019). Environmental DNA
 947 provides information on sediment sources: A study in catchments affected by Fukushima
 948 radioactive fallout. *Science of The Total Environment*, 665, 873-881.
- 949 Fernanda Nardi, C., Alfredo Fernandez, D., Alberto Vanella, F. & Chalde, T. (2019). The expansion of
 950 exotic Chinook salmon (*Oncorhynchus tshawytscha*) in the extreme south of Patagonia: an
 951 environmental DNA approach. *Biol Invasions*, 21, 1415-1425.
- 952 Ficetola, G.F., Manenti, R. & Taberlet, P. (2019). Environmental DNA and metabarcoding for the study of
 953 amphibians and reptiles: species distribution, the microbiome, and much more. *Amphibia-*
 954 *Reptilia*, 40, 129-148.
- 955 Ficetola, G.F., Miaud, C., Pompanon, F. & Taberlet, P. (2008). Species detection using environmental
 956 DNA from water samples. *Biol Letters*, 4, 423-425.
- 957 Ficetola, G.F., Pansu, J., Bonin, A., Coissac, E., Giguët-Covex, C., De Barba, M. *et al.* (2015). Replication
 958 levels, false presences and the estimation of the presence/absence from eDNA metabarcoding
 959 data. *Mol Ecol Resour*, 15, 543-556.
- 960 Ficetola, G.F., Taberlet, P. & Coissac, E. (2016). How to limit false positives in environmental DNA and
 961 metabarcoding? *Mol Ecol Resour*, 16, 604-607.
- 962 Fonseca, V.G. (2018). "Pitfalls in relative abundance estimation using eDNA metabarcoding". *Mol Ecol*
 963 *Resour*, 18, 923-926.
- 964 Franklin, T.W., McKelvey, K.S., Golding, J.D., Mason, D.H., Dysthe, J.C., Pilgrim, K.L. *et al.* (2019). Using
 965 environmental DNA methods to improve winter surveys for rare carnivores: DNA from snow and
 966 improved noninvasive techniques. *Biol Conserv*, 229, 50-58.
- 967 Fraser, C.I., Connell, L., Lee, C.K. & Cary, S.C. (2018). Evidence of plant and animal communities at
 968 exposed and subglacial (cave) geothermal sites in Antarctica. *Polar Biol*, 41, 417-421.
- 969 Fritts, A.K., Knights, B.C., Larson, J.H., Amberg, J.J., Merkes, C.M., Tajjioui, T. *et al.* (2019). Development
 970 of a quantitative PCR method for screening ichthyoplankton samples for bigheaded carps. *Biol*
 971 *Invasions*, 21, 1143-1153.
- 972 Fujii, K., Doi, H., Matsuoka, S., Nagano, M., Sato, H. & Yamanaka, H. (2019). Environmental DNA
 973 metabarcoding for fish community analysis in backwater lakes: A comparison of capture
 974 methods. *PLoS one*, 14, e0210357-e0210357.

- 975 Fukumoto, S., Ushimaru, A. & Minamoto, T. (2015). A basin-scale application of environmental DNA
 976 assessment for rare endemic species and closely related exotic species in rivers: a case study of
 977 giant salamanders in Japan. *J Appl Ecol*, 52, 358-365.
- 978 Galan, M., Pages, M. & Cosson, J.F. (2012). Next-Generation Sequencing for Rodent Barcoding: Species
 979 Identification from Fresh, Degraded and Environmental Samples. *Plos One*, 7.
- 980 Galan, M., Pons, J.-B., Tournayre, O., Pierre, E., Leuchtman, M., Pontier, D. *et al.* (2018). Metabarcoding
 981 for the parallel identification of several hundred predators and their prey: Application to bat
 982 species diet analysis. *Mol Ecol Resour*, 18, 474-489.
- 983 Gillet, B., Cottet, M., Destanque, T., Kue, K., Descloux, S., Chanudet, V. *et al.* (2018). Direct fishing and
 984 eDNA metabarcoding for biomonitoring during a 3-year survey significantly improves number of
 985 fish detected around a South East Asian reservoir. *PLoS one*, 13, e0208592-e0208592.
- 986 Goldberg, C.S., Pilliod, D.S., Arkle, R.S. & Waits, L.P. (2011). Molecular Detection of Vertebrates in
 987 Stream Water: A Demonstration Using Rocky Mountain Tailed Frogs and Idaho Giant
 988 Salamanders. *Plos One*, 6.
- 989 Goldberg, C.S., Sepulveda, A., Ray, A., Baumgardt, J. & Waits, L.P. (2013). Environmental DNA as a new
 990 method for early detection of New Zealand mudsnails (*Potamopyrgus antipodarum*). *Freshw Sci*,
 991 32, 792-800.
- 992 Goldberg, C.S., Strickler, K.M. & Fremier, A.K. (2018). Degradation and dispersion limit environmental
 993 DNA detection of rare amphibians in wetlands: Increasing efficacy of sampling designs. *Science
 994 of The Total Environment*, 633, 695-703.
- 995 Goldberg, C.S., Turner, C.R., Deiner, K., Klymus, K.E., Thomsen, P.F., Murphy, M.A. *et al.* (2016). Critical
 996 considerations for the application of environmental DNA methods to detect aquatic species.
 997 *Methods Ecol Evol*, 7, 1299-1307.
- 998 Gomes, G.B., Hutson, K.S., Domingos, J.A., Villamil, S.I., Huerlimann, R., Miller, T.L. *et al.* (2019). Parasitic
 999 protozoan interactions with bacterial microbiome in a tropical fish farm. *Aquaculture*, 502, 196-
 1000 201.
- 1001 Gómez-Zurita, J., Cardoso, A., Coronado, I., De la Cadena, G., Jurado-Rivera, J.A., Maes, J.-M. *et al.* (2016).
 1002 High-throughput biodiversity analysis: Rapid assessment of species richness and ecological
 1003 interactions of Chrysomelidae (Coleoptera) in the tropics. *Zookeys*, 3-26.
- 1004 Graham, S.E., Chariton, A.A. & Landis, W.G. (2019). Using Bayesian networks to predict risk to estuary
 1005 water quality and patterns of benthic environmental DNA in Queensland. *Integrated
 1006 Environmental Assessment and Management*, 15, 93-111.
- 1007 Guillera-Aroita, G., Lahoz-Monfort, J.J., van Rooyen, A.R., Weeks, A.R. & Tingley, R. (2017). Dealing with
 1008 false-positive and false-negative errors about species occurrence at multiple levels. *Methods
 1009 Ecol Evol*, 8, 1081-1091.
- 1010 Günther, B., Kneibelsberger, T., Neumann, H., Laakmann, S. & Martínez Arbizu, P. (2018). Metabarcoding
 1011 of marine environmental DNA based on mitochondrial and nuclear genes. *Scientific Reports*, 8,
 1012 14822.
- 1013 Hanfling, B., Handley, L.L., Read, D.S., Hahn, C., Li, J.L., Nichols, P. *et al.* (2016). Environmental DNA
 1014 metabarcoding of lake fish communities reflects long-term data from established survey
 1015 methods. *Mol Ecol*, 25, 3101-3119.
- 1016 Harper, K.J., Goodwin, K.D., Harper, L.R., LaCasella, E.L., Frey, A. & Dutton, P.H. (2020). Finding Crush:
 1017 Environmental DNA Analysis as a Tool for Tracking the Green Sea Turtle *Chelonia mydas* in a
 1018 Marine Estuary. *Frontiers in Marine Science*, 6.
- 1019 Harper, L.R., Griffiths, N.P., Handley, L.L., Sayer, C.D., Read, D.S., Harper, K.J. *et al.* (2019). Development
 1020 and application of environmental DNA surveillance for the threatened crucian carp (*Carassius
 1021 carassius*). *Freshwater Biol*, 64, 93-107.

