Development of a standardized method for the detection of freshwater target species via environmental DNA

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Abstract: Over the last decades we witnessed a significant increase in the use of methods based on the analysis of environmental DNA (eDNA). All organisms continuously release their DNA in the environment, which can be used for conservation purposes, such as the rapid and non-invasive detection of threatened taxa and for monitoring the presence of alien and pathogenic ones of which these are vectors. This approach, compared to the canonical monitoring methods, offers a lot of advantages not only in terms of time and resources used, but also at the analytical level, since it is very sensitive and scalable (from target-specific to untargeted assays, from area-specific to wide scale monitoring).

The purpose of this study is to create a standardized approach, based on the use of taxa-specific probes, which allows the identification of 26 italian freshwater target species and of 3 pathogen infecting amphibians and fishes (*Batrachochytrium dendrobatidis*, *Saprolegnia* spp. and *Ranavirus*). The investigated target species encompass amphibians, reptiles, mammals, fishes, mollusks, decapods, and odonata. For 16 of these, there is not a detection test already developed. During this project, tissue and environmental samples belonging to the taxa of interest were collected in various freshwater sites of Lombardia and Lazio, Italy. The different primer pairs used for the detection, made *ex novo* or already developed, were first tested *in silico* (using bioinformatics tools), then *in vitro* (on tissue samples) and finally in an operative environment (on water samples).

The results obtained show that this approach is effective in the identification of target organisms even with very low DNA concentrations and in presence of sympatric congeneric species. However, detection limits have also emerged, especially for species with an exoskeleton, which release low DNA amounts in the environment. Using multi-marker approaches and the creation of new reference molecular datasets will improve the development of more sensitive and specific assays.