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## Multi-marker metabarcoding approach to study mesozooplankton at basin scale

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Zooplankton plays a pivotal role in marine ecosystems and the characterisation of its biodiversity still represents a challenge for marine ecologists. In this study, mesozooplankton composition from 46 samples collected in summer along the western Adriatic Sea, was retrieved by DNA metabarcoding analysis. For the first time, the highly variable fragments of the mtDNA COI and the V9 region of 18S rRNA genes were used in a combined matrix to compile an inventory of mesozooplankton at basin scale. The number of sequences retrieved after quality filtering were 824,148 and 223,273 for COI and 18S (V9), respectively. The taxonomical assignment against reference sequences, using 95% (for COI) and 97% (for 18S) similarity thresholds, recovered 234 taxa. NMDS plots and cluster analysis divided coastal from offshore samples and the most representative species of these clusters were distributed according to the dominant surface current pattern of the Adriatic for the summer period. For selected sampling sites, mesozooplankton species were also identified under a stereo microscope providing insights on the strength and weakness of the two approaches. In addition, DNA metabarcoding was shown to be helpful for the monitoring of non-indigenous marine metazoans and spawning areas of commercial fish species. We defined pros and cons of applying this approach at basin scale and the benefits of combining the datasets from two genetic markers.

The complexity of taxonomic composition, morphology, size range, life cycle and trophic role of zooplankton are probably unique in the marine world. Zooplankton is very important in the food webs of both marine- and fresh- water ecosystems, supporting fisheries and mediating fluxes of nutrients and chemical elements. The availability of zooplankton, food for fish larvae, is believed to be an essential factor in determining the success of fish recruitment. Any event, a decline in the zooplankton population may have far-reaching effects on the ecosystem and the economy<sup>1</sup>. Moreover, zooplankton can contribute to the role of marine systems as sources or sinks of CO<sub>2</sub> and other greenhouse gasses<sup>2</sup>. Zooplankton communities are highly diverse and vary in their susceptibility to environmental stressors, such as exposure to toxic chemicals, acidification, eutrophication and oxygen depletion, or changes in temperature. As a result, the knowledge of species assemblages of the zooplankton is crucial for providing insights on the status of marine ecosystems. However, deriving qualitative and quantitative information on zooplankton composition requires intensive and highly trained labour. Classical microscopy methods are time consuming and require a high degree of taxonomic expertise and, in several cases, species level identification cannot be achieved, especially where early life stages are difficult to connect with adult forms. During the last few years, DNA barcodes provided a support to taxonomists that traditionally rely on a complex array of morphological characters to describe and discriminate species<sup>3</sup>. In this context, the growing database of DNA barcodes linked to species names and morphological characters for marine zooplankton may be considered as a “Rosetta Stone” for decoding patterns of species diversity in the pelagic realm<sup>4</sup>. DNA barcodes are also useful to discover new species, reveal cryptic species, and assess taxonomically significant variation within species with broad or disjointed distributions<sup>5</sup>.

The introduction of high-throughput sequencing (HTS) technology based on loop array sequencing allows for analysis of a large number of samples simultaneously. Its large scale sequencing capacity and low costs holds

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