- 1022 Harper, L.R., Lawson Handley, L., Hahn, C., Boonham, N., Rees, H.C., Gough, K.C. *et al.* (2018). Needle in
 1023 a haystack? A comparison of eDNA metabarcoding and targeted qPCR for detection of the great
 1024 crested newt (*Triturus cristatus*). *Ecol Evol*, 8, 6330-6341.
- 1025 Harrer, L.E.F. & Levi, T. (2018). The primacy of bears as seed dispersers in salmon-bearing ecosystems.
 1026 *Ecosphere*, 9.
- 1027 Harrison, J.B., Sunday, J.M. & Rogers, S.M. (2019). Predicting the fate of eDNA in the environment and
 1028 implications for studying biodiversity. *Proceedings of the Royal Society B: Biological Sciences*,
 1029 286, 20191409.
- 1030 Hemery, L.G., Politano, K.K. & Henkel, S.K. (2017). Assessing differences in macrofaunal assemblages as a
 1031 factor of sieve mesh size, distance between samples, and time of sampling. *Environ Monit Assess*,
 1032 189.
- 1033 Hering, D., Borja, A., Jones, J.I., Pont, D., Boets, P., Bouchez, A. *et al.* (2018). Implementation options for
 1034 DNA-based identification into ecological status assessment under the European Water
 1035 Framework Directive. *Water Research*, 138, 192-205.
- 1036 Hinlo, R., Furlan, E., Sutor, L. & Gleeson, D. (2017). Environmental DNA monitoring and management of
 1037 invasive fish: comparison of eDNA and fyke netting. *Manag Biol Invasion*, 8, 89-100.
- 1038 Hobbs, J., Round, J.M., Allison, M.J. & Helbing, C.C. (2019). Expansion of the known distribution of the
 1039 coastal tailed frog, *Ascaphus truei*, in British Columbia, Canada, using robust eDNA detection
 1040 methods. *Plos One*, 14.
- 1041 Hopken, M.W., Orning, E.K., Young, J.K. & Piaggio, A.J. (2016). Molecular forensics in avian conservation:
 1042 a DNA-based approach for identifying mammalian predators of ground-nesting birds and eggs.
 1043 *BMC Research Notes*, 9, 14.
- 1044 Hunter, M.E., Ferrante, J.A., Meigs-Friend, G. & Ulmer, A. (2019). Improving eDNA yield and inhibitor
 1045 reduction through increased water volumes and multi-filter isolation techniques. *Sci Rep-Uk*, 9.
- 1046 Huver, J.R., Koprivnikar, J., Johnson, P.T.J. & Whyard, S. (2015). Development and application of an
 1047 eDNA method to detect and quantify a pathogenic parasite in aquatic ecosystems. *Ecol Appl*, 25,
 1048 991-1002.
- 1049 Itakura, H., Wakiya, R., Yamamoto, S., Kaifu, K., Sato, T. & Minamoto, T. (2019). Environmental DNA
 1050 analysis reveals the spatial distribution, abundance, and biomass of Japanese eels at the river-
 1051 basin scale. *Aquat Conserv*, 29, 361-373.
- 1052 Jane, S.F., Wilcox, T.M., McKelvey, K.S., Young, M.K., Schwartz, M.K., Lowe, W.H. *et al.* (2015). Distance,
 1053 flow and PCR inhibition: eDNA dynamics in two headwater streams. *Mol Ecol Resour*, 15, 216-
 1054 227.
- 1055 Jerde, C.L., Chadderton, W.L., Mahon, A.R., Renshaw, M.A., Corush, J., Budny, M.L. *et al.* (2013).
 1056 Detection of Asian carp DNA as part of a Great Lakes basin-wide surveillance program. *Can J Fish*
 1057 *Aquat Sci*, 70, 522-526.
- 1058 Jerde, C.L., Mahon, A.R., Chadderton, W.L. & Lodge, D.M. (2011a). "Sight-unseen" detection of rare
 1059 aquatic species using environmental DNA. *Conserv Lett*, 4, 150-157.
- 1060 Jerde, C.L., Mahon, A.R., Chadderton, W.L. & Lodge, D.M. (2011b). "Sight-unseen" detection of rare
 1061 aquatic species using environmental DNA. *Conservation Letters*, 4, 150-157.
- 1062 Jerde, C.L., Olds, B.P., Shogren, A.J., Andruszkiewicz, E.A., Mahon, A.R., Bolster, D. *et al.* (2016). Influence
 1063 of Stream Bottom Substrate on Retention and Transport of Vertebrate Environmental DNA.
 1064 *Environ Sci Technol*, 50, 8770-8779.
- 1065 Jeunen, G.J., Knapp, M., Spencer, H.G., Lamare, M.D., Taylor, H.R., Stat, M. *et al.* (2019). Environmental
 1066 DNA (eDNA) metabarcoding reveals strong discrimination among diverse marine habitats
 1067 connected by water movement. *Mol Ecol Resour*, 19, 426-438.

1068 Ji, Y., Baker, C.C., Li, Y., Popescu, V.D., Wang, Z., Wang, J. *et al.* (2020). Large-scale Quantification of
1069 Vertebrate Biodiversity in Ailaoshan Nature Reserve from Leech iDNA. *bioRxiv*,
1070 2020.2002.2010.941336.

1071 Ji, Y., Huotari, T., Roslin, T., Martin-Schmidt, N., Wang, J., Yu, D. *et al.* (2019). SPIKEPIPE: A metagenomic
1072 pipeline for the accurate quantification of eukaryotic species occurrences and abundances using
1073 DNA barcodes or mitogenomes. *bioRxiv*, 533737.

1074 Jia, Q., Wang, X., Zhang, Y., Cao, L. & Fox, A.D. (2018). Drivers of waterbird communities and their
1075 declines on Yangtze River floodplain lakes. *Biol Conserv*, 218, 240-246.

1076 Jo, T., Murakami, H., Masuda, R., Sakata, M.K., Yamamoto, S. & Minamoto, T. (2017). Rapid degradation
1077 of longer DNA fragments enables the improved estimation of distribution and biomass using
1078 environmental DNA. *Mol Ecol Resour*, 17, e25-e33.

1079 Judo, M.S., Wedel, A.B. & Wilson, C. (1998). Stimulation and suppression of PCR-mediated
1080 recombination. *Nucleic Acids Res*, 26, 1819-1825.

1081 Jusino, M.A., Banik, M.T., Palmer, J.M., Wray, A.K., Xiao, L., Pelton, E. *et al.* (2019). An improved method
1082 for utilizing high-throughput amplicon sequencing to determine the diets of insectivorous
1083 animals. *Mol Ecol Resour*, 19, 176-190.

1084 Kebschull, J.M. & Zador, A.M. (2015). Sources of PCR-induced distortions in high-throughput sequencing
1085 data sets. *Nucleic Acids Res*, 43.

1086 Kemp, B.M. & Smith, D.G. (2005). Use of bleach to eliminate contaminating DNA from the surface of
1087 bones and teeth. *Forensic Sci Int*, 154, 53-61.

1088 Kessler, E.J., Ash, K.T., Barratt, S.N., Larson, E.R. & Davis, M.A. (2020). Radiotelemetry reveals effects of
1089 upstream biomass and UV exposure on environmental DNA occupancy and detection for a large
1090 freshwater turtle. *Environmental DNA*, 2, 13-23.

1091 Khelifa, R. (2019). Sensitivity of biodiversity indices to life history stage, habitat type and landscape in
1092 Odonata community. *Biol Conserv*, 237, 63-69.

1093 Klein, C.J., Beher, J., Chaloupka, M., Hamann, M., Limpus, C. & Possingham, H.P. (2017). Prioritization of
1094 Marine Turtle Management Projects: A Protocol that Accounts for Threats to Different Life
1095 History Stages. *Conserv Lett*, 10, 547-554.

1096 Klymus, K.E., Richter, C.A., Chapman, D.C. & Paukert, C. (2015). Quantification of eDNA shedding rates
1097 from invasive bighead carp *Hypophthalmichthys nobilis* and silver carp *Hypophthalmichthys*
1098 *molitrix*. *Biol Conserv*, 183, 77-84.

