1 Environmental (e)DNA: what's behind the term? Clarifying the terminology and
2 recommendations for its future use in biomonitoring

3 An opinion paper

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Abstract

The last decade brought a spectacular development of so-called environmental (e)DNA studies. In general, “environmental DNA” is defined as DNA isolated from environmental samples, in opposition to genomic DNA that is extracted directly from specimens. However, the variety of different sources of eDNA and the range of taxonomic groups that are targeted by eDNA studies is large, which has led to some discussion about the breadth of the eDNA concept. In particular, there is a recent trend to restrict the use of the term “eDNA” to the DNA of macro-organisms, which are not physically present in environmental samples. In this paper, we argue that such a distinction may not be ideal, because the eDNA signal can come from organisms across the whole tree of life. Consequently, we advocate that the term “eDNA” should be used in its generic sense, as originally defined, encompassing the DNA of all organisms present in environmental samples, including microbial, meiofaunal, and macrobial taxa. We first suggest specifying the environmental origin of the DNA sample, such as water eDNA, sediment eDNA, or soil eDNA. A second specification would then define the taxonomic group targeted through PCR amplification, such as fish eDNA, invertebrate eDNA, bacterial eDNA. This terminology does also not require assumptions about the specific state of the DNA sampled (intracellular or extracellular). We hope that such terminology will help better define the scope of eDNA studies, especially for environmental managers, who use them as reference in routine biomonitoring and bioassessment.

The importance of environmental DNA studies

During the last decade we observe a rapidly increasing number of studies that are using DNA isolated from the environment, especially for aquatic ecosystems, both freshwater and marine. These ecosystems are under immense anthropogenic pressures, and the biodiversity and

associated ecosystem processes and services are heavily and negatively affected (Dudgeon, 2019; Reid et al., 2019). Consequently, effective management is needed, and this itself depends on accurate, timely, and reliable assessments of the state and change of the organismal communities, either by describing their biodiversity or using them for calculating indices as proxies describing the environmental state (Jackson et al., 2016; Pawlowski et al., 2018). A major limitation of past assessment methods, however, was their high cost, methodological diversity across taxonomic groups, as well as the inability to upscale the methods in time and space. Highly resolved biomonitoring data, however, are crucially needed, possibly depending on novel technologies. An example of such an advancement is the use of molecular techniques, and the study of eDNA in particular, which have been proposed to be a game-changer for bioassessment and monitoring of biodiversity (Altermatt et al., 2020; Deiner et al., 2017; Kelly et al., 2014; Pawlowski et al., 2018; Pfrender et al., 2010; Taberlet et al., 2018). Within few years, many studies on bioassessment in aquatic systems started to use and develop eDNA tools. However, the objectives, the methodologies, the source of eDNA, and the organisms targeted by these studies can be very different. In parallel, these novel techniques and the use of eDNA for bioassessment have raised high expectations, especially from stakeholders, and they are progressively implemented in ongoing biodiversity monitoring programs and bioassessment studies (e.g., Herder et al., 2014; Pawlowski et al., 2020; Thomsen & Willerslev, 2015). While many of the expectations are probably realistic, there is also regular misunderstandings and misconceptions on the potential but also limitations (or at least boundary-conditions) of eDNA studies, which is further fuelled by different uses of terminology in the research field itself.

Here, we advocate for a common terminology, which specifies where the DNA comes from (i.e., from which environment), and which organisms are looked for (i.e., which organisms' DNA is targeted with PCR). Our proposed terminology links to the original definition of

“environmental DNA” (Taberlet et al., 2012). It would clarify issues concerning the state of the DNA sampled and would also resolve discussions about the inclusion/exclusion of certain organismal groups based on their size only.

The evolution of the eDNA concept: from microbial to macrobial studies

At the beginning, the technical concept of environmental DNA (eDNA) was used principally to explore microbial diversity. At its basis laid a ground-breaking idea that the analysis of RNA or DNA extracted from environmental samples can be used to assess the natural diversity of microorganisms (Pace et al., 1986). The early studies were based on RNA isolated from environmental samples (Olsen et al., 1986; Stahl et al., 1984). However, very rapidly environmental DNA became the focus of studies on microbial diversity and several papers were published on how to recover DNA from environmental samples (Ogram et al., 1987; Paul & Myers, 1982; Somerville et al., 1989; Steffan et al., 1988). At that time, the authors either used a descriptive term “the DNA isolated from environmental samples” (Somerville et al., 1989) or specifically referred to targeted organisms quoting “bacterial DNA” (Steffan et al., 1988) or “microbial DNA” (Ogram et al., 1987; Paul & Myers, 1982). To our knowledge the term “environmental DNA” was used for the first time by Ogram et al. (1987) in a figure describing the protocol for the isolation of microbial DNA from sediments. Later, Somerville et al. (1989) used it in reference to the work of Pace et al. (1986).

