

COMMENTARY

Title:

Steps forward in Biomonitoring 2.0: eDNA metabarcoding and community-level modelling allow the assessment of complex drivers of Neotropical fish diversity

Running Title: Steps forward in Biomonitoring 2.0

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The way we inventory and monitor biodiversity globally has been revolutionized by new molecular and ecological methods (Pawlowski et al 2021). Nonetheless, the Neotropical realm still represents a great challenge to our understanding of species distribution patterns and the role played by different drivers of biodiversity. The paper by Coutant et al. (2022) is a great advance towards a holistic approach to quantifying the contribution of environmental and anthropogenic factors that drive community assembly in the Amazon, and how we can apply such knowledge to guide future monitoring programmes. Disentangling the relative roles played by multiple drivers of biodiversity allowed them to also highlight hotspot areas hosting unique freshwater fish diversity and to pinpoint conservation priorities. Toward this end, the authors applied an innovative framework that included the use of environmental DNA (eDNA) to conduct fish inventories combined with community-level modelling. Their multifaceted approach accounting for taxonomic and functional measures of biodiversity allowed a thorough evaluation of ecological patterns. They reported a mismatch between functional and taxonomic biodiversity in their responses to geographical, environmental, and anthropogenic factors. Functional diversity was influenced by environmental and anthropogenic factors, while fish taxonomic diversity was determined by dispersal restrictions at both intra and inter-basin scales in the Guiana Shield. The role played by dispersal limitation and human activities in shaping fish diversity in large rivers is very relevant considering that Amazonian freshwater ecosystems are facing large-scale degradation due to human activities greatly impairing river connectivity (Castello & Macedo, 2016).

Our knowledge of the patterns and drivers of species distribution in Amazonian freshwater ecosystems is constantly expanding. Recent studies revealed some surprises, highlighting how historical and ecological processes are complex and intertwined in these tropical watercourses. Oberdorff et al. (2019) found that, contrary to all expectations, fish species richness increases towards the headwaters of the Amazon River. This reverse richness gradient is attributed to the history of the river, as until the rise of the Andes, the Amazon River flowed towards the Pacific Ocean. In Amazonian small streams, evidence shows high levels of compositional dissimilarity in fish assemblages, even within the same river basin, mostly driven by species turnover (Leal et al., 2018). Beta diversity patterns in these small streams appear to be the result of both habitat filtering and dispersal limitation (Benone et al., 2020). And now, using a state-of-the-art eDNA approach, Coutant et al. (2021), add one more piece to the puzzle for the rivers of the Guiana Shield. Besides the novelty of their study in showing the greater role played by dispersal limitation in shaping fish diversity in rivers, their approach also emphasizes the need for multifaceted approaches to measuring biodiversity. Both functional and taxonomic diversity are essential for our understanding of the ecological patterns of Amazonian fish.

The use of eDNA to detect species in freshwater systems has been widely applied, including in the Neotropics (Cilleros et al 2019; Sales et al 2021). However, the large-scale implementation of eDNA techniques in hyperdiverse tropical regions still faces multiple challenges. For instance, molecular identification of the highly diverse ichthyofauna is still not achievable for most taxa. This is particularly true for the Amazon River basin, which hosts more than 2,700 fish species and several other thousands yet to be discovered (Dagosta & De Pinna, 2019). Moreover, the use of a short eDNA fragment to identify specimens to the species level is challenging, since a curated reference library is needed while the selected molecular marker should also have enough variation to discriminate the entire ichthyofauna. Nonetheless, in some regions such as the river basins studied by Coutant et al. (2022), knowledge from previous studies, including a reference database, traditional fish inventories and ecological studies (Cilleros et al 2019) together with testing for eDNA protocol robustness (Coutant et al 2021), helped overcome such challenges. For instance, 92% of the 400 freshwater fish species described in French Guiana are represented in their local and curated database of the 12S mtDNA fragment, and only 9 species could not be differentiated to the species level (Coutant et al. 2022).

Environmental DNA (eDNA) metabarcoding has great potential for biomonitoring of complex biodiversity, a use for which the term Biomonitoring 2.0 has been christened (Baird & Hajibabaei 2012). Traditional assessments of biodiversity can be logistically challenging and consequently restricted in their spatial and temporal

resolution. To bypass current limitations in taxonomic assignments in eDNA approaches due to incomplete or erroneous reference databases, taxonomy-free approaches are considered an option for biomonitoring at the level of operational taxonomic units (OTUs). Decision-making and best practices for taxonomy-free environmental DNA metabarcoding have been proposed (Mächler, E., Walsler, J. C., & Altermatt, F. 2021). However, distinct bioinformatic treatments and filtering thresholds may affect conclusions resulting in large differences in diversity estimates. Moreover, the use of taxonomy-free approaches instead of species level identification of the eDNA output would hamper the use of the Coutant et al. (2022) eDNA framework to estimate and predict taxonomic and functional biodiversity distribution in species-rich environments and unexplored areas. The Coutant et al. (2022) approach highlights the importance of the species level resolution in eDNA studies and a proper local curated database.

It is unlikely that eDNA will replace traditional biodiversity assessments that provide unique and valuable ecological information, especially in biodiverse-rich ecosystems. However, Coutant et al. (2022) shed light on the great potential of combining knowledge from traditional and state-of-the-art methods in assessing biodiversity. In addition to being a valuable tool for unraveling distribution patterns of diversity in the Amazon, eDNA also has great potential for scaling up the monitoring of freshwater biodiversity throughout the Amazon biome. The framework proposed by Coutant et al. (2022) will inspire future studies that will improve our understanding of complex ecological systems, as well as assist in conservation planning of freshwater ecosystems. This is urgently needed in Tropical regions, particularly the Amazon, which host most of the global freshwater biodiversity and are undergoing rapid change due to human activities.

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