1099 Knudsen, S.W., Ebert, R.B., Hesselsoe, M., Kuntke, F., Hassingboe, J., Mortensen, P.B. *et al.* (2019).
1100 Species-specific detection and quantification of environmental DNA from marine fishes in the
1101 Baltic Sea. *J Exp Mar Biol Ecol*, 510, 31-45.

1102 Koziol, A., Stat, M., Simpson, T., Jarman, S., DiBattista, J.D., Harvey, E.S. *et al.* (2019). Environmental DNA
1103 metabarcoding studies are critically affected by substrate selection. *Mol Ecol Resour*, 19, 366-
1104 376.

1105 Kraaijeveld, K., De Weger, L.A., Garcia, M.V., Buermans, H., Frank, J., Hiemstra, P.S. *et al.* (2015). Efficient
1106 and sensitive identification and quantification of airborne pollen using next-generation DNA
1107 sequencing. *Mol Ecol Resour*, 15, 8-16.

1108 Kuzmina, M.L., Braukmann, T.W.A. & Zakharov, E.V. (2018). Finding the pond through the weeds: eDNA
1109 reveals underestimated diversity of pondweeds. *Appl Plant Sci*, 6.

1110 Lacoursière-Roussel, A., Howland, K., Normandeau, E., Grey, E.K., Archambault, P., Deiner, K. *et al.*
1111 (2018). eDNA metabarcoding as a new surveillance approach for coastal Arctic biodiversity. *Ecol*
1112 *Evol*, 8, 7763-7777.

- 1113 Lacoursière-Roussel, A., Rosabal, M. & Bernatchez, L. (2016). Estimating fish abundance and biomass
 1114 from eDNA concentrations: variability among capture methods and environmental conditions.
 1115 *Mol Ecol Resour*, 16, 1401-1414.
- 1116 Lahoz-Monfort, J.J., Guillera-Arroita, G. & Tingley, R. (2016). Statistical approaches to account for false-
 1117 positive errors in environmental DNA samples. *Mol Ecol Resour*, 16, 673-685.
- 1118 Laramie, M.B., Pilliod, D.S. & Goldberg, C.S. (2015). Characterizing the distribution of an endangered
 1119 salmonid using environmental DNA analysis. *Biol Conserv*, 183, 29-37.
- 1120 Laroche, O., Wood, S.A., Tremblay, L.A., Lear, G., Ellis, J.I. & Pochon, X. (2017). Metabarcoding
 1121 monitoring analysis: the pros and cons of using co-extracted environmental DNA and RNA data
 1122 to assess offshore oil production impacts on benthic communities. *Peerj*, 5.
- 1123 Lecaudey, L.A., Schletterer, M., Kuzovlev, V.V., Hahn, C. & Weiss, S.J. (2019). Fish diversity assessment in
 1124 the headwaters of the Volga River using environmental DNA metabarcoding. *Aquat Conserv*.
- 1125 Leempoel, K., Hebert, T. & Hadly, E.A. (2020). A comparison of eDNA to camera trapping for assessment
 1126 of terrestrial mammal diversity. *Proceedings of the Royal Society B: Biological Sciences*, 287,
 1127 20192353.
- 1128 Leray, M. & Knowlton, N. (2017). Random sampling causes the low reproducibility of rare eukaryotic
 1129 OTUs in Illumina COI metabarcoding. *Peerj*, 5, e3006.
- 1130 Leray, M., Yang, J.Y., Meyer, C.P., Mills, S.C., Agudelo, N., Ranwez, V. *et al.* (2013). A new versatile primer
 1131 set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan
 1132 diversity: application for characterizing coral reef fish gut contents. *Front Zool*, 10, 34.
- 1133 Levi, T., Allen, J.M., Bell, D., Joyce, J., Russell, J.R., Tallmon, D.A. *et al.* (2019). Environmental DNA for the
 1134 enumeration and management of Pacific salmon. *Molecular Ecology Resources*, 19, 597-608.
- 1135 Levy-Booth, D.J., Campbell, R.G., Gulden, R.H., Hart, M.M., Powell, J.R., Klironomos, J.N. *et al.* (2007).
 1136 Cycling of extracellular DNA in the soil environment. *Soil Biol Biochem*, 39, 2977-2991.
- 1137 Liang, Z. & Keeley, A. (2013a). Filtration Recovery of Extracellular DNA from Environmental Water
 1138 Samples. *Environmental Science & Technology*, 47, 9324-9331.
- 1139 Liang, Z.B. & Keeley, A. (2013b). Filtration Recovery of Extracellular DNA from Environmental Water
 1140 Samples. *Environ Sci Technol*, 47, 9324-9331.
- 1141 Lim, N.K.M., Tay, Y.C., Srivathsan, A., Tan, J.W.T., Kwik, J.T.B., Baloglu, B. *et al.* (2016). Next-generation
 1142 freshwater bioassessment: eDNA metabarcoding with a conserved metazoan primer reveals
 1143 species-rich and reservoir-specific communities. *Roy Soc Open Sci*, 3.
- 1144 Lin, M.X., Zhang, S. & Yao, M. (2019). Effective detection of environmental DNA from the invasive
 1145 American bullfrog. *Biol Invasions*, 21, 2255-2268.
- 1146 Lindahl, T. (1993). Instability and Decay of the Primary Structure of DNA. *Nature*, 362, 709-715.
- 1147 Liu, S., Zhou, X., Zhou, C. & Yang, C. (2017). Filling reference gaps via assembling DNA barcodes using
 1148 high-throughput sequencing—moving toward barcoding the world. *Gigascience*, 6.
- 1149 Lugg, W.H., Griffiths, J., van Rooyen, A.R., Weeks, A.R. & Tingley, R. (2018). Optimal survey designs for
 1150 environmental DNA sampling. *Methods in Ecology and Evolution*, 9, 1049-1059.
- 1151 Ma, H.J., Stewart, K., Loughheed, S., Zheng, J.S., Wang, Y.X. & Zhao, J.F. (2016). Characterization,
 1152 optimization, and validation of environmental DNA (eDNA) markers to detect an endangered
 1153 aquatic mammal. *Conserv Genet Resour*, 8, 561-568.
- 1154 Machida, R.J., Leray, M., Ho, S.L. & Knowlton, N. (2017). Metazoan mitochondrial gene sequence
 1155 reference datasets for taxonomic assignment of environmental samples. *Sci Data*, 4, 170027.
- 1156 Mahe, F., Rognes, T., Quince, C., de Vargas, C. & Dunthorn, M. (2014). Swarm: robust and fast clustering
 1157 method for amplicon-based studies. *Peerj*, 2.
- 1158 Mahe, F., Rognes, T., Quince, C., de Vargas, C. & Dunthorn, M. (2015). Swarm v2: highly-scalable and
 1159 high-resolution amplicon clustering. *Peerj*, 3.

