Environmental DNA Sampling of Zooplankton Improves upon Arthropod Biodiversity Identification

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INTRODUCTION

Biodiversity assessment, including a complete accounting of species richness, is required to preserve what is known about our natural world (Appeltans et al. 2012, Mora et al. 2011). Marine zooplankton, including holopelagic and invertebrate larvae, are estimated to be undercounted by as much as 70-90% in most studies because of lack of taxonomic expert knowledge and the presence of many cryptic or new species (Maslakova et al 2022). Traditional methods relying on hand sorting and morphological identification is tedious and time consuming, leaving a need for more efficient technology, such as eDNA to assist in rapid documentation of biodiversity before it is lost to climate change.

Even for arthropods, the most abundant phylum in near-shore surface zooplankton, increased documentation is needed to account for the high amounts of species with undescribed larvae. In Pacific Northwest waters, nearly one third of decapod species have no larval descriptions and for Cirripedia only 12 of the total 29 species identified in this region have larval stage descriptions (Shanks 2001). To support this needed discovery, we applied a new method using eDNA to examine its utility for identification and documentation of zooplankton. We applied this method to the Arthropod fraction of zooplankton collected at Friday Harbor Laboratories (FHL), WA because it has well-known marine invertebrate fauna.

Figure 1. *Balanus sp*., a nauplius larva that may be one of the many unidentified species of barnacle larvae.

Figure 2. Pinnixia sp. zoea with extra long rostral spine collected at night. Presently unknown to any genetic databases!

SPECIES RICHNESS BY TIME

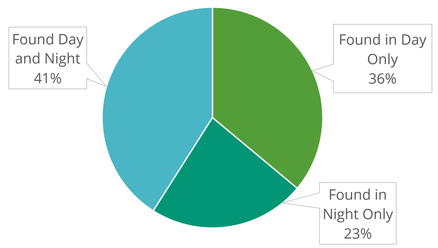

Figure 3. Species richness for arthropods collected in day and night samples.

METHODS

Plankton Collection: Samples were collected on June 28, 2021, at noon and at 11:30pm in Friday Harbor, WA by hand tow of a 153 µm mesh plankton net. Tows were taken directly parallel to the University of Washington's Friday Harbor Labs dock and along the entrance to Beaverton Cove.

Sample Preparation: Each tow was gently mixed and divided in half by volume, one half preserved for metabarcoding, and the other half manually sorted. The samples designated for eDNA were filtered with a Strivex filter and frozen in preparation for sequencing. PCR of the metabarcode and direct sequence samples were performed by the Smithsonian Institution's Laboratory of Analytical Biology. Cytochrome oxidase subunit one was used as the gene marker due to its high conservation across animals and the extensive databases of previously identified sequences (Bucklin et al 2011).

Direct Sequencing and Sorting: Invertebrate Zoology students sorted zooplankton into morphological categories with unique morphospecies imaged and preserved in genetic grade ethanol for direct sequencing.

Data Processing: Reads produced from the eDNA and direct sequencing were trimmed and edited using Geneious Prime 2.0 with default settings. Contigs were filtered by quality with length set to ≥250 base pairs with 95% sequence identity match. Resulting contigs were compared through several BLAST searches to NOAA's COI-arbitor database, Midori, GenBank, and prior marine invertebrate eDNA results from FHL to identify operational taxonomic (OTU). Species richness was identified from the results for the total sample and for arthropods.

RESULTS

We found that a total of 198 OTU's were recovered between both the day and night samples of which 30% (n=59) were arthropods. Arthropoda were the most abundant phylum regardless of identification technique or time of day.

- There were distinct differences between the species present in the day and night samples; 36% (n=22) of the arthropods were only found in the day sample while 23% (n=14) was found at night only (Figure 3). There was only a 41% (n=25) overlap of OTU's between the two times of day.
- Hand sorting recovered 22% of the arthropods not found in metabarcoding with direct sequencing of arthropod plankton doing a better job on large larvae such as zoea and megalopae (Figure 4).

We found that 36% of identified unique arthropod OTU's are currently unknown to existing databases, which is surprising considering the long history of invertebrate larval study and DNA barcoding at FHL (Figure 5).

SPECIES RICHNESS BY METHODS

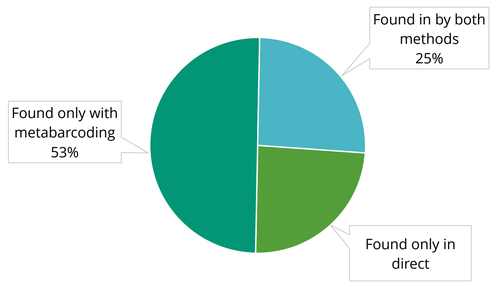

Figure 4. Species richness by eDNA and metabarcoding or hand sorting and direct sequencing.

ARTHROPOD SPECIES RICHNESS

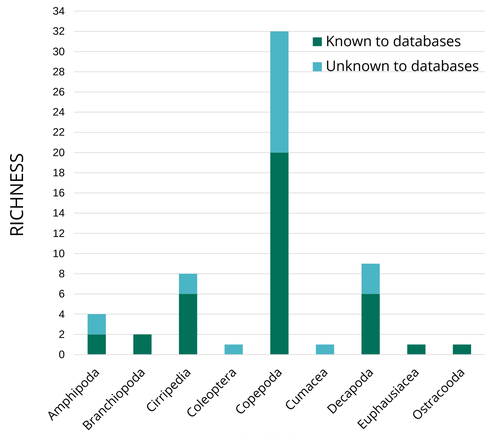

Figure 5. Total species richness identified both samples and techniques. OTU's currently known to existing sequence banks are shown in dark green and OTU's unknown to databases are shown in light blue for each arthropod order.

CONCLUSION

The richness results for both samples and techniques support the validity of eDNA in accurately documenting the full content of zooplankton samples (Figure 4). The overlap and differences between the samples collected at night and during the day also support its recognition of changing plankton assemblages and ecosystems depending on the time of day (Figure 3).

While we found support of the use of eDNA for arthropod zooplankton, our results also call out the need for hand sorting and imaging to improve existing databases and make more accurate assessments. This was highlighted by the decapods which were missed or absent in the eDNA but were captured by direct sequencing. Increasing our understanding of which arthropod species are present in plankton is valuable for monitoring commercially valuable species and for species such as copepods that serve as a key primary consumer.

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