Testing COI primers for ichthyoplankton metabarcoding and their capability to assess local mesozooplankton communities

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Abstract

DNA metabarcoding is particularly helpful for monitoring taxonomically complex communities and hard to identify morphologically, such as several zoo and ichthyoplankton, which contain eggs and larval stages of unknown species. However, the efficiency of metabarcoding in diversity recovery is dependent on the targeted genetic markers and primers employed. In this work, we compared the performance of three different primer pairs from cytochrome oxidase subunit I (COI) genetic marker in species detection from marine mesozooplankton samples and its potential to be implemented in biomonitoring programs. We employed the mlCOIintF/LoboR1 primer combination targeting marine metazoans, and two newly designed fish-specific primer cocktails for targeting the ichthyoplankton. Mesozooplankton samples were collected at 4 locations on the Portuguese coast – 1 in the northwest (Viana do Castelo) and 3 in the south (coastal lagoons of Ria de Alvor and Ria Formosa, and in the river Guadiana estuary). Bulk community DNA was extracted using a non-destructive protocol and amplicon libraries produced for the 3 primers combinations. After quality-filtering bioinformatic steps, we obtained 3.04×10^5 usable sequences, of which 76.26% were clustered into OTUs (operational taxonomic units) and 46.30% were identified at species level - corresponding to 103 taxa from 8 different metazoan Phyla. The most diverse classes were *Malacostraca*, *Actinopterygii,* and *Copepoda*. As expected, the generic primer pair for marine metazoa (mlCOIintF/LoboR1) retrieved a higher number of species (94) compared with the fish-specific primer cocktails (30). Nevertheless, 9 % of the total species were identified exclusively by the cocktails, of which 42% were fish. These results confirmed the potential of metabarcoding as a tool for profiling zooplankton communities and to assess ichthyoplankton diversity. Multiple primers pairs increased

species detection from different taxonomic groups, being the protocol optimization for fish-specific primer cocktails, the next step for its implementation in fish stock assessments.

Keywords

metabarcoding, ichthyoplankton, mesozooplankton, cytochrome oxidase subunit I, biomonitoring

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Conflicts of interest