- 1160 Mahon, A.R., Jerde, C.L., Galaska, M., Bergner, J.L., Chadderton, W.L., Lodge, D.M. *et al.* (2013).
 1161 Validation of eDNA Surveillance Sensitivity for Detection of Asian Carps in Controlled and Field
 1162 Experiments. *Plos One*, 8.
- 1163 Majaneva, M., Diserud, O.H., Eagle, S.H.C., Boström, E., Hajibabaei, M. & Ekrem, T. (2018).
 1164 Environmental DNA filtration techniques affect recovered biodiversity. *Scientific Reports*, 8,
 1165 4682.
- 1166 Manfrin, C., Souty-Grosset, C., Anastacio, P.M., Reynolds, J. & Giulianini, P.G. (2019). Detection and
 1167 Control of Invasive Freshwater Crayfish: From Traditional to Innovative Methods. *Diversity-Basel*,
 1168 11.
- 1169 Marshall, N.T. & Stepien, C.A. (2019). Invasion genetics from eDNA and thousands of larvae: A targeted
 1170 metabarcoding assay that distinguishes species and population variation of zebra and quagga
 1171 mussels. *Ecol Evol*, 9, 3515-3538.
- 1172 Maruyama, A., Nakamura, K., Yamanaka, H., Kondoh, M. & Minamoto, T. (2014). The Release Rate of
 1173 Environmental DNA from Juvenile and Adult Fish. *PLOS ONE*, 9, e114639.
- 1174 Maruyama, A., Nakamura, K., Yamanaka, H., Kondoh, M. & Minamoto, T. (2019). The Release Rate of
 1175 Environmental DNA from Juvenile and Adult Fish (vol 9, e114639, 2014). *Plos One*, 14.
- 1176 Maruyama, A., Sugatani, K., Watanabe, K., Yamanaka, H. & Imamura, A. (2018). Environmental DNA
 1177 analysis as a non-invasive quantitative tool for reproductive migration of a threatened endemic
 1178 fish in rivers. *Ecol Evol*, 8, 11964-11974.
- 1179 Mata, V.A., Amorim, F., Corley, M.F.V., McCracken, G.F., Rebelo, H. & Beja, P. (2016). Female dietary bias
 1180 towards large migratory moths in the European free-tailed bat (*Tadarida teniotis*). *Biol Letters*,
 1181 12.
- 1182 Mata, V.A., Rebelo, H., Amorim, F., McCracken, G.F., Jarman, S. & Beja, P. (2019). How much is enough?
 1183 Effects of technical and biological replication on metabarcoding dietary analysis. *Mol Ecol*, 28,
 1184 165-175.
- 1185 Matter, A.N., Falke, J.A., Lopez, J.A. & Saveriede, J.W. (2018). A Rapid-Assessment Method to Estimate
 1186 the Distribution of Juvenile Chinook Salmon in Tributary Habitats Using eDNA and Occupancy
 1187 Estimation. *N Am J Fish Manage*, 38, 223-236.
- 1188 Minamoto, T., Uchii, K., Takahara, T., Kitayoshi, T., Tsuji, S., Yamanaka, H. *et al.* (2017). Nuclear internal
 1189 transcribed spacer-1 as a sensitive genetic marker for environmental DNA studies in common
 1190 carp *Cyprinus carpio*. *Mol Ecol Resour*, 17, 324-333.
- 1191 Minamoto, T., Yamanaka, H., Takahara, T., Honjo, M.N. & Kawabata, Z. (2012). Surveillance of fish
 1192 species composition using environmental DNA. *Limnology*, 13, 193-197.
- 1193 Montagna, M., Berruti, A., Bianciotto, V., Cremonesi, P., Giannico, R., Gusmeroli, F. *et al.* (2018).
 1194 Differential biodiversity responses between kingdoms (plants, fungi, bacteria and metazoa)
 1195 along an Alpine succession gradient. *Mol Ecol*, 27, 3671-3685.
- 1196 Mora, A.J., Prosse, S.W.J. & Mora, J.A. (2019). DNA metabarcoding allows non-invasive identification of
 1197 arthropod prey provisioned to nestling Rufous hummingbirds (*Selasphorus rufus*). *Peerj*, 7.
- 1198 Moraes, C.T. (2001). What regulates mitochondrial DNA copy number in animal cells? *Trends Genet*, 17,
 1199 199-205.
- 1200 Morin, P.A., Chambers, K.E., Boesch, C. & Vigilant, L. (2001). Quantitative polymerase chain reaction
 1201 analysis of DNA from noninvasive samples for accurate microsatellite genotyping of wild
 1202 chimpanzees (*Pan troglodytes verus*). *Mol Ecol*, 10, 1835-1844.
- 1203 Morley, S.A. & Nielsen, B.L. (2016). Chloroplast DNA Copy Number Changes during Plant Development in
 1204 Organelle DNA Polymerase Mutants. *Front Plant Sci*, 7.

- 1205 Moushomi, R., Wilgar, G., Carvalho, G., Creer, S. & Seymour, M. (2019). Environmental DNA size sorting
1206 and degradation experiment indicates the state of *Daphnia magna* mitochondrial and nuclear
1207 eDNA is subcellular. *Scientific Reports*, 9, 12500.
- 1208 Moyer, G.R., Diaz-Ferguson, E., Hill, J.E. & Shea, C. (2014). Assessing Environmental DNA Detection in
1209 Controlled Lentic Systems. *Plos One*, 9.
- 1210 Muha, T.P., Robinson, C.V., Garcia de Leaniz, C. & Consuegra, S. (2019). An optimised eDNA protocol for
1211 detecting fish in lentic and lotic freshwaters using a small water volume. *PLOS ONE*, 14,
1212 e0219218.
- 1213 Murakami, H., Yoon, S., Kasai, A., Minamoto, T., Yamamoto, S., Sakata, M.K. *et al.* (2019). Dispersion and
1214 degradation of environmental DNA from caged fish in a marine environment. *Fisheries Sci*, 85,
1215 327-337.
- 1216 Nardi, C.F., Fernandez, D.A., Vanella, F.A. & Chalde, T. (2019). The expansion of exotic Chinook salmon
1217 (*Oncorhynchus tshawytscha*) in the extreme south of Patagonia: an environmental DNA
1218 approach. *Biol Invasions*, 21, 1415-1425.
- 1219 Nichols, R.V., Vollmers, C., Newsom, L.A., Wang, Y., Heintzman, P.D., Leighton, M. *et al.* (2018).
1220 Minimizing polymerase biases in metabarcoding. *Mol Ecol Resour*, 18, 927-939.
- 1221 O'Donnell, J.L., Kelly, R.P., Shelton, A.O., Samhouri, J.F., Lowell, N.C. & Williams, G.D. (2017). Spatial
1222 distribution of environmental DNA in a nearshore marine habitat. *Peerj*, 5.
- 1223 O'Donnell, J.L., Kelly, R.P., Lowell, N.C. & Port, J.A. (2016). Indexed PCR Primers Induce Template-Specific
1224 Bias in Large-Scale DNA Sequencing Studies. *PLOS ONE*, 11, e0148698.
- 1225 O'Donnell, J.L., Kelly, R.P., Shelton, A.O., Samhouri, J.F., Lowell, N.C. & Williams, G.D. (2017). Spatial
1226 distribution of environmental DNA in a nearshore marine habitat. *Peerj*, 5, e3044.
- 1227 Olds, B.P., Jerde, C.L., Renshaw, M.A., Li, Y.Y., Evans, N.T., Turner, C.R. *et al.* (2016). Estimating species
1228 richness using environmental DNA. *Ecol Evol*, 6, 4214-4226.
- 1229 Olson, Z.H., Briggler, J.T. & Williams, R.N. (2012a). An eDNA approach to detect eastern hellbenders
1230 (*Cryptobranchus a. alleganiensis*) using samples of water. *Wildlife Research*, 39, 629-636,
1231 628.
- 1232 Olson, Z.H., Briggler, J.T. & Williams, R.N. (2012b). An eDNA approach to detect eastern hellbenders
1233 (*Cryptobranchus a. alleganiensis*) using samples of water. *Wildlife Res*, 39, 629-636.
- 1234 Orzechowski, S.C.M., Frederick, P.C., Dorazio, R.M. & Hunter, M.E. (2019). Environmental DNA sampling
1235 reveals high occupancy rates of invasive Burmese pythons at wading bird breeding aggregations
1236 in the central Everglades. *Plos One*, 14.
- 1237 Outhwaite, C.L., Gregory, R.D., Chandler, R.E., Collen, B. & Isaac, N.J.B. (2020). Complex long-term
1238 biodiversity change among invertebrates, bryophytes and lichens. *Nature Ecology & Evolution*, 4,
1239 384-392.
- 1240 Ovaskainen, O., Moliterno de Camargo, U. & Somervuo, P. (2018). Animal Sound Identifier (ASI):
1241 software for automated identification of vocal animals. *Ecol Lett*, 21, 1244-1254.
- 1242 Piaggio, A.J., Engeman, R.M., Hopken, M.W., Humphrey, J.S., Keacher, K.L., Bruce, W.E. *et al.* (2014).
1243 Detecting an elusive invasive species: a diagnostic PCR to detect Burmese python in Florida
1244 waters and an assessment of persistence of environmental DNA. *Mol Ecol Resour*, 14, 374-380.
- 1245 Piggott, M.P. (2016). Evaluating the effects of laboratory protocols on eDNA detection probability for an
1246 endangered freshwater fish. *Ecol Evol*, 6, 2739-2750.
- 1247 Pilliod, D.S., Goldberg, C.S., Arkle, R.S. & Waits, L.P. (2013). Estimating occupancy and abundance of
1248 stream amphibians using environmental DNA from filtered water samples. *Can J Fish Aquat Sci*,
1249 70, 1123-1130.
- 1250 Pilliod, D.S., Goldberg, C.S., Arkle, R.S. & Waits, L.P. (2014). Factors influencing detection of eDNA from a
1251 stream-dwelling amphibian. *Mol Ecol Resour*, 14, 109-116.

