COMMENT

Trade-offs between reducing complex terminology and producing accurate interpretations from environmental DNA: Comment on "Environmental DNA: What's behind the term?" by Pawlowski *et al.* (2020)

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Abstract

In a recent paper, "Environmental DNA: What's behind the term? Clarifying the terminology and recommendations for its future use in biomonitoring", Pawlowski *et al.* argue that the term eDNA should be used to refer to the pool of DNA isolated from environmental samples, as opposed to only extra-organismal DNA from macro-organisms. We agree with this view. However, we are concerned that their proposed two-level terminology specifying sampling environment and targeted taxa is overly simplistic and might hinder rather than improve clear communication about environmental DNA and its use in biomonitoring. Not only is this terminology based on categories that are often difficult to assign and uninformative, but it ignores what is in our opinion the most important distinction within eDNA: the type of DNA (organismal or extra-organismal) from which ecological interpretations are derived.

Keywords: clear terminology, organismal DNA, extra-organismal DNA, ecology of eDNA

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1. eDNA should be used to refer to the total pool of DNA isolated from the environment

Use of unclear terminologies in science communication inevitably causes confusion, inefficiencies, and misinterpretation of information, with potential costly ramifications. Hence, we applaud Pawlowski *et al.* (2020) for highlighting inconsistencies in the use of the term "environmental DNA" (eDNA) and their implications for biomonitoring. As described by the authors, these inconsistencies stem from some practitioners using the term to refer to any DNA collected from an environmental sample without first isolating targeted organisms (*e.g.* Stat *et al.* (2017)), while others use it to refer only to extra-

87 organismal DNA released by macro-organisms into the environment (*e.g.* Fraija-
88 Fernández *et al.* (2020)). Although some of us have previously advocated for eDNA to
89 be defined as extra-organismal DNA, the value of which is effectively refuted by
90 Pawlowski *et al.* (2020).We therefore agree with Pawlowski *et al.* (2020) that
91 environmental DNA should be defined in the broadest sense.

92 However, the recommendation to employ a standard two-level terminology in eDNA
93 studies, first indicating the environmental origin of the DNA collected (e.g., water,
94 sediment, biofilm, soil) and second indicating the taxa (e.g., fish, diatom, bacteria)
95 targeted by polymerase chain reaction (PCR), does not align with their overall purpose
96 of improving clarity in eDNA biomonitoring. The reason is that it does not account for
97 the distinction between the different types of eDNA (organismal and extra-organismal),
98 which is the level of classification that can have strong impact on eDNA data
99 interpretation. While Pawlowski *et al.* (2020) discounts this, we argue there is a need to
100 be clear about the type of eDNA that is being evaluated in any given study and this is
101 the reason for why the term has been described in the broad and narrow sense.

102 **2. eDNA is composed of organismal and extra-organismal DNA**

103 Environmental DNA can be classified into two types (Figure 1a): organismal DNA and
104 extra-organismal DNA, the latter also including extracellular DNA (Barnes & Turner
105 2016; Bohmann *et al.* 2014; Taberlet *et al.* 2012; Torti *et al.* 2015). Organismal DNA is
106 sourced from whole individuals most likely alive at the time of sampling; as such, this
107 type of eDNA is typically of high quality and significant quantity. In contrast, extra-
108 organismal DNA originates from a variety of sources and thus is of highly variable
109 quality and quantity. For example, extra-organismal DNA can come from biological
110 material shed from an organism as part of tissue replacement or metabolic waste (Allan
111 *et al.* 2020), as biologically active propagules such as gametes, pollen, seeds or spores
112 (Stewart 2019), or as a result of cell-lysis or cell extrusion (Pietramellara *et al.* 2009).
113 The latter processes results in extracellular DNA, which can persist in the environment

on its own or be adsorbed onto surface-reactive particles such as humic substances, clay, silt, or sand (Levy-Booth *et al.* 2007; Pietramellara *et al.* 2009). Environmental DNA samples are therefore composed of a complex mixture of both types of DNA (*i.e.*, organismal and extra-organismal) from various sources and in varying proportions (Taberlet *et al.* 2012).

