ARPHA Conference Abstracts 4: e65460
doi: 10.3897/aca.4.e65460

Conference Abstract

Optimization of eDNA metabarcoding methodology for the biomontoring of the ichthyofauna in the Eastern Mediterranean Sea

Panagiotis Kasapidis‡, Christina Karli‡

‡ Hellenic Centre for Marine Research, Heraklion, Greece

Corresponding author: Panagiotis Kasapidis (kasapidi@hcmr.gr)

Received: 03 Mar 2021 | Published: 03 Mar 2021

Citation: Kasapidis P, Karli C (2021) Optimization of eDNA metabarcoding methodology for the biomontoring of the ichthyofauna in the Eastern Mediterranean Sea. ARPHA Conference Abstracts 4: e65460. https://doi.org/10.3897/aca.4.e65460

Abstract

Environmental DNA (eDNA) metabarcoding is a relatively new methodology for the detection of organisms in an environmental sample, with emerging applications in the fields of ecology, conservation, invasive biology, biomonitoring and more. Several studies are using eDNA nowadays, yet in the Mediterranean marine ecosystems, its use is limited mostly on plankton studies so far. The icthyofauna of the Eastern Mediterranean is undergoing major changes due to biological invasions, mainly form the Red Sea, in combination with the climate change, and a reliable high-throughput biomonitoring tool is essential to monitor these changes.

The main goal of this study was to develop a reliable eDNA metabarcoding protocol to study and monitor fish biodiversity in the oligotrophic ecosystems of the Eastern Mediterranean. The study had two parts: a) standardization of the method by testing two different sets of primers in aquaria with known fish species assemblages, and b) estimation of the heterogeneity of fish eDNA distribution in different habitats within a coastal area, in order to determine the most efficient sampling strategy. In both cases, samples were analysed through Next Generation Sequencing on a Illumina MiSeq platform.

To standardize the method, we sampled and filtered water from two tanks of the 'Cretaquarium'. We tested two different sets of primers, one for 16S rRNA and the MiFish

primers of Miya et al. (2015) for 12S rRNA, in order to estimate their efficiency in assessing species' composition both qualitatively and semi-quantitatively. Both primer sets performed well and most taxa in both tanks were detected up to species level, with 16S marker exhibiting higher resolution. A rather weak correlation was also detected between actual fish biomass and relative abundance as estimated by eDNA metabarcoding.

To estimate the eDNA heterogeneity in natural ecosystems, we sampled water in a coastal ecosystem over three distinct types of habitats: hard substrate, soft substrate, *Posidonia* meadows, as well as in the mid of the water column. Three samples per habitat were collected, two PCRs per DNA extract were performed and results were obtained only for the 16S marker. A total of 69 taxa were detected, with 55 of them distinguished at the species level, while in each sample the number of taxa detected ranged from 13 to 27. *Posidonia* meadows and the water column samples showed the greatest heterogeneity, in contrast to the hard and soft substrate samples that showed little differentiation both within and between habitat type. Based on these results, an improved protocol should include more technical PCR replicates per sample (at least 3 PCRs), at least one sample per habitat in each area, and a larger volume of water filtered per sample or alternatively, more samples mixed together in order to achieve better representation of the community.

Moreover, it was apparent the need of a more complete and curated reference database for the Mediterranean fishes for the aforementioned markers, in order to be able to reliably identify fishes of the Mediterranean ecosystems at species level.

In conclusion, the method seems to work well, and with some small improvements, as well as with the complementation of the respective reference databases, it can be used as a reliable tool for the study of biodiversity and biomonitoring of fish communities of the oligotrophic ecosystems of the Eastern Mediterranean Sea.

Presenting author

Panagiotis Kasapidis

Presented at

1st DNAQUA International Conference (March 9-11, 2021)

Hosting institution

Institute of Marine Biology, Biotechnology and Aquaculture - Hellenic Centre for Marine Research

Author contributions

PK conceived and supervised the project and