- 1252 Piñol, J., Senar, M.A. & Symondson, W.O.C. (2019). The choice of universal primers and the
 1253 characteristics of the species mixture determine when DNA metabarcoding can be quantitative.
 1254 *Mol Ecol*, 28, 407-419.
- 1255 Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D.S., Jarman, S.N. & Taberlet, P. (2012). Who is
 1256 eating what: diet assessment using next generation sequencing. *Mol Ecol*, 21, 1931-1950.
- 1257 Porter, T.M. & Hajibabaei, M. (2018). Over 2.5 million COI sequences in GenBank and growing. *PLOS*
 1258 *ONE*, 13, e0200177.
- 1259 Preissler, K., Watzal, A.D., Vences, M. & Steinfartz, S. (2019). Detection of elusive fire salamander larvae
 1260 (*Salamandra salamandra*) in streams via environmental DNA. *Amphibia-Reptilia*, 40, 55-64.
- 1261 Prince, A.M. & Andrus, L. (1992). Pcr - How to Kill Unwanted DNA. *Biotechniques*, 12, 358-&.
- 1262 Qu, C. & Stewart, K.A. (2019a). Evaluating monitoring options for conservation: comparing traditional
 1263 and environmental DNA tools for a critically endangered mammal. *The Science of Nature*, 106, 9.
- 1264 Qu, C. & Stewart, K.A. (2019b). Evaluating monitoring options for conservation: comparing traditional
 1265 and environmental DNA tools for a critically endangered mammal. *Sci Nat-Heidelberg*, 106.
- 1266 Radstrom, P., Knutsson, R., Wolffs, P., Lovenklev, M. & Lofstrom, C. (2004). Pre-PCR processing -
 1267 Strategies to generate PCR-compatible samples. *Mol Biotechnol*, 26, 133-146.
- 1268 Rajan, S.C., Athira, K., Jaishanker, R., Sooraj, N.P. & Sarojkumar, V. (2019). Rapid assessment of
 1269 biodiversity using acoustic indices. *Biodivers Conserv*, 28, 2371-2383.
- 1270 Ratnasingham, S. & Hebert, P.D. (2007). bold: The Barcode of Life Data System
 1271 (<http://www.barcodinglife.org>). *Mol Ecol Notes*, 7, 355-364.
- 1272 Rees, H.C., Baker, C.A., Gardner, D.S., Maddison, B.C. & Gough, K.C. (2017). The detection of great
 1273 crested newts year round via environmental DNA analysis. *BMC Research Notes*, 10, 327.
- 1274 Reinhardt, T., van Schingen, M., Windisch, H.S., Nguyen, T.Q., Ziegler, T. & Fink, P. (2019). Monitoring a
 1275 loss: Detection of the semi-aquatic crocodile lizard (*Shinisaurus crocodilurus*) in inaccessible
 1276 habitats via environmental DNA. *Aquat Conserv*, 29, 353-360.
- 1277 Renshaw, M.A., Olds, B.P., Jerde, C.L., McVeigh, M.M. & Lodge, D.M. (2015a). The room temperature
 1278 preservation of filtered environmental DNA samples and assimilation into a phenol-chloroform-
 1279 isoamyl alcohol DNA extraction. *Mol Ecol Resour*, 15, 168-176.
- 1280 Renshaw, M.A., Olds, B.P., Jerde, C.L., McVeigh, M.M. & Lodge, D.M. (2015b). The room temperature
 1281 preservation of filtered environmental DNA samples and assimilation into a phenol-chloroform-
 1282 isoamyl alcohol DNA extraction. *Molecular Ecology Resources*, 15, 168-176.
- 1283 Rice, C.J., Larson, E.R. & Taylor, C.A. (2018). Environmental DNA detects a rare large river crayfish but
 1284 with little relation to local abundance. *Freshwater Biol*, 63, 443-455.
- 1285 Riggio, J., Kija, H., Masenga, E., Mbwilo, F., Van de Perre, F. & Caro, T. (2018). Sensitivity of Africa's larger
 1286 mammals to humans. *J Nat Conserv*, 43, 136-145.
- 1287 Rivera, S.F., Vasselon, V., Bouchez, A. & Rimet, F. (2020). Diatom metabarcoding applied to large scale
 1288 monitoring networks: Optimization of bioinformatics strategies using Mothur software. *Ecol*
 1289 *Indic*, 109, 105775.
- 1290 Rodriguez-Estrella, R., Estrada, C.G., Alvarez-Castaneda, S.T. & Ferrer-Sanchez, Y. (2019). Comparing
 1291 individual raptor species and coarse taxonomic groups as biodiversity surrogates in desert
 1292 ecosystems. *Biodivers Conserv*, 28, 1225-1244.
- 1293 Rose, J.P., Wademan, C., Weir, S., Wood, J.S. & Todd, B.D. (2019). Traditional trapping methods
 1294 outperform eDNA sampling for introduced semi-aquatic snakes. *Plos One*, 14.
- 1295 Roussel, J.-M., Paillisson, J.-M., Tréguier, A. & Petit, E. (2015). The downside of eDNA as a survey tool in
 1296 water bodies. *Journal of Applied Ecology*, 52, 823-826.
- 1297 Rudko, S.P., Turnbull, A., Reimink, R.L., Froelich, K. & Hanington, P.C. (2019). Species-specific qPCR
 1298 assays allow for high-resolution population assessment of four species avian schistosome that

1299 cause swimmer's itch in recreational lakes. *International journal for parasitology. Parasites and*
1300 *wildlife*, 9, 122-129.

1301 Rytkonen, S., Vesterinen, E.J., Westerduin, C., Leviakangas, T., Vatka, E., Mutanen, M. *et al.* (2019). From
1302 feces to data: A metabarcoding method for analyzing consumed and available prey in a bird-
1303 insect food web. *Ecol Evol*, 9, 631-639.

1304 Saha, A., McRae, L., Dodd, C.K., Gadsden, H., Hare, K.M., Lukoschek, V. *et al.* (2018). Tracking Global
1305 Population Trends: Population Time-Series Data and a Living Planet Index for Reptiles. *J Herpetol*,
1306 52, 259-268.

1307 Sales, N.G., Wangenstein, O.S., Carvalho, D.C., Deiner, K., Præbel, K., Coscia, I. *et al.* (2020). Space-time
1308 dynamics in monitoring neotropical fish communities using eDNA metabarcoding. *bioRxiv*,
1309 2020.2002.2004.933366.

1310 Sansom, B.J. & Sassoubre, L.M. (2017). Environmental DNA (eDNA) Shedding and Decay Rates to Model
1311 Freshwater Mussel eDNA Transport in a River. *Environ Sci Technol*, 51, 14244-14253.