The invention of PCR amplification (Saiki et al., 1988) contributed to the rapid development of studies exploring microbial diversity in environmental samples. These studies totally changed our perception of bacterial diversity, revealing huge numbers of uncultivable species in the ocean (Giovannoni et al., 1990; Schmidt et al., 1991) and in the soil (Picard et al. 1992; Torsvik et al., 1990). Yet, the term “environmental DNA” was only sporadically used in these

early days of bacterial diversity exploration (Suzuki et al., 1997). In parallel, the term was sometimes used to refer to the “free” DNA released by the lysis of dying cells (Kloos et al., 1994), corresponding to the “extracellular DNA”. However, the research focus of this and previous similar studies (Deflaun et al., 1986, Paul et al. 1987) was on the dynamics and biological potential of “extracellular DNA” rather than its use for biodiversity monitoring or bioassessment.

Since 2000, the term “environmental DNA” became much more commonly used (Fig. 1) in a variety of studies and often in a biodiversity context, such as in the description of new environmental microbial phyla (Huber et al., 2002), or the exploration of microbial diversity in the extreme environments (Gordon et al., 2000). The term was also generally adopted in early studies exploring microbial eukaryotes diversity (Bass & Cavalier-Smith, 2004; Berney et al., 2004; Holzmann et al., 2003). In all of these studies, the term did not make a specific assumption about the state of the DNA sampled, and was mostly linked to the microbial organisms being directly sampled. Shortly after, the scope of environmental DNA studies was expanded to the detection of large animals, such as fish or amphibians, whose DNA traces are preserved in water for a certain time, as demonstrated in a seminal paper by Ficetola et al. (2008). This new application constituted a turning point in the research field on environmental DNA, prompting a series of studies using eDNA to monitor biological invasions and/or endangered species in aquatic environments (e.g., Darling & Mahon 2011; Macher et al., 2018; Mächler et al., 2014; Thomsen et al., 2012). In parallel, the development of high-throughput sequencing opened new perspectives to apply the eDNA approach to survey the community of species at very high yield and relatively low cost compared to the traditional cloning approach. Rapid increases of the number of eDNA studies in the first and second decades of the 21st Century called for clarification of eDNA terminology. This was done by Taberlet et al. (2012) in a special issue of *Molecular Ecology*, where eDNA was defined as “DNA that can be

114 *extracted from environmental samples (such as soil, water or air), without first isolating any*
115 *target organisms*". Importantly, this definition does not make assumptions on the state of the
116 DNA sampled (extracellular or intracellular, tissue fragments, gametes, etc.), and is also not
117 restricted to any specific group of organisms. The authors also clarified the differences between
118 DNA barcoding, DNA metabarcoding (referring to analysis of bulk samples), and eDNA
119 metabarcoding (defined as a study that allow identification of multiple taxa using
120 environmental DNA as a template material) (Taberlet et al., 2012). It was also proposed to
121 expand the concept of eDNA and to include DNA extracts from gut content or from faeces,
122 both containing a mixture of genomic DNA from different organisms (Yoccoz et al., 2012).
123 However, this latter suggestion was rarely followed by authors using DNA metabarcoding for
124 diet analysis (Srivathsan et al., 2015).

125 The recent massive increase of eDNA studies applied to conservation biology and biodiversity
126 assessments targeting mainly macro-organisms prompted some authors to redefine
127 environmental DNA as "*a mixture of potentially degraded DNA from many different*
128 *organisms*" (Bohmann et al., 2014; Cristescu, 2014) or "*genetic material obtained directly*
129 *from environmental samples (...) without any obvious signs of biological source material*"
130 (Thomsen & Willerslev, 2015). The latter definition was introduced to highlight the fact that
131 the nature of DNA present as traces in environmental samples is different from the DNA
132 derived from living microorganisms or meiofauna that can be present in eDNA samples
133 (Goldberg et al., 2015). Although the authors assume that the macrobial eDNA exists
134 predominantly inside mitochondria and cells (Turner et al., 2014), they state that part of it may
135 have extracellular origin. The importance of subcellular origin of eDNA has also been
136 suggested by other studies (Moushomi et al. 2019). This creates an additional confusion
137 between the terms "environmental DNA" and "extracellular DNA". The latter is also used for
138 biodiversity surveys (Corinaldesi et al., 2018; Guardiola et al., 2015; Pearman et al., 2016;

Taberlet et al., 2012), and can be abbreviated as “eDNA”, although mainly in studies related to microbial biofilm formation (Harmsen et al., 2010).