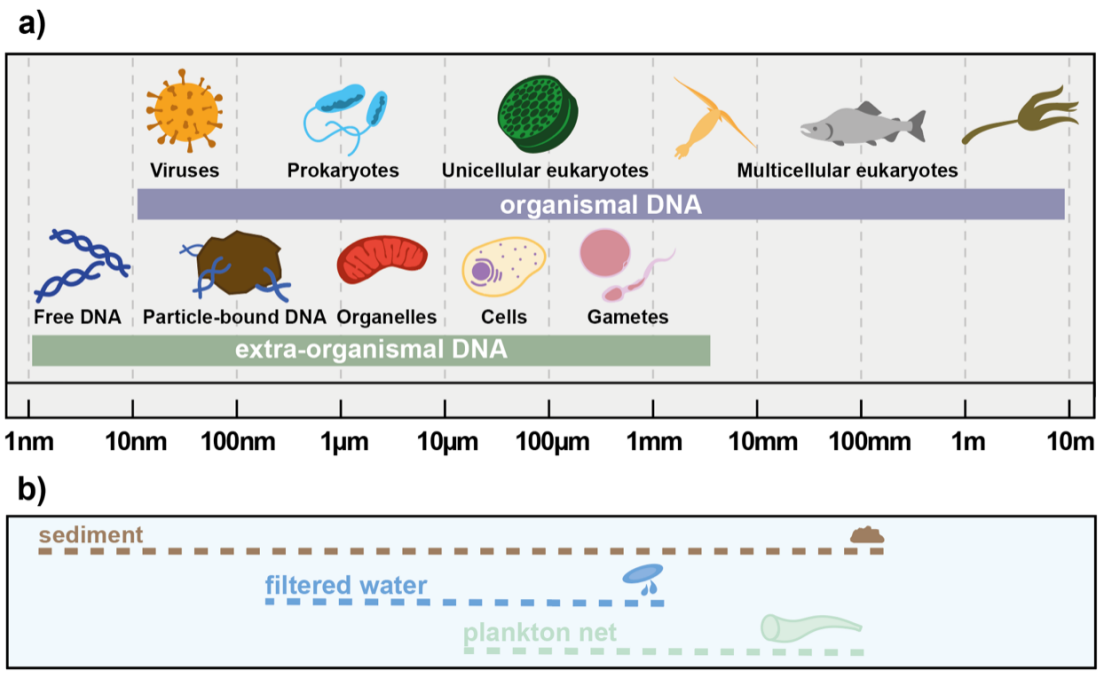

Figure 1. a) Types of environmental DNA (organismal and extra-organismal, including extracellular) with possible sources and approximate size ranges. b) Illustrative examples of sampling methods with intended captured particle size ranges.

3. eDNA can be enriched for different sources and types

Generally, not all DNA present in a studied environment is required to address a given research question or is used for an application, and successive steps of enrichment for specific types or sources of eDNA are usually applied. For example, eDNA from a large variety of taxonomic groups can be found as organismal or extra-organismal DNA (types) in the environment (Figure 1a) and can be obtained in many ways from aquatic,

aerial and terrestrial environments (Deiner *et al.* 2017). The first step is performed at the sampling level, where typically the collected material is passed through filters, meshes or nets to retain organisms, organismal debris or particles of a desired size (Figure 1b). Notably, this step does not imply a separation of DNA types or taxonomic groups because different sources and types of DNA overlap in size (Figure 1a) and because of the "sticky" nature of eDNA to bind other particles (Barnes *et al.* 2020). A subsequent enrichment can be performed during laboratory work through PCR or sequence capture using taxon-specific primers or probes (Jensen *et al.* 2020). However, this step is not perfect; a fraction of non-target taxa DNA can also be amplified, and target taxa DNA can be missed. Finally, DNA sequences from particular taxa can be selected at the analysis/interpretation step by considering only those sequences belonging to a given taxonomic group.

The particular methods applied at each of these enrichment steps will determine the final dataset used for ecological inferences, but these methods evolve and are not in themselves completely deterministic. For example, "water eDNA amplified for metazoans" could refer to either organismal DNA collected through a plankton net containing fish larvae and zooplankton, or to extra-organismal DNA collected through a 0.45 μm pore size filter containing tissue, scales or cellular debris from fish and zooplankton.

4. Ecological interpretations should consider DNA type

While it is currently impractical to separate and independently analyze organismal and extra-organismal DNA, the distinction between the two types is nonetheless crucial for ecological hypothesis-testing and data interpretation. Organismal DNA is often targeted when a living community of organisms is studied, asking questions about specific habitat, functional role of communities or community assembly processes driven by abiotic factors and biotic interactions. Here, chances of misleading data (i.e., the species was not in that environment at that time and place) are likely minimal. Instead,

work focusing on extra-organismal DNA is more prone to misinterpretations about organismal distribution due to potential long-distance transport from source populations (Lacoursière-Roussel & Deiner, 2019). The processes regulating the presence of extra-organismal DNA in the environment and its detection in the laboratory are more stochastic. As a result, studies targeting this type of eDNA require a sampling design with in-depth replication, extra attention to potential sources of contamination and need to be cognizant that the results are less likely to be definitive about species presence or absence at the time of sampling.