1312 Sassoubre, L.M., Yamahara, K.M., Gardner, L.D., Block, B.A. & Boehm, A.B. (2016). Quantification of
1313 Environmental DNA (eDNA) Shedding and Decay Rates for Three Marine Fish. *Environ Sci*
1314 *Technol*, 50, 10456-10464.

1315 Sawaya, N.A., Djurhuus, A., Closek, C.J., Hepner, M., Olesin, E., Visser, L. *et al.* (2019). Assessing
1316 eukaryotic biodiversity in the Florida Keys National Marine Sanctuary through environmental
1317 DNA metabarcoding. *Ecol Evol*, 9, 1029-1040.

1318 Schmidt, B.R., Kery, M., Ursenbacher, S., Hyman, O.J. & Collins, J.P. (2013). Site occupancy models in the
1319 analysis of environmental DNA presence/absence surveys: a case study of an emerging
1320 amphibian pathogen. *Methods Ecol Evol*, 4, 646-653.

1321 Schrader, C., Schielke, A., Ellerbroek, L. & Johne, R. (2012). PCR inhibitors – occurrence, properties and
1322 removal. *J Appl Microbiol*, 113, 1014-1026.

1323 Schumer, G., Crowley, K., Maltz, E., Johnston, M., Anders, P. & Blankenship, S. (2019). Utilizing
1324 environmental DNA for fish eradication effectiveness monitoring in streams. *Biol Invasions*, 21,
1325 3415-3426.

1326 Sekercioglu, C.H., Mendenhall, C.D., Oviedo-Brenes, F., Horns, J.J., Ehrlich, P.R. & Daily, G.C. (2019).
1327 Long-term declines in bird populations in tropical agricultural countryside. *P Natl Acad Sci USA*,
1328 116, 9903-9912.

1329 Sengupta, M.E., Hellstrom, M., Kariuki, H.C., Olsen, A., Thomsen, P.F., Mejer, H. *et al.* (2019).
1330 Environmental DNA for improved detection and environmental surveillance of schistosomiasis. *P*
1331 *Natl Acad Sci USA*, 116, 8931-8940.

1332 Seymour, M. (2019). Rapid progression and future of environmental DNA research. *Communications*
1333 *Biology*, 2, 80.

1334 Seymour, M., Durance, I., Cosby, B.J., Ransom-Jones, E., Deiner, K., Ormerod, S.J. *et al.* (2018). Acidity
1335 promotes degradation of multi-species environmental DNA in lotic mesocosms. *Communications*
1336 *Biology*, 1, 4.

1337 Shelton, A.O., Kelly, R.P., O'Donnell, J.L., Park, L., Schwenke, P., Greene, C. *et al.* (2019). Environmental
1338 DNA provides quantitative estimates of a threatened salmon species. *Biol Conserv*, 237, 383-391.

1339 Sigsgaard, E.E., Carl, H., Møller, P.R. & Thomsen, P.F. (2015a). Monitoring the near-extinct European
1340 weather loach in Denmark based on environmental DNA from water samples. *Biological*
1341 *Conservation*, 183, 46-52.

1342 Sigsgaard, E.E., Carl, H., Møller, P.R. & Thomsen, P.F. (2015b). Monitoring the near-extinct European
1343 weather loach in Denmark based on environmental DNA from water samples. *Biological*
1344 *Conservation*, 183, 46-52.

- 1345 Singer, D., Kosakyan, A., Seppey, C.V.W., Pillonel, A., Fernández, L.D., Fontaneto, D. *et al.* (2018).
 1346 Environmental filtering and phylogenetic clustering correlate with the distribution patterns of
 1347 cryptic protist species. *Ecology*, 99, 904-914.
- 1348 Singer, G.A.C., Fahner, N.A., Barnes, J.G., McCarthy, A. & Hajibabaei, M. (2019). Comprehensive
 1349 biodiversity analysis via ultra-deep patterned flow cell technology: a case study of eDNA
 1350 metabarcoding seawater. *Scientific Reports*, 9, 5991.
- 1351 Sirois, S.H. & Buckley, D.H. (2019). Factors governing extracellular DNA degradation dynamics in soil.
 1352 *Environmental Microbiology Reports*, 11, 173-184.
- 1353 Smart, A.S., Weeks, A.R., van Rooyen, A.R., Moore, A., McCarthy, M.A. & Tingley, R. (2016). Assessing
 1354 the cost-efficiency of environmental DNA sampling. *Methods Ecol Evol*, 7, 1291-1298.
- 1355 Song, J.W., Small, M.J. & Casman, E.A. (2017). Making sense of the noise: The effect of hydrology on
 1356 silver carp eDNA detection in the Chicago area waterway system. *Sci Total Environ*, 605, 713-720.
- 1357 Spear, S.F., Groves, J.D., Williams, L.A. & Waits, L.P. (2015). Using environmental DNA methods to
 1358 improve detectability in a hellbender (*Cryptobranchus alleganiensis*) monitoring program. *Biol
 1359 Conserv*, 183, 38-45.
- 1360 Spens, J., Evans, A.R., Halfmaerten, D., Knudsen, S.W., Sengupta, M.E., Mak, S.S.T. *et al.* (2017).
 1361 Comparison of capture and storage methods for aqueous microbial eDNA using an optimized
 1362 extraction protocol: advantage of enclosed filter. *Methods in Ecology and Evolution*, 8, 635-645.
- 1363 Srinivasan, U. (2019). Morphological and Behavioral Correlates of Long-Term Bird Survival in Selectively
 1364 Logged Forest. *Frontiers in Ecology and Evolution*, 7.
- 1365 Stadhouders, R., Pas, S.D., Anber, J., Voermans, J., Mes, T.H.M. & Schutten, M. (2010). The effect of
 1366 primer-template mismatches on the detection and quantification of nucleic acids using the 5'
 1367 nuclease assay. *J Mol Diagn*, 12, 109-117.
- 1368 Steenweg, R., Hebblewhite, M., Kays, R., Ahumada, J., Fisher, J.T., Burton, C. *et al.* (2017). Scaling-up
 1369 camera traps: monitoring the planet's biodiversity with networks of remote sensors. *Front Ecol
 1370 Environ*, 15, 26-34.
- 1371 Stewart, K., Ma, H.J., Zheng, J.S. & Zhao, J.F. (2017). Using environmental DNA to assess population-wide
 1372 spatiotemporal reserve use. *Conserv Biol*, 31, 1173-1182.
- 1373 Stewart, K.A. (2019). Understanding the effects of biotic and abiotic factors on sources of aquatic
 1374 environmental DNA. *Biodiversity and Conservation*, 28, 983-1001.
- 1375 Stoeckle, B.C., Kuehn, R. & Geist, J. (2016). Environmental DNA as a monitoring tool for the endangered
 1376 freshwater pearl mussel (*Margaritifera margaritifera* L.): a substitute for classical monitoring
 1377 approaches? *Aquat Conserv*, 26, 1120-1129.
- 1378 Stoeckle, M.Y., Soboleva, L. & Charlop-Powers, Z. (2017). Aquatic environmental DNA detects seasonal
 1379 fish abundance and habitat preference in an urban estuary. *Plos One*, 12.
- 1380 Strickland, G.J. & Roberts, J.H. (2019). Utility of eDNA and occupancy models for monitoring an
 1381 endangered fish across diverse riverine habitats. *Hydrobiologia*, 826, 129-144.
- 1382 Strickler, K.M., Fremier, A.K. & Goldberg, C.S. (2015). Quantifying effects of UV-B, temperature, and pH
 1383 on eDNA degradation in aquatic microcosms. *Biological Conservation*, 183, 85-92.
- 1384 Sutter, M. & Kinziger, A.P. (2019). Rangewide tidewater goby occupancy survey using environmental
 1385 DNA. *Conserv Genet*, 20, 597-613.
- 1386 TABERLET, P., COISSAC, E., HAJIBABAEI, M. & RIESEBERG, L.H. (2012a). Environmental DNA. *Mol Ecol*, 21,
 1387 1789-1793.
- 1388 Taberlet, P., Prud'homme, S.M., Campione, E., Roy, J., Miquel, C., Shehzad, W. *et al.* (2012b). Soil
 1389 sampling and isolation of extracellular DNA from large amount of starting material suitable for
 1390 metabarcoding studies. *Mol Ecol*, 21, 1816-1820.