Currently, two definitions of environmental DNA are used in ecological studies in parallel. On the one hand, the definition of eDNA *sensu lato* is used in global biodiversity surveys that analyse microbial, meiofauna and macrofauna communities, focusing on their ecological interactions (Deiner et al., 2016; Djurhuus et al., 2020; Zhang et al., 2020) and temporal and spatial dynamics (Altermatt et al., 2020; Bálint et al., 2018; Carraro et al., 2020). Such definition is also commonly used in environmental biomonitoring studies that target different groups of bioindicators to infer or predict biotic indices (Cordier et al., 2018, 2019; Li et al., 2018; Pawlowski et al., 2018; Stoeck et al., 2018). This definition also recognizes that samples of environmental DNA contain both intra- and extracellular DNA of microbial and macrobial species, and that the type of DNA captured may depend on capture method (Deiner et al., 2015). On the other hand, the definition of eDNA *sensu stricto* only referring to (mostly or even exclusively extracellular) DNA of macrobial organisms is especially used in conservation biology to monitor invasive and/or endangered species (Borrell et al., 2018; Lacoursière-Roussel & Deiner, 2019; O’Sullivan & al., 2020), as well as in ecology to survey animal and plants communities and to study biodiversity patterns in aquatic ecosystems (see Deiner et al., 2016; Nguyen et al., 2020). Sometimes, the eDNA concept also includes the DNA extracted from bulk samples (see e.g., Lynggaard et al. 2019, Nielsen et al. 2019, both working on bulk samples but published in a journal dedicated to environmental DNA). Hence, the same term is used in slightly different ways for different types of studies, which can lead to misunderstandings or confusion.

Recommended eDNA terminology

Here, we suggest maintaining the original concept of environmental DNA, defined as **a total pool of DNA isolated from environmental samples** (Taberlet et al., 2012, 2018). This general concept assumes that the environmental DNA is defined primarily by its origin and not by its taxonomic composition nor its specific structural state (intra- or extracellular). Indeed, such definition covers the DNA of various taxonomic origins, including living microorganisms and meiofauna-size taxa, as well as macrofauna traces, possible larval stages or gametes, as well as extracellular DNA. The eDNA can be isolated from various types of material, principally soil, sediment, and water, but also from air, biofilm and organic remains, such as faeces that may contain DNA of different origin. In principle, such definition precludes any type of size-based physical preselection of target taxa, such as sieving or kicknet sampling.

We think that restricting eDNA definition to the traces of large-size macro-organisms, which are not physically present in environmental DNA samples is unnecessary and possibly confusing. First and most importantly from a semantic point of view, such definition refers to the target DNA that is amplified from the environmental sample, not to the DNA that is isolated from the environment. It is incorrect to say that “macrobial DNA is isolated directly from an environmental sample”, as the separation between microbial and macrobial DNA occurs only later in processing of eDNA samples. It is often forgotten that the macrobial DNA represents only a very small fraction of the total DNA recovered from the environment, which is of microbial origins mainly (Stat et al. 2017). Secondly, such a definition does also not take in consideration the eggs, larvae or other small stages of macrofauna life cycles can be present in environmental samples and have been suspected to contribute to some of the eDNA signals observed. Finally, it implicitly assumes that the structural state of macrobial eDNA is different because it originates from DNA traces, while microbial or meiofaunal eDNA might derive from whole organisms. However, as demonstrated by numerous extracellular DNA studies, the microorganisms and meiofauna are represented there as much as macro-organisms.

We recommend that the eDNA studies adopt a **two level terminology** that clearly specifies the origin of environmental samples and the target taxa. At a first level, specific terms, such as water eDNA, sediment eDNA, or soil eDNA, would provide information about the environmental source of eDNA and/or the sampling methodology. Given that it is an environmental sample already, the “e” of the environment is basically defined by the specific environment given, and its use as a prefix of DNA might be considered as redundant but may be clearer. A second level of specification would then provide information about the taxonomic groups targeted in environmental samples, that is their DNA amplified and sequenced, such as fish eDNA, invertebrate eDNA, diatom eDNA, bacterial eDNA. In this case, the use of “eDNA” would clearly separate it from DNA extracted from tissues or cultures of these organisms, as well as from the DNA extracted from bulk samples that are not considered as eDNA here. Our proposed terminology does not require assumptions about the specific state of the DNA sampled (e.g., from cells, tissue fragments, gametes or free-floating), which is also generally not assessed, nor can it be told apart based on the sequence information. Obviously, this second level of the terminology is not required in the case of studies based on PCR-free approaches.

