

## Environmental DNA as a cetacean monitoring tool in the Northern Coast of Continental Portugal

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In recent decades, environmental DNA (eDNA) has emerged as a utopian monitoring tool in the marine conservation landscape, for its potential to collect data on presence and abundance of biological communities with insufficient knowledge and/or difficult access. In the ATLANTIDA Project, this tool is being optimized with the ultimate goal of detecting and identifying cetacean species on the northern coast of continental Portugal without relying on visual monitoring. To this end, a molecular biology-based methodology is being developed and tested in positive control samples, consisting of a mixture of DNA extracted directly from muscle tissue or gums of cetaceans with eDNA samples collected in ATLANTIDA dedicated at-sea surveys. Firstly, *in silico* analysis using metagenomic data was performed to assess the pair of primers directed to mitochondrial DNA presenting the highest variability and, consequently, a higher probability of distinction among the target species. For that purpose, different sets of primers previously described in the bibliography were analysed and unique single nucleotide polymorphisms between the species of interest were counted. After the selection of the most suitable primer set, several optimization tests have been conducted, through conventional PCR, in order to identify the most efficient DNA amplification protocol for the gene of interest. From these tests, we were able to identify the ideal annealing temperature, the best TAQ polymerase enzyme, and the detection of cetacean DNA up to very low concentrations (~5ng/μL) was achieved, with salt water not inhibiting the reaction. In environmental samples, we were still not able to detect cetacean DNA, probably because of its concentration below our detection limit in the samples tested. In conclusion, although the effectiveness of resorting to eDNA for cetacean monitoring programs remains unclear, these results represent a step forward towards that goal.