- 1391 Takahara, T., Iwai, N., Yasumiba, K. & Igawa, T. Comparison of the detection of 3 endangered frog
1392 species by eDNA and acoustic surveys across 3 seasons. *Freshwater Science*, 0, 000-000.
- 1393 Takahara, T., Iwai, N., Yasumiba, K. & Igawa, T. (2020). Comparison of the detection of 3 endangered
1394 frog species by eDNA and acoustic surveys across 3 seasons. *Freshw Sci*, 39, 18-27.
- 1395 Takahara, T., Minamoto, T., Yamanaka, H., Doi, H. & Kawabata, Z. (2012). Estimation of Fish Biomass
1396 Using Environmental DNA. *Plos One*, 7.
- 1397 Takeuchi, A., Iijima, T., Kakuzen, W., Watanabe, S., Yamada, Y., Okamura, A. *et al.* (2019a). Release of
1398 eDNA by different life history stages and during spawning activities of laboratory-reared
1399 Japanese eels for interpretation of oceanic survey data. *Scientific Reports*, 9, 6074.
- 1400 Takeuchi, A., Iijima, T., Kakuzen, W., Watanabe, S., Yamada, Y., Okamura, A. *et al.* (2019b). Release of
1401 eDNA by different life history stages and during spawning activities of laboratory-reared
1402 Japanese eels for interpretation of oceanic survey data. *Sci Rep-Uk*, 9.
- 1403 Takeuchi, A., Watanabe, S., Yamamoto, S., Miller, M.J., Fukuba, T., Miwa, T. *et al.* (2019c). First use of
1404 oceanic environmental DNA to study the spawning ecology of the Japanese eel *Anguilla japonica*.
1405 *Mar Ecol Prog Ser*, 609, 187-196.
- 1406 Thomas, A.C., Howard, J., Nguyen, P.L., Seimon, T.A. & Goldberg, C.S. (2018). ANDe (TM): A fully
1407 integrated environmental DNA sampling system. *Methods Ecol Evol*, 9, 1379-1385.
- 1408 Thomas, A.C., Nguyen, P.L., Howard, J. & Goldberg, C.S. (2019). A self-preserving, partially biodegradable
1409 eDNA filter. *Methods in Ecology and Evolution*, 10, 1136-1141.
- 1410 Thomsen, P.F., Kielgast, J., Iversen, L.L., Møller, P.R., Rasmussen, M. & Willerslev, E. (2012a). Detection
1411 of a Diverse Marine Fish Fauna Using Environmental DNA from Seawater Samples. *Plos One*, 7.
- 1412 Thomsen, P.F., Kielgast, J., Iversen, L.L., Møller, P.R., Rasmussen, M. & Willerslev, E. (2012b). Detection
1413 of a Diverse Marine Fish Fauna Using Environmental DNA from Seawater Samples. *PLOS ONE*, 7,
1414 e41732.
- 1415 Thomsen, P.F., Kielgast, J., Iversen, L.L., Wiuf, C., Rasmussen, M., Gilbert, M.T.P. *et al.* (2012c).
1416 Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology*,
1417 21, 2565-2573.
- 1418 Thomsen, P.F. & Sigsgaard, E.E. (2019). Environmental DNA metabarcoding of wild flowers reveals
1419 diverse communities of terrestrial arthropods. *Ecol Evol*, 9, 1665-1679.
- 1420 Thomsen, P.F. & Willerslev, E. (2015). Environmental DNA - An emerging tool in conservation for
1421 monitoring past and present biodiversity. *Biol Conserv*, 183, 4-18.
- 1422 Tillotson, M.D., Kelly, R.P., Duda, J.J., Hoy, M., Kralj, J. & Quinn, T.P. (2018). Concentrations of
1423 environmental DNA (eDNA) reflect spawning salmon abundance at fine spatial and temporal
1424 scales. *Biol Conserv*, 220, 1-11.
- 1425 Tingley, R., Greenlees, M., Oertel, S., van Rooyen, A.R. & Weeks, A.R. (2019). Environmental DNA
1426 sampling as a surveillance tool for cane toad *Rhinella marina* introductions on offshore islands.
1427 *Biol Invasions*, 21, 1-6.
- 1428 Togaki, D., Doi, H. & Katano, I. (2020). Detection of freshwater mussels (*Sinanodonta* spp.) in artificial
1429 ponds through environmental DNA: a comparison with traditional hand collection methods.
1430 *Limnology*, 21, 59-65.
- 1431 Trebitz, A.S., Hoffman, J.C., Darling, J.A., Pilgrim, E.M., Kelly, J.R., Brown, E.A. *et al.* (2017). Early
1432 detection monitoring for aquatic non-indigenous species: Optimizing surveillance, incorporating
1433 advanced technologies, and identifying research needs. *J Environ Manage*, 202, 299-310.
- 1434 Tsuji, S., Ushio, M., Sakurai, S., Minamoto, T. & Yamanaka, H. (2017). Water temperature-dependent
1435 degradation of environmental DNA and its relation to bacterial abundance. *PLOS ONE*, 12,
1436 e0176608.

- 1437 Tsukamoto, K., Chow, S., Otake, T., Kurogi, H., Mochioka, N., Miller, M.J. *et al.* (2011). Oceanic spawning
1438 ecology of freshwater eels in the western North Pacific. *Nat Commun*, 2, 179.
- 1439 Turner, C.R., Barnes, M.A., Xu, C.C.Y., Jones, S.E., Jerde, C.L. & Lodge, D.M. (2014a). Particle size
1440 distribution and optimal capture of aqueous microbial eDNA. *Methods Ecol Evol*, 5, 676-684.
- 1441 Turner, C.R., Miller, D.J., Coyne, K.J. & Corush, J. (2014b). Improved Methods for Capture, Extraction,
1442 and Quantitative Assay of Environmental DNA from Asian Bigheaded Carp (*Hypophthalmichthys*
1443 spp.). *Plos One*, 9.
- 1444 Turner, C.R., Uy, K.L. & Everhart, R.C. (2015). Fish environmental DNA is more concentrated in aquatic
1445 sediments than surface water. *Biol Conserv*, 183, 93-102.
- 1446 Ulibarri, R.M., Bonar, S.A., Rees, C., Amberg, J., Ladell, B. & Jackson, C. (2017). Comparing Efficiency of
1447 American Fisheries Society Standard Snorkeling Techniques to Environmental DNA Sampling
1448 Techniques. *N Am J Fish Manage*, 37, 644-651.
- 1449 Unnithan, V.V., Unc, A., Joe, V. & Smith, G.B. (2014). Short RNA indicator sequences are not completely
1450 degraded by autoclaving. *Scientific reports*, 4, 4070-4070.
- 1451 Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P.F. *et al.* (2016a). Next-generation
1452 monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Mol Ecol*, 25, 929-
1453 942.
- 1454 Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P.F. *et al.* (2016b). Next-generation
1455 monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Mol Ecol*, 25, 929-
1456 942.
- 1457 van der Heyde, M., Bunce, M., Wardell-Johnson, G., Fernandes, K., White, N.E. & Nevill, P. Testing
1458 multiple substrates for terrestrial biodiversity monitoring using environmental DNA (eDNA)
1459 metabarcoding. *Mol Ecol Resour*, n/a.
- 1460 van der Heyde, M., Bunce, M., Wardell-Johnson, G., Fernandes, K., White, N.E. & Nevill, P. Testing
1461 multiple substrates for terrestrial biodiversity monitoring using environmental DNA
1462 metabarcoding. *Mol Ecol Resour*, n/a.
- 1463 Vimercati, G., Labadesse, M., Dejean, T. & Secondi, J. (2020). Assessing the effect of landscape features
1464 on pond colonisation by an elusive amphibian invader using environmental DNA. *Freshwater*
1465 *Biol*, 65, 502-513.
- 1466 Voros, J., Marton, O., Schmidt, B.R., Gal, J.T. & Jelic, D. (2017). Surveying Europe's Only Cave-Dwelling
1467 Chordate Species (*Proteus anguinus*) Using Environmental DNA. *Plos One*, 12.
- 1468 Walsh, J.R., Spear, M.J., Shannon, T.P., Krysan, P.J. & Vander Zanden, M.J. (2019). Using eDNA, sediment
1469 subfossils, and zooplankton nets to detect invasive spiny water flea (*Bythotrephes longimanus*).
1470 *Biol Invasions*, 21, 377-389.
- 1471 Watson, R.J. & Blackwell, B. (2000). Purification and characterization of a common soil component which
1472 inhibits the polymerase chain reaction. *Can J Microbiol*, 46, 633-642.
- 1473 Wei, N., Nakajima, F. & Tobino, T. (2018). Effects of treated sample weight and DNA marker length on
1474 sediment eDNA based detection of a benthic invertebrate. *Ecol Indic*, 93, 267-273.
- 1475 Weltz, K., Lyle, J.M., Ovenden, J., Morgan, J.A.T., Moreno, D.A. & Semmens, J.M. (2017). Application of
1476 environmental DNA to detect an endangered marine skate species in the wild. *Plos One*, 12.
- 1477 Wilcox, T.M., McKelvey, K.S., Young, M.K., Jane, S.F., Lowe, W.H., Whiteley, A.R. *et al.* (2013). Robust
1478 detection of rare species using environmental DNA: the importance of primer specificity. *Plos*
1479 *One*, 8, e59520-e59520.
- 1480 Wilcox, T.M., McKelvey, K.S., Young, M.K., Sepulveda, A.J., Shepard, B.B., Jane, S.F. *et al.* (2016).
1481 Understanding environmental DNA detection probabilities: A case study using a stream-dwelling
1482 char *Salvelinus fontinalis*. *Biol Conserv*, 194, 209-216.