In eDNA studies, extra-organismal DNA is increasingly targeted for the indirect detection of (often macro-) organisms without destroying their natural habitats or harming individuals; for example, detecting fish taxa from eDNA extracted from water (Antognazza *et al.* 2019; Fraija-Fernández *et al.* 2020). Here, any link between the presence of a species' DNA and the presence of a living individual or population in the local area is implied. While a recent meta-analysis found that fish diversity estimated using eDNA agrees closely with estimates using conventional methods of capturing or observing the fish (McElroy *et al.* 2020), absolute conclusions about space and time inferences made from extra-organismal DNA are not yet possible. To make such a link accurate, an understanding of the 'ecology' of extra-organismal DNA (Barnes & Turner 2016) is crucial, which requires knowledge of the often site-specific processes governing its production, transportation and degradation rate in the environment.

While separating the different eDNA types in practice remains a challenge, researchers using eDNA need to be clear about their intent. Specifically, we need to clearly report the methodological choices made to target one type of eDNA or another (whether by sampling, laboratory treatment, or bioinformatics), make informed speculations about the likelihood of succeeding with that target, and acknowledge the limitations of the data we generate. If we target extra-organismal DNA, we also need to consider what process(es) we hypothesize govern the transport between the temporal and spatial

bounds of detected DNA and what inferences we can therefore make from its detection.

5. Pawlowski et al.´s proposed two-level terminology does not contribute to improving clarity in eDNA studies

We argue that Pawlowski *et al.* (2020) not only obviate the most important distinction within eDNA by disregarding the type of DNA sampled (organismal, extra-organismal), but also introduce two distinctions that lead to confusion rather than clarity. First, their suggestion for environmental-origin classification represents a simplistic view of environmental samples. In any ecosystem, eDNA flows between mediums, and is subjected to multiple processes, such as decay (Lance *et al.* 2017) transport (Deiner *et al.* 2016), sedimentation (Turner *et al.* 2015) or resuspension (Shogren et al., 2017) in aquatic and aerial systems, or burial in soils and sediments (Haile *et al.* 2007). For example, eDNA in a water sample represents the complex amalgamation of DNA sources from sediment, biofilm and water within both aquatic and terrestrial environments (Shogren et al., 2017). As such, this proposed level attempts to make a definitive delineation where, in practice, the line is not clear with current technologies. Second, Pawlowski *et al*.´s classification based on targeted taxonomic group using PCR is also problematic because it is overly simplistic. Designing primers to detect higher taxonomic ranks (e.g., family- or phylum-level) does not preclude amplification of not-target taxa. Conversely, not all taxa targeted by a particular primer set will be amplified due to primer bias (Bakker *et al.* 2017). Further, this level of classification is irrelevant to PCR-free approaches, which are now increasingly used (Cowart *et al.* 2018; Jensen *et al.* 2020). Defining a field by current (PCR-based) technologies risks becoming rapidly outdated and perhaps even incompatible with future innovations. Lastly, PCR cannot distinguish between a DNA molecule from organismal or extra-organismal DNA, thus setting a false expectation.

In summary, we agree with Pawlowski *et al.* (2020) that eDNA should be defined in the broadest sense, but do not agree that the formal adoption of their additional proposed nomenclature by will improve clarity in communication or reduce confusion around the use of the term eDNA. We suggest instead that scientists carefully and clearly identify the type of DNA being targeted for analysis (Figure 1) based on the existing terminology of organismal and extra-organismal DNA. This explicit stated intention would then clearly inform study design, sampling strategies, analytical choices and data interpretation to avoid potential biases and promote valid inferences. Because none of these choices and strategies are perfect in their detection of a particular type of DNA, we also suggest that the sampling strategy be clearly described including the targeted size classes and taxa and whether taxa were targeted in any way during sampling, laboratory analysis (PCR, capture), data analysis (sequence selection) or some combination thereof. We feel that improvement of the field is a shared responsibility among researchers, reviewers, editors and managers and support the development and application of best practices in the acquisition and reporting of eDNA data (Goldberg *et al.* 2016) as the best way to improve clarity. We endorse a holistic approach whereby the research question being asked remains a central element of studies utilizing eDNA rather than new terminologies, acronyms or different definitions emerging from technical novelty, rather than biological reality.

Acknowledgements

NRE has been supported by the Spanish Ministry of Science and Innovation (project EDAMAME – Environmental DNA based approaches for marine and aquatic monitoring and evaluation; CTM2017-89500-R). LEH was supported by the Natural Environmental Research Council (grant number NE/L002531/1). KD has been supported by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (Grant agreement No. 852621). We would like to

thank Kristine Bohmann for providing comments on an earlier version of this manuscript. This collaboration was a result of an initial collaboration of us on Twitter.

