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Conference Abstract

The use of multiple markers and internal positive controls significantly improves species eDNA detection rates and data reliability

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Abstract

In recent years, environmental DNA analyses became increasingly integrated to detect and monitor the presence and abundance of rare organisms, especially in inaccessible aquatic habitats. Although it is generally proven that detection probabilities of eDNA surveys exceed those obtained via conventional techniques, these molecular approaches are, however, also subjected to detection limitations and levels of uncertainty. Besides improvements that can be made in terms of sampling design, volumes of filtered water, and the effective quantity of DNA that is finally analysed, the sensitivity of eDNA surveys is inherently determined by the number of target eDNA copies suspended in the water column. Here we show that multiplexing different primer/probe assays for the same species, but targeting amplicons situated at different loci, is a surprisingly overlooked aspect that can substantially contribute to reduce these limitations and increase the sensitivity of single-species detections. By empirically testing a large number of natural eDNA samples via ddPCR, we reveal that the use of multiple markers can significantly lower the LOD and LOQ of rare and elusive species, such as the invasive American bullfrog and the endangered European weather loach in a variety of different water bodies, such as ponds, lakes, streams, canals, etc. Especially at very low eDNA concentrations of both target species, our results showed that analysing mulitple loci significantly increased

detection probabilities and lowered stochasticity effects, and thus ultimately reduces PCR costs when analysed in multiplex. The validation and use of more than one assay taregtting a single species, may further increase the confidence of positive detections. Finally, we illustrate that the implementation of internal positive controls (IPC's), is an absolute must for accurate validation of eDNA workflows and reliable interpretation of the generated data. IPC’s not only help to track down degraded and inhibited samples, to avoid false-negative detections, it also offers insights into extraction efficiency, indispensable for accurate quantification of population densities. Overall, our findings provide strong support that the multiplexing of multiple markers on different loci in combination with the use of internal positive controls ensures increased detection rates at very low eDNA concentrations and generates more robust and reliable data.

Keywords

ddPCR, LOD, LOQ, primer/probe assays, single-species detection.

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