In our opinion, our proposed two-level terminology will contribute to clarify the scope of eDNA studies. Given the rapidly increasing number of studies using eDNA for biomonitoring and bioassessment, it is important to be as precise as possible regarding their objectives and outcomes, for example by specifying that the particular study was conducted using water eDNA and focusing on fish eDNA. A terminological clarity is particularly important for environmental managers who are not always aware about the various opportunities offered by new technology. Restricting the use of the term solely to tracing the large-sized organisms is drawing attention away from what we see as the most prominent application of eDNA technology, namely being a unique tool capable of providing a global assessment of ecological

status including different biological quality elements at a time. We are convinced that retaining the original broad definition of eDNA highlighting its universal character will contribute to expanding the field eDNA research and its successful application.

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227 **Figure captions:**

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229 Fig. 1. The number of publications by years referring to environmental DNA studies targeting
 230 microbial diversity, macrobial diversity, or both. Microbial diversity encompasses bacterial
 231 and viral diversity as well as eukaryotic micro- and meiofauna. The figure is based on a
 232 PubMed NCBI search (on May 5th, 2020) of titles and abstract containing the term
 233 “Environmental DNA”, excluding studies containing “medical” or “cancer”. This resulted in
 234 1009 papers. After manual inspection, 192 papers were removed from this list because they
 235 clearly were outside a biodiversity context. The full list of all papers considered is available
 236 upon request.

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Fig. 2. Principal types of environmental samples and target taxonomic groups commonly used in biomonitoring and bioassessment. This figure shows the two levels at which environmental DNA term can be specified. The first level indicates where the DNA comes from (the type of environmental substrate sampled, such as soil, sediment, biofilm, or water). The second level is then specifying what taxonomic group is targeted by PCR amplification (based on the specific choice of primers), including bacteria, protists, fungi, diatoms, meiofauna, arthropods, molluscs, amphibians and fish. The width of the line corresponds qualitatively to the common usage of particular eDNA extracted for each taxonomic group. Other types of environments, such as air or faeces, were not included for simplicity.

References

- Altermatt, F., Little, C. J., Mächler, E., Wang, S., Zhang, X., & Blackman, R. C. (2020). Uncovering the complete biodiversity structure in spatial networks: the example of riverine systems. *Oikos*, 129(5), 607–618. doi: 10.1111/oik.06806
- Bálint, M., Pfenninger, M., Grossart, H.-P., Taberlet, P., Vellend, M., Leibold, M. A., ... Bowler, D. (2018). Environmental DNA Time Series in Ecology. *Trends in Ecology & Evolution*, 33(12), 945–957. doi: 10.1016/j.tree.2018.09.003
- Bass, D., & Cavalier-Smith, T. (2004). Phylum-specific environmental DNA analysis reveals remarkably high global biodiversity of Cercozoa (Protozoa). *International Journal of Systematic and Evolutionary Microbiology*, 54(Pt 6), 2393–2404. doi: 10.1099/ijs.0.63229-0
- Berney, C., Fahrni, J., & Pawlowski, J. (2004). How many novel eukaryotic “kingdoms”? Pitfalls and limitations of environmental DNA surveys. *BMC Biology*, 2, 13. doi: 10.1186/1741-7007-2-13
- Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., ... de Bruyn, M. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology & Evolution*, 29(6), 358–367. doi: 10.1016/j.tree.2014.04.003
- Borrell, Y. J., Miralles, L., Martínez-Marqués, A., Semeraro, A., Arias, A., Carleos, C. E., & García-Vázquez, E. (2018). Metabarcoding and post-sampling strategies to discover non-indigenous species: A case study in the estuaries of the central south Bay of Biscay. *Journal for Nature Conservation*, 42, 67–74. doi: 10.1016/j.jnc.2017.07.002
- Carraro, L., Mächler, E., Wüthrich, R. & Altermatt, F. (2020). Environmental DNA allows upscaling spatial patterns of biodiversity in freshwater ecosystems. *Nature Communications* 11, 3585. doi: [10.1038/s41467-020-17337-8](https://doi.org/10.1038/s41467-020-17337-8)
- Cordier, T., Forster, D., Dufresne, Y., Martins, C. I. M., Stoeck, T., & Pawlowski, J. (2018). Supervised machine learning outperforms taxonomy-based environmental DNA metabarcoding applied to biomonitoring. *Molecular Ecology Resources*, 18(6), 1381–1391. doi: 10.1111/1755-0998.12926
- Cordier, T., Lanzén, A., Apothéloz-Perret-Gentil, L., Stoeck, T., & Pawlowski, J. (2019). Embracing Environmental Genomics and Machine Learning for Routine Biomonitoring. *Trends in Microbiology*, 27(5), 387–397. doi: 10.1016/j.tim.2018.10.012
- Corinaldesi, C., Tangherlini, M., Manea, E., & Dell’Anno, A. (2018). Extracellular DNA as a genetic recorder of microbial diversity in benthic deep-sea ecosystems. *Scientific Reports*, 8(1), 1–9. doi: 10.1038/s41598-018-20302-7
- Cristescu, M. E. (2014). From barcoding single individuals to metabarcoding biological communities: towards an integrative approach to the study of global biodiversity. *Trends in Ecology & Evolution*, 29(10), 566–571. doi: 10.1016/j.tree.2014.08.001
- Darling, J. A., & Mahon, A. R. (2011). From molecules to management: adopting DNA-based methods for monitoring biological invasions in aquatic environments. *Environmental Research*, 111(7), 978–988. doi: 10.1016/j.envres.2011.02.001

