Environmental versus extra-organismal DNA

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We are very pleased that our opinion paper "Environmental DNA: What's behind the term?" (Pawlowski et al. 2020) stimulated a lively discussion and we are grateful for the comments on proposed terminology (Rodriguez-Ezpeleta et al. 2021). The clarity of scientific terms is essential for both fundamental and applied research and any debate on this issue is very important, especially in the early days of a new field. A major requirement of clarity is that terminology refers to measurable and implementable classifications. To recall the context of this debate, the aim of our paper was to restore a broad definition of environmental DNA (eDNA) as referring to all organisms present in environmental samples, including both macrobial as well as microbial organisms (Taberlet et al. 2012).

We are very glad that our proposition to adopt a broad definition of eDNA was accepted by the Rodriguez-Ezpeleta et al. (2021). However, we cannot agree with their opinion that our two-level terminology is oversimplistic. Our terminology refers to the two basic steps of any eDNA metabarcoding study, defining firstly the material (i.e., environmental sample) taken for DNA extraction and secondly the taxonomic group targeted by PCR. This may be simple but is also directly and clearly applicable, thereby clarifying the aims and targets of eDNA studies in general. We privilege the choice of material and method over the potential outputs of a study. According to our view, even if sediment samples are used as source of information about present or past surface plankton, these are still sediment eDNA studies (e.g., Morard et al. 2017, Monchamp et al. 2018). Similarly, if taxon-specific PCR primers amplify also other taxa, the target taxon should be mentioned, rather than PCR by-products (e.g., Mächler et al. 2019).

It is important to highlight that our proposed classification remains totally open to a more detailed specification of the eDNA study. We think that targeting extra-organismal DNA corresponds to such complementary information and this information can be included in the description of the study. As correctly emphasized by Rodriguez-Ezpeleta et al. (2021), the ecological interpretation of extra-organismal DNA data must take in consideration many factors specific to this type of DNA. Nevertheless, in practice such distinction concerns only the organisms over a certain size at best. As shown in Fig. 1 of Rodriguez-Ezpeleta et al. (2021), there is overlap over at least six orders of magnitude in size between intra and extra-organismal DNA, and even they conclude that "it is currently impractical to separate and independently analyse organismal and extra-organismal DNA". Given the continuous occurrence and transition of DNA from living organisms, to within tissues or cells (living or dead), to organelles and truly free DNA, we also think such separation is methodologically challenging if not impossible, and thus not directly applicable. While smaller-sized organisms (microbes or small animals such as rotifers) may be indeed often sampled in their living state, they still can also be recorded through DNA from degraded cells or organisms. In analogy, large organisms, such as mussels, may be largely recorded by extra-organismal DNA, but the occurrence of veliger larvae in water eDNA samples may go unnoticed and not be separable. Indeed, the complex mixture of different origin (or "types") of DNA may be a Gordian knot hard to resolve, and we recommend sticking to the directly applicable, technical terminology proposed by us. We fully understand the importance of eDNA for the detection and monitoring of aquatic vertebrates, such as amphibians or fish, and the inferences implied. However, from a semantic perspective it is hard not to think that a water eDNA study targeting extra-organismal fish DNA is just a pleonasm – and nothing else.

References

Mächler E, Little CJ, Wüthrich R, Alther R, Fronhofer EA, Gounand I, Harvey E, Hürlemann S, Walser J-C, Altermatt F (2019) Assessing different components of biodiversity across a river network using eDNA. *Environmental DNA* **1**, 290-301.

Monchamp M-E, Spaak P, Domaizon I, Dubois N, Bouffard D, Pomati F (2018) Homogenization of lake cyanobacterial communities over a century of climate change and eutrophication. *Nature Ecology & Evolution* **2**, 317-324.

Morard R, Lejzerowicz F, Darling KF, Lecroq-Bennet B, Pedersen MW, Orlando L, Pawlowski J, Mulitza S, de Vargas C, Kucera M (2017) Planktonic foraminifera-derived environmental DNA extracted from abyssal sediments preserves patterns of plankton macroecology. Biogeosciences 14(11): 2741.

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.

Rodriguez-Ezpeleta N, Morissette O, Bean CW, Manu S, Banerjee P, Laoursière-Roussel A, Beng KC, Alters SE, Roger F, Holman LE, Stewart KA, Monaghan MT, Mauvisseau Q, Mirimin L, Wangensteen OS, Antognazza CM, Helyar SJ, de Boer H, Monchamp M-E, Nijland R, Abbott CL, Doi H, Barnes MA, Leray M, Hablützel PI, Deiner K (2021) 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). *Molecular Ecology*. This issue

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