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*Abstract*

# Evaluating Environmental DNA Efficiency in the Detection of Freshwater Species in a System with High Endemism †

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**Abstract:** Freshwater fish diversity is highly imperiled due to environmental change, habitat destruction, over-fishing and invasive species. Consequently, it is crucial to develop efficient and easy-to-standardize tools to describe these communities. Traditional fishing methods might not be able to capture all-size and well-hidden organisms, which can lead to an underestimation of biodiversity. In theory, it is possible to detect all species present in a certain locality by sequencing DNA traces left in the environment (eDNA). This approach is especially promising in freshwater systems, which may function as eDNA reservoirs of their respective drainage area. In this context, eDNA metabarcoding is the established method where amplicons for a specific locus are sequenced. However, primers affinity towards certain taxa can lead to false negatives and biased species abundances estimates. In this study, we tested eDNA ability and efficiency in detecting fish species in a threatened and highly endemic ecosystem: the Portuguese area occupied by the Tagus Basin. DNA present in the collected water samples from the Tagus River was amplified with 12S rRNA gene primers and sequenced with Illumina MiSeq technology. Primer choice was previously carried out by in silico PCR test which evaluates primers taxonomic coverage and species identification reliability. The obtained sequences were converted to 312 Amplicon Sequence Variance (ASV), filtered to decrease contamination errors, and compared to different reference databases. Obtained results were compared with conventional methods, such as electric fishing, considering that differences might shed light on which factors constrain species detection using eDNA metabarcoding. Preliminary results show congruences in fish species distributions between electrofishing and eDNA metabarcoding. However, eDNA metabarcoding detected exotic species that had not been previously detected in the Tagus basin, but which are known to exist in other Iberian basins: the South American cichlid—***ustraloheros facetus***—and a European leuciscid—***Squalius cephalus***. Nevertheless, traditional methods were more efficient in describing fish communities at some sites along the basin, showing the importance of using both approaches in a complementary way.

**Keywords:** eDNA metabarcoding; freshwater biodiversity; Tagus basin

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