290 Deflaun, M. F., Paul, J. H., & Davis, D. (1986). Simplified method for dissolved DNA
 291 determination in aquatic environments. *Applied and Environmental Microbiology*, 52(4),
 292 654–659.

293 Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., ...
 294 Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey
 295 animal and plant communities. *Molecular Ecology*, 26(21), 5872–5895. doi:
 296 10.1111/mec.14350

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

300 Deiner, K., Fronhofer, E. A., Mächler, E., Walser, J.-C., & Altermatt, F. (2016).
 301 Environmental DNA reveals that rivers are conveyor belts of biodiversity information.
 302 *Nature Communications*, 7, 12544. doi: 10.1038/ncomms12544

303 Djurhuus, A., Closek, C. J., Kelly, R. P., Pitz, K. J., Michisaki, R. P., Starks, H. A., ... others.
 304 (2020). Environmental DNA reveals seasonal shifts and potential interactions in a marine
 305 community. *Nature Communications*, 11(1), 1–9.

306 Dudgeon, D. (2019). Multiple threats imperil freshwater biodiversity in the Anthropocene.
 307 *Current Biology*, 29(19), R960–R967. doi: 10.1016/j.cub.2019.08.002

308 Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using
 309 environmental DNA from water samples. *Biology Letters*, 4(4), 423–425. doi:
 310 10.1098/rsbl.2008.0118

311 Giovannoni, S. J., Britschgi, T. B., Moyer, C. L., & Field, K. G. (1990). Genetic diversity in
 312 Sargasso Sea bacterioplankton. *Nature*, 345(6270), 60–63. doi: 10.1038/345060a0

313 Goldberg, C. S., Strickler, K. M., & Pilliod, D. S. (2015). Moving environmental DNA
 314 methods from concept to practice for monitoring aquatic macroorganisms. *Biological*
 315 *Conservation*, 183, 1–3. doi: 10.1016/j.biocon.2014.11.040

316 Gordon, D. A., Priscu, J., & Giovannoni, S. (2000). Origin and Phylogeny of Microbes
 317 Living in Permanent Antarctic Lake Ice. *Microbial Ecology*, 39(3), 197–202. doi:
 318 10.1007/s002480000016

319 Guardiola, M., Uriz, M. J., Taberlet, P., Coissac, E., Wangenstein, O. S., & Turon, X. (2015).
 320 Deep-Sea, Deep-Sequencing: Metabarcoding Extracellular DNA from Sediments of
 321 Marine Canyons. *PloS One*, 10(10), e0139633. doi: 10.1371/journal.pone.0139633

322 Harmsen, M., Yang, L., Pamp, S. J., & Tolker-Nielsen, T. (2010). An update on
 323 *Pseudomonas aeruginosa* biofilm formation, tolerance, and dispersal. *FEMS Immunology*
 324 *and Medical Microbiology*, 59(3), 253–268. doi: 10.1111/j.1574-695X.2010.00690.x

325 Herder, J., Valentini, A., Bellemain, E., Dejean, T., Delft, J., Thomsen, P., & Taberlet, P.
 326 (2014). Environmental DNA - a review of the possible applications for the detection of
 327 (invasive) species. doi: 10.13140/RG.2.1.4002.1208

328 Holzmann, M., Habura, A., Giles, H., Bowser, S. S., & Pawlowski, J. (2003). Freshwater
 329 foraminiferans revealed by analysis of environmental DNA samples. *The Journal of*
 330 *Eukaryotic Microbiology*, 50(2), 135–139.

