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Methodological developments and integration of environmental DNA metabarcoding in fisheries research and augmented fish community monitoring

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Abstract

Environmental DNA (eDNA) analysis has been increasingly used in the past decade to study both terrestrial and aquatic ecosystems. In particular, eDNA metabarcoding presents itself as an extremely useful tool for minimally invasive species richness monitoring, finding applications in both conservation and fisheries science. Moreover, novel technologies such as portable high throughput sequencers are enabling near-real time and on site eDNA analysis, thus significantly reducing the sampling-to-results time and facilitating molecular analysis in remote field conditions. However, eDNA-based methods still face limitations, both methodological and in the interpretation of results, thus protocols must be optimised before the routine use of eDNA in megafauna monitoring and stock assessments. The work presented in this thesis focused on methodological aspects of the eDNA metabarcoding workflow and its applications to study fish communities. Decontamination practices for eDNA sampling aboard research vessels were examined, showing that residual bleach can impact the recovery of DNA quantity and species richness in environmental samples. Quality check steps, including inhibition testing and accurate quantification of fish DNA, were applied, providing recommendations towards methodological standardisation. In addition, more commonly used (Illumina) and novel (Nanopore) sequencing platforms were compared, as well as two metabarcoding mitochondrial genes, 12S rRNA and Cytochrome Oxidase *c* Subunit I (COI), commonly used for fish community profiling. The latest Nanopore kits were deemed equivalent to Illumina sequencing in terms of species richness, with a long 12S marker (~650 bp) providing high taxonomic resolution. Moreover, Nanopore sequencing was applied to investigate a mesopelagic fish layer in the Northeast Atlantic Ocean. Combining molecular and fishing data the layer was described as mainly composed by a key mesopelagic fish, *Maurolicus muelleri*, with ancillary species described by both methods. Similarly, eDNA data was integrated with underwater imaging data from the cabled OBSEA observatory in the Mediterranean Sea. eDNA data identified both economically and ecologically important species with different living/foraging trends and the integration of both methods led to an augmented community assessment. Knowledge produced by this research has highlighted challenges and advances of eDNA metabarcoding. The presented workflow using novel portable technology shows potential to be included in the further developments of tools for *in situ* near-real time eDNA metabarcoding. Ultimately, these tools, combined with cabled observatories and automated samplers, will be pivotal for the integration of eDNA in augmented biodiversity and ecological monitoring, as well as fisheries science.