Authors Contribution

NRE and KD conceived the idea. All authors contributed to the discussion and wrote the manuscript. LEH constructed the figure with input from all authors.

Data Availability Statement

Not applicable.

References

Allan EA, Zhang WG, C. Lavery A, F. Govindarajan A (2020) Environmental DNA shedding and decay rates from diverse animal forms and thermal regimes. *Environmental DNA* **n/a**.

Antognazza CM, Britton JR, Potter C*, et al.* (2019) Environmental DNA as a non-invasive sampling tool to detect the spawning distribution of European anadromous shads (Alosa spp.). *Aquatic Conservation: Marine and Freshwater Ecosystems* **29**, 148-152.

Bakker J, Wangensteen OS, Chapman DD*, et al.* (2017) Environmental DNA reveals tropical shark diversity in contrasting levels of anthropogenic impact. *Scientific reports* **7**.

Barnes MA, Chadderton WL, Jerde CL*, et al.* (2020) Environmental conditions influence eDNA particle size distribution in aquatic systems. *Environmental DNA* **n/a**.

Barnes MA, Turner CR (2016) The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics* **17**, 1-17.

Bohmann K, Evans A, Gilbert MTP*, et al.* (2014) Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology & Evolution* **29**, 358-367.

Cowart DA, Murphy KR, Cheng CHC (2018) Metagenomic sequencing of environmental DNA reveals marine faunal assemblages from the West Antarctic Peninsula. *Marine Genomics* **37**, 148-160.

Deiner K, Bik HM, Mächler E*, et al.* (2017) Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology* **26**, 5872-5895.

Deiner K, Fronhofer EA, Mächler E, Walser J-C, Altermatt F (2016) Environmental DNA reveals that rivers are conveyer belts of biodiversity information. *Nature Communications* **7**, 12544.

Fraija-Fernández N, Bouquieaux M-C, Rey A*, et al.* (2020) Marine water environmental DNA metabarcoding provides a comprehensive fish diversity assessment and reveals spatial patterns in a large oceanic area. *Ecology and Evolution* **10**, 7560-7584.

Goldberg CS, Turner CR, Deiner K*, et al.* (2016) Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution* **7**, 1299-1307.

Haile J, Holdaway R, Oliver K*, et al.* (2007) Ancient DNA Chronology within Sediment Deposits: Are Paleobiological Reconstructions Possible and Is DNA Leaching a Factor? *Molecular Biology and Evolution* **24**, 982-989.

Jensen MR, Sigsgaard EE, Liu S*, et al.* (2020) Genome-scale target capture of mitochondrial and nuclear environmental DNA from water samples. *Molecular ecology resources* **n/a**.

Lance RF, Klymus KE, Richter CA*, et al.* (2017) Experimental observations on the decay of environmental DNA from bighead and silver carps. *Management of Biological Invasions* **8**, 343-359.

Levy-Booth DJ, Campbell RG, Gulden RH*, et al.* (2007) Cycling of extracellular DNA in the soil environment. *Soil Biology and Biochemistry* **39**, 2977-2991.

McElroy ME, Dressler TL, Titcomb GC*, et al.* (2020) Calibrating Environmental DNA Metabarcoding to Conventional Surveys for Measuring Fish Species Richness. *Frontiers in Ecology and Evolution* **8**.

Pawlowski J, Apothéloz-Perret-Gentil L, Altermatt F (2020) Environmental DNA: What's behind the term? Clarifying the terminology and recommendations for its future use in biomonitoring. *Molecular Ecology* **29**, 4258-4264.

Pietramellara G, Ascher J, Borgogni F*, et al.* (2009) Extracellular DNA in soil and sediment: fate and ecological relevance. *Biology and Fertility of Soils* **45**, 219-235.

Stat M, Huggett MJ, Bernasconi R*, et al.* (2017) Ecosystem biomonitoring with eDNA: metabarcoding across the tree of life in a tropical marine environment. *Scientific reports* **7**.

Stewart KA (2019) Understanding the effects of biotic and abiotic factors on sources of aquatic environmental DNA. *Biodiversity and Conservation* **28**, 983-1001.

Taberlet P, Coissac E, Hajibabaei M, Rieseberg LH (2012) Environmental DNA. *Molecular Ecology* **21**, 1789-1793.

Torti A, Lever MA, Jørgensen BB (2015) Origin, dynamics, and implications of extracellular DNA pools in marine sediments. *Marine Genomics* **24**, 185-196.

Turner CR, Karen LU, Robert CE (2015) Fish environmental DNA is more concentrated in aquatic sediments than surface water. *Biological Conservation* **183**, 93-102.