331 Huber, H., Hohn, M. J., Rachel, R., Fuchs, T., Wimmer, V. C., & Stetter, K. O. (2002). A
 332 new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. *Nature*,
 333 417(6884), 63–67. doi: 10.1038/417063a

334 Jackson, M. C., Weyl, O. L. F., Altermatt, F., Durance, I., Friberg, N., Dumbrell, A. J., ...
 335 Woodward, G. (2016). Chapter Twelve - Recommendations for the Next Generation of
 336 Global Freshwater Biological Monitoring Tools. In A. J. Dumbrell, R. L. Kordas, & G.
 337 Woodward (Eds.), *Advances in Ecological Research* (pp. 615–636). doi:
 338 10.1016/bs.aecr.2016.08.008

339 Kelly, R. P., Port, J. A., Yamahara, K. M., Martone, R. G., Lowell, N., Thomsen, P. F., ...
 340 Crowder, L. B. (2014). Harnessing DNA to improve environmental management. *Science*,
 341 344(6191), 1455–1456. doi: 10.1126/science.1251156

342 Kloos, D. U., Strätz, M., Güttler, A., Steffan, R. J., & Timmis, K. N. (1994). Inducible cell
 343 lysis system for the study of natural transformation and environmental fate of DNA
 344 released by cell death. *Journal of Bacteriology*, 176(23), 7352–7361. doi:
 345 10.1128/jb.176.23.7352-7361.1994

346 Lacoursière-Roussel A & Deiner K (2019) Environmental DNA is not the tool by itself.
 347 *Journal of Fish Biology*, <https://doi.org/10.1111/jfb.14177>

348 Li, F., Peng, Y., Fang, W., Altermatt, F., Xie, Y., Yang, J., & Zhang, X. (2018). Application
 349 of Environmental DNA Metabarcoding for Predicting Anthropogenic Pollution in Rivers.
 350 *Environmental Science & Technology*, 52(20), 11708–11719. doi:
 351 10.1021/acs.est.8b03869

352 Lorenz, M. G., & Wackernagel, W. (1987). Adsorption of DNA to sand and variable
 353 degradation rates of adsorbed DNA. *Applied and Environmental Microbiology*, 53(12),
 354 2948–2952.

355 Lynggaard, C., Nielsen, M., Santos-Bay, L., Gastauer, M., Oliveira, G., & Bohmann, K.
 356 (2019). Vertebrate diversity revealed by metabarcoding of bulk arthropod samples from
 357 tropical forests. *Environmental DNA*, 1(4), 329–341.

358 Macher J-N, Vivancos A, Piggott JJ, Centeno FC, Matthaei CD & Leese F (2018)
 359 Comparison of environmental DNA and bulk-sample metabarcoding using highly
 360 degenerate cytochrome c oxidase I primers. *Molecular Ecology Resources*, **18**: 1456-1468.

361 Mächler E, Deiner K, Steinmann P & Altermatt F (2014) Utility of environmental DNA for
 362 monitoring rare and indicator macroinvertebrate species. *Freshwater Science*, **33**(4): 1174-
 363 1183.

364 Moushomi, R., Wilgar, G., Carvalho, G., Creer, S. & Seymour, M. (2019) Environmental
 365 DNA size sorting and degradation experiment indicates the state of *Daphnia magna*
 366 mitochondrial and nuclear eDNA is subcellular. *Scientific Reports*. 9, 12500.

367 Nguyen, B. N., Shen, E. W., Seemann, J., Correa, A. M. S., O'Donnell, J. L., Altieri, A. H.,
 368 ... Leray, M. (2020). Environmental DNA survey captures patterns of fish and
 369 invertebrate diversity across a tropical seascape. *Scientific Reports*, 10(1), 1–14. doi:
 370 10.1038/s41598-020-63565-9

371 Nielsen, M., Gilbert, M. T. P., Pape, T., & Bohmann, K. (2019). A simplified DNA
372 extraction protocol for unsorted bulk arthropod samples that maintains exoskeletal
373 integrity. *Environmental DNA*, 1(2), 144–154.

