Zooplankton Biodiversity is enhanced by eDNA

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ABSTRACT

The microscopic plankton community is an understudied, yet vital component of pelagic food webs. Much of the summertime zooplankton community consists of indirect developing larvae that feed on other plankton and are dispersed by currents. When the zooplankton community is included in biodiversity estimates of marine invertebrates through direct sequencing of unique larval morphotypes, biodiversity and species richness is greatly increased. However, direct sequencing and identification of individual zooplankton is a time-consuming and labor-intensive process. This is unsustainable if we want to understand Earth's biodiversity before climate change fully alters ecosystems. We used a new tool for biodiversity sampling, environmental DNA (eDNA) with metabarcoding, in comparison with manually picking and direct sequencing unique zooplankton morphotypes. Half our samples were sorted for unique morphotypes, imaged and then directly sequenced for Cytochrome oxidase subunit one (COI). The other half of our samples (eDNA) were submitted for next generation sequencing of COI and metabarcode analysis. We found that metabarcoding is superior to traditional hand-picked, directly sequenced morphotypes, recovering 70% more OTUs than direct sequencing and a higher Shannon-Wiener index value. However, traditional methods also found 25 additional morphospecies not recovered by metabarcoding. Both methods are useful and important for accurate biodiversity documentation.

annelid plankton voucher image

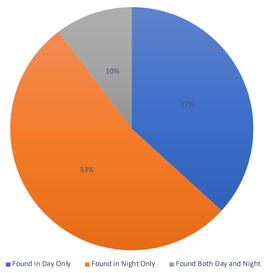

Figure 1. Shows the species richness for annelids found in only the day/night or both

METHODS

Plankton Collection: Samples were collected from just below sea surface by hand tow of a plankton net with 153 um mesh on July 1, 2021, at noon and at 11:30pm in Friday Harbor Laboratories, San Juan Island, WA. Tows were taken directly parallel to the University of Washington's Friday Harbor Labs dock. Each tow was a 5-minute surface tow (≤ 1m).

Sample Preparation:
Each tow was gently mixed and divided in half by volume. One half was manually sorted for unique zooplankton morphotypes which were imaged and preserved in molecular grade ethanol for direct sequencing. The other half of the sample was processed for eDNA and metabarcoding by filtering through a Strivex filter, which was submerged in 100% EtOH. The preserved samples were handled by Smithsonian Institute's Laboratories of Analytical Biology (SILAB), which performed the extractions, PCR and NGS sample preparation. We chose to target the Cytochrome Oxidase Subunit I (COI) mitochondrial gene, also known as the animal barcode gene, due to its broad conservation across animals, the utility of a barcode gap to identify putative species (Herbert et al 2003, Bucklin et al 2011), and the extensive database of previously identified sequences we could use for comparison.

Data Processing
Reads returned by SILAB were trimmed and edited using Geneious Prime 2.0 with BBDuK plugins and default settings. Contigs were filtered by quality and length to ensure correct operational taxonomic unit (OUT) assignment, which was set to 95% identity and a sequence length of 200 bp. Resulting contigs were compared through several levels of hierarchical blast searches including a data base of marine invertebrates from the FHL docks and surrounding environment, Midori, a data base of all COI animal sequences released in February 2022, and GenBank. Sequences which could not be classified by any of these three data bases were run through an NCBI-hosted Blast nr-search. Contigs were classified if they had 95% or greater identity to a known sequence.

RESULTS

198 morphospecies and/or OTUs were recovered of which 23% (n=46), which is the second most species rich phylum in this study. There were distinct differences between the species present in the day and night samples (Figures 2, 3) ; 37% (n=25) of the annelids were only found in the day sample, with 12 of those being new to the database. 53% (n=36) found at night only, with 17 of those being new. Only 10% were found both day and night (n= 17).

eDNA and metabarcode analysis recovered 27% of our annelid OTUs, including invasive species of the Pseudopolydora which were not found by manually sorting through the plankton. Hand sorting and direct sequencing recovered only 4% of the annelids not found in metabarcoding demonstrating the need for both techniques. eDNA picked up OTUs that were missed because of rarity or crypsis. Finally, we found that at last 63% (n=29) of our annelids were either new species or had never been sequenced and deposited in public databases or represent cryptic species.

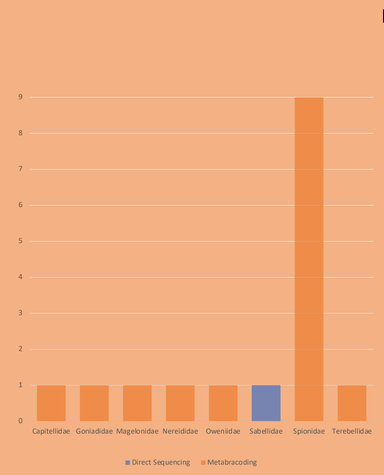

Figure 2. Compares the direct sequencing to metabarcoding for annelids by phyla during the day.

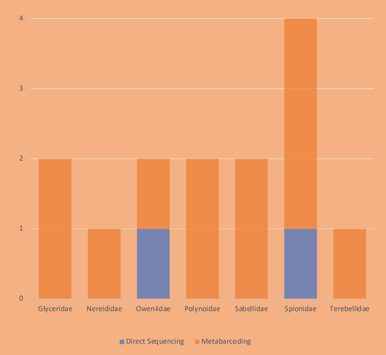

Figure 3. Compares the direct sequencing to metabarcoding for annelid by phyla during for the night.

DISCUSSION

The results of the abundance percentages for both samples and techniques support the validity of eDNA in accurately documenting the full content of zooplankton samples. The differences between the samples and minimal overlap between the OTU's identified collected at night and during the day also support its recognition of changing plankton assemblages and ecosystems depending on the time of day. While this supports the use of eDNA for zooplankton it also shows the need for continued biodiversity documentation.
In monitoring changes in zooplankton we recovered 46 different species of annelids, only 21 of which we were able to assign to a scientific name, including two species of invasive polychaetes from Peter the Great Bay and the Sea of Japan. As databases of COI barcode sequences are added metabarcoding will be increasingly useful for monitoring zooplankton communities during climate change and for the detection of invasive species.

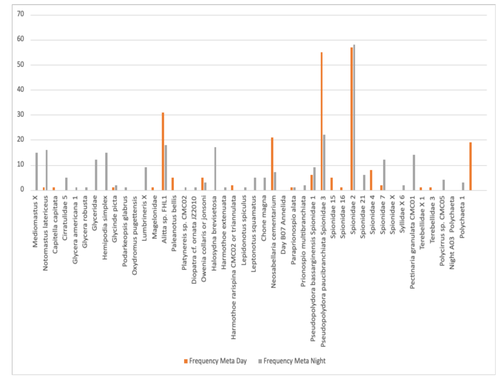

Figure 4. Graph shows the species richness of Annelida found at Friday Harbor

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