- 1483 Wilcox, T.M., Zarn, K.E., Piggott, M.P., Young, M.K., McKelvey, K.S. & Schwartz, M.K. (2018). Capture
 1484 enrichment of aquatic environmental DNA: A first proof of concept. *Mol Ecol Resour*, 18, 1392-
 1485 1401.
- 1486 Williams, K.E., Huyvaert, K.P. & Piaggio, A.J. (2016). No filters, no fridges: a method for preservation of
 1487 water samples for eDNA analysis. *BMC Research Notes*, 9, 298.
- 1488 Willoughby, J.R., Wijayawardena, B.K., Sundaram, M., Swihart, R.K. & Dewoody, J.A. (2016). The
 1489 importance of including imperfect detection models in eDNA experimental design. *Mol Ecol*
 1490 *Resour*, 16, 837-844.
- 1491 Wilson, C.C., Wozney, K.M. & Smith, C.M. (2016). Recognizing false positives: synthetic oligonucleotide
 1492 controls for environmental DNA surveillance. *Methods Ecol Evol*, 7, 23-29.
- 1493 Wineland, S.M., Welch, S.M., Pauley, T.K., Apodaca, J.J., Olszack, M., Mosher, J.J. *et al.* (2019). Using
 1494 environmental DNA and occupancy modelling to identify drivers of eastern hellbender
 1495 (*Cryptobranchus alleganiensis alleganiensis*) extirpation. *Freshwater Biol*, 64, 208-221.
- 1496 Wood, S.A., Pochon, X., Ming, W., von Ammon, U., Woods, C., Carter, M. *et al.* (2019). Considerations for
 1497 incorporating real-time PCR assays into routine marine biosecurity surveillance programmes: a
 1498 case study targeting the Mediterranean fanworm (*Sabella spallanzanii*) and club tunicate (*Styela*
 1499 *clava*). *Genome*, 62, 137-146.
- 1500 Wu, Q.Q., Kawano, K., Uehara, Y., Okuda, N., Hongo, M., Tsuji, S. *et al.* (2018). Environmental DNA
 1501 reveals nonmigratory individuals of *Palaemon paucidens* overwintering in Lake Biwa shallow
 1502 waters. *Freshw Sci*, 37, 307-314.
- 1503 Xia, Z.Q., Zhan, A.B., Gao, Y.C., Zhang, L., Haffner, G.D. & MacIsaac, H.J. (2018). Early detection of a
 1504 highly invasive bivalve based on environmental DNA (eDNA). *Biol Invasions*, 20, 437-447.
- 1505 Xiong, W. & Zhan, A.B. (2018). Testing clustering strategies for metabarcoding-based investigation of
 1506 community-environment interactions. *Mol Ecol Resour*, 18, 1326-1338.
- 1507 Yamahara, K.M., Preston, C.M., Birch, J., Walz, K., Marin, R., Jensen, S. *et al.* (2019). In situ Autonomous
 1508 Acquisition and Preservation of Marine Environmental DNA Using an Autonomous Underwater
 1509 Vehicle. *Frontiers in Marine Science*, 6.
- 1510 Yamamoto, S., Masuda, R., Sato, Y., Sado, T., Araki, H., Kondoh, M. *et al.* (2017). Environmental DNA
 1511 metabarcoding reveals local fish communities in a species-rich coastal sea. *Sci Rep-Uk*, 7.
- 1512 Yamanaka, H., Minamoto, T., Matsuura, J., Sakurai, S., Tsuji, S., Motozawa, H. *et al.* (2017). A simple
 1513 method for preserving environmental DNA in water samples at ambient temperature by
 1514 addition of cationic surfactant. *Limnology*, 18, 233-241.
- 1515 Yang, J. & Zhang, X. (2020). eDNA metabarcoding in zooplankton improves the ecological status
 1516 assessment of aquatic ecosystems. *Environment International*, 134, 105230.
- 1517 Yoccoz, N.G., Brathen, K.A., Gielly, L., Haile, J., Edwards, M.E., Goslar, T. *et al.* (2012). DNA from soil
 1518 mirrors plant taxonomic and growth form diversity. *Mol Ecol*, 21, 3647-3655.
- 1519 Young, M.R., Proctor, H.C., deWaard, J.R. & Hebert, P.D.N. DNA Barcodes Expose Unexpected Diversity
 1520 in Canadian Mites. *Mol Ecol*, 0.
- 1521 Zeale, M.R.K., Butlin, R.K., Barker, G.L.A., Lees, D.C. & Jones, G. (2011). Taxon-specific PCR for DNA
 1522 barcoding arthropod prey in bat faeces. *Mol Ecol Resour*, 11, 236-244.
- 1523 Zhang, S., Lu, Q., Wang, Y., Wang, X., Zhao, J. & Yao, M. (2020a). Assessment of fish communities using
 1524 environmental DNA: Effect of spatial sampling design in lentic systems of different sizes. *Mol*
 1525 *Ecol Resour*, 20, 242-255.
- 1526 Zhang, Y., Pavlovska, M., Stoica, E., Prekrasna, I., Yang, J., Slobodnik, J. *et al.* (2020b). Holistic pelagic
 1527 biodiversity monitoring of the Black Sea via eDNA metabarcoding approach: From bacteria to
 1528 marine mammals. *Environment International*, 135, 105307.

- 1529 Zhou, X., Li, Y., Liu, S., Yang, Q., Su, X., Zhou, L. *et al.* (2013). Ultra-deep sequencing enables high-fidelity
1530 recovery of biodiversity for bulk arthropod samples without PCR amplification. *Gigascience*, 2, 4.
1531 Zinger, L., Taberlet, P., Schimann, H., Bonin, A., Boyer, F., De Barba, M. *et al.* (2019). Body size
1532 determines soil community assembly in a tropical forest. *Mol Ecol*, 28, 528-543.
- 1533
- 1534