374 Ogram, A., Sayler, G. S., & Barkay, T. (1987). The extraction and purification of microbial
375 DNA from sediments. *Journal of Microbiological Methods*, 7(2), 57–66. doi:
376 10.1016/0167-7012(87)90025-X

377 Olsen, G. J., Lane, D. J., Giovannoni, S. J., Pace, N. R., & Stahl, D. A. (1986). Microbial
378 ecology and evolution: a ribosomal RNA approach. *Annual Review of Microbiology*, 40,
379 337–365. doi: 10.1146/annurev.mi.40.100186.002005

380 O’Sullivan, A. M., Samways, K. M., Perreault, A., Hernandez, C., Gautreau, M. D., Curry, R.
381 A., & Bernatchez, L. (2020). Space invaders: Searching for invasive Smallmouth Bass
382 (*Micropterus dolomieu*) in a renowned Atlantic Salmon (*Salmo salar*) river. *Ecology and*
383 *Evolution*, 10(5), 2588–2596. doi: 10.1002/ece3.6088

384 Pace, N. R., Stahl, D. A., Lane, D. J., & Olsen, G. J. (1986). The Analysis of Natural
385 Microbial Populations by Ribosomal RNA Sequences. In K. C. Marshall (Ed.), *Advances*
386 *in Microbial Ecology* (pp. 1–55). doi: 10.1007/978-1-4757-0611-6_1

387 Paul, J. H., Jeffrey, W. H., & DeFlaun, M. F. (1987). Dynamics of extracellular DNA in the
388 marine environment. *Applied and Environmental Microbiology*, 53(1), 170–179.

389 Paul, J. H., & Myers, B. (1982). Fluorometric determination of DNA in aquatic
390 microorganisms by use of hoechst 33258. *Applied and Environmental Microbiology*,
391 43(6), 1393–1399.

392 Pawlowski J, Apothéloz-Perret-Gentil L, Mächler E, & Altermatt F. (2020) Guidelines to
393 environmental DNA applications in biomonitoring and bioassessment of aquatic
394 ecosystems. Guidelines. Federal Office for the Environment, Bern. Environmental Studies.
395 no. 2010.

396 Pawlowski, J., Kelly-Quinn, M., Altermatt, F., Apothéloz-Perret-Gentil, L., Beja, P.,
397 Boggero, A., ... Kahlert, M. (2018). The future of biotic indices in the ecogenomic era:
398 Integrating (e)DNA metabarcoding in biological assessment of aquatic ecosystems. *The*
399 *Science of the Total Environment*, 637–638, 1295–1310. doi:
400 10.1016/j.scitotenv.2018.05.002

401 Pearman, J. K., Irigoien, X., & Carvalho, S. (2016). Extracellular DNA amplicon sequencing
402 reveals high levels of benthic eukaryotic diversity in the central Red Sea. *Marine*
403 *Genomics*, 26, 29–39. doi: 10.1016/j.margen.2015.10.008

404 Pfrender, M. E., Hawkins, C. P., Bagley, M., Courtney, G. W., Creutzburg, B. R., Epler, J.
405 H., ... Whiting, M. (2010). Assessing macroinvertebrate biodiversity in freshwater
406 ecosystems: advances and challenges in DNA-based approaches. *The Quarterly Review of*
407 *Biology*, 85(3), 319–340. doi: 10.1086/655118

408 Picard, C., Ponsonnet, C., Paget, E., Nesme, X., & Simonet, P. (1992). Detection and
409 enumeration of bacteria in soil by direct DNA extraction and polymerase chain reaction.
410 *Applied and Environmental Microbiology*, 58(9), 2717–2722.

411 Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., ...
 412 Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for
 413 freshwater biodiversity. *Biological Reviews*, 94(3), 849–873. doi: 10.1111/brv.12480

414 Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., ... Erlich, H.
 415 A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA
 416 polymerase. *Science (New York, N.Y.)*, 239(4839), 487–491. doi:
 417 10.1126/science.2448875

418 Schmidt, T. M., DeLong, E. F., & Pace, N. R. (1991). Analysis of a marine picoplankton
 419 community by 16S rRNA gene cloning and sequencing. *Journal of Bacteriology*, 173(14),
 420 4371–4378. doi: 10.1128/jb.173.14.4371-4378.1991

421 Sigsgaard, E. E., Jensen, M. R., Winkelmann, I. E., Møller, P. R., Hansen, M. M., &
 422 Thomsen, P. F. (2020). Population-level inferences from environmental DNA—Current
 423 status and future perspectives. *Evolutionary Applications*, 13(2), 245–262. doi:
 424 10.1111/eva.12882

425 Somerville, C. C., Knight, I. T., Straube, W. L., & Colwell, R. R. (1989). Simple, rapid
 426 method for direct isolation of nucleic acids from aquatic environments. *Applied and*
 427 *Environmental Microbiology*, 55(3), 548–554.

