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The "MetaCopepod" project: Designing an integrated DNA metabarcoding and image analysis approach to study and monitor the diversity of zooplanktonic copepods and cladocerans in the Mediterranean Sea.

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The timely and accurate analysis of marine zooplankton diversity is a challenge in ecological and monitoring studies. Morphology-based identification of taxa, which requires taxonomy experts, is time consuming and cannot provide accurate resolution at species level in several cases (e.g. immature stages, cryptic species, broken specimens). The "MetaCopepod" project is aimed at overcoming these limitations by developing a high-throughput and cost effective methodology that integrates DNA metabarcoding and image analysis. Utilizing the accuracy of DNA metabarcoding in species recognition and the quantitative results of image analysis, zooplankton diversity (mainly of copepods and cladocerans) is assessed both qualitatively (species' composition) and quantitatively (abundance, biomass and size-distribution). To achieve this goal, bulk zooplankton samples are first scanned and analyzed with ZooImage and then massively sequenced for a selected fragment of the mitochondrial 16S rRNA gene. Through a bioinformatic pipeline, sequences are compared to a reference genetic database, constructed within the project, and identified at species-level. The methodology was calibrated by using both mock and taxonomically identified samples and demonstrated on samples collected monthly from monitoring stations across the Mediterranean Sea. It is currently optimized for higher integration and accuracy and is expected to become a powerful tool for monitoring zooplankton in the long term and for early warning of bioinvasions and other ecosystem changes.

Keywords: DNA metabarcoding, image analysis, Mediterranean Sea, zooplankton, Copepoda, Cladocera

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