428 Srivathsan, A., Sha, J. C. M., Vogler, A. P., & Meier, R. (2015). Comparing the effectiveness
 429 of metagenomics and metabarcoding for diet analysis of a leaf-feeding monkey (*Pygathrix*
 430 *nemaeus*). *Molecular Ecology Resources*, 15(2), 250–261. doi: 10.1111/1755-0998.12302

431 Stahl, D. A., Lane, D. J., Olsen, G. J., & Pace, N. R. (1984). Analysis of hydrothermal vent-
 432 associated symbionts by ribosomal RNA sequences. *Science (New York, N.Y.)*,
 433 224(4647), 409–411. doi: 10.1126/science.224.4647.409

434 Stat, M., Huggett, M.J., Bernasconi, R., DiBattista, J.D., Berry, T.E., Newman, S.J., Harvey,
 435 E.S. & Bunce, M. (2017) Ecosystem biomonitoring with eDNA: metabarcoding across the
 436 tree of life in a tropical marine environment. *Scientific Reports* 7, 12240. doi:
 437 10.1038/s41598-017-12501-5.

438 Steffan, R. J., Goksøyr, J., Bej, A. K., & Atlas, R. M. (1988). Recovery of DNA from soils
 439 and sediments. *Applied and Environmental Microbiology*, 54(12), 2908–2915.

440 Stoeck, T., Fröhe, L., Forster, D., Cordier, T., Martins, C. I. M., & Pawlowski, J. (2018).
 441 Environmental DNA metabarcoding of benthic bacterial communities indicates the
 442 benthic footprint of salmon aquaculture. *Marine Pollution Bulletin*, 127, 139–149. doi:
 443 10.1016/j.marpolbul.2017.11.065

444 Suzuki, M. T., Rappé, M. S., Haimberger, Z. W., Winfield, H., Adair, N., Ströbel, J., &
 445 Giovannoni, S. J. (1997). Bacterial diversity among small-subunit rRNA gene clones and
 446 cellular isolates from the same seawater sample. *Applied and Environmental*
 447 *Microbiology*, 63(3), 983–989.

448 Taberlet, P., Bonin, A., Coissac, E., & Zinger, L. (2018). *Environmental DNA: For*
 449 *Biodiversity Research and Monitoring*. Oxford University Press.

450 Taberlet, P., Coissac, E., Hajibabaei, M., & Rieseberg, L. H. (2012). Environmental DNA.
 451 *Molecular Ecology*, 21(8), 1789–1793. doi: 10.1111/j.1365-294X.2012.05542.x

452 Thomsen, P. F., Kielgast, J., Iversen, L. L., Wiuf, C., Rasmussen, M., Gilbert, M. T. P., ...
 453 Willerslev, E. (2012). Monitoring endangered freshwater biodiversity using environmental
 454 DNA. *Molecular Ecology*, 21(11), 2565–2573. doi: 10.1111/j.1365-294X.2011.05418.x

455 Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA – An emerging tool in
 456 conservation for monitoring past and present biodiversity. *Biological Conservation*, 183,
 457 4–18. doi: 10.1016/j.biocon.2014.11.019

458 Torsvik, V., Goksøyr, J., & Daae, F. L. (1990). High diversity in DNA of soil bacteria.
 459 *Applied and Environmental Microbiology*, 56(3), 782–787.

460 Tsuji, S., Maruyama, A., Miya, M., Ushio, M., Sato, H., Minamoto, T., & Yamanaka, H.
 461 (2020). Environmental DNA analysis shows high potential as a tool for estimating
 462 intraspecific genetic diversity in a wild fish population. *Molecular Ecology Resources*.
 463 doi: 10.1111/1755-0998.13165

464 Turner, C. R., Barnes, M. A., Xu, C. C. Y., Jones, S. E., Jerde, C. L., & Lodge, D. M. (2014).
 465 Particle size distribution and optimal capture of aqueous microbial eDNA. *Methods in*
 466 *Ecology and Evolution*, 5(7), 676–684. doi: 10.1111/2041-210X.12206

467 Yoccoz, N.G. (2012) The future of environmental DNA in ecology. *Molecular Ecology*
 468 21(8), 2031-2038. doi: 10.1111/j.1365-294X.2012.05505.x.

469 Zhang, Y., Pavlovska, M., Stoica, E., Prekrasna, I., Yang, J., Slobodnik, J., ... Dykyi, E.
 470 (2020). Holistic pelagic biodiversity monitoring of the Black Sea via eDNA
 471 metabarcoding approach: From bacteria to marine mammals. *Environment International*,
 472 135, 105307. doi: 10.1016/j.envint.2019.105307

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