Integrating Environmental DNA and Traditional DNA Barcoding Reveals Higher Zooplankton Species Richness

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Introduction

Zooplankton are integral to marine food webs, energy transfer, and nutrient cycling, yet their biodiversity remains poorly documented (Maslakova et al. 2022). Environmental DNA (eDNA) metabarcoding offers a rapid way to assess species richness, but its performance relative to traditional approaches remains under evaluation (Ohnesorge et al. 2023). We compared hand-sorted zooplankton samples that were DNA barcoded to bulk eDNA samples that were metabarcoded at Friday Harbor Laboratories, WA, USA. Previous sampling data from 2021 was run through updated databases revealing new and previously unidentified species at Friday Harbor Laboratories.

Both methods recovered diverse operational taxonomic units (OTUs), but each captured unique subsets of the community. eDNA was highly effective for broad biodiversity discovery, while hand sorting with direct sequencing added taxa and generated voucher specimens with images. Reanalysis revealed new species not thought to be present before. Combining these complementary approaches yields a more comprehensive picture of local zooplankton diversity and strengthens future eDNA-based biodiversity assessments.

Materials and Methods

Surface plankton tows (153 µm mesh, 5 min) were performed midday and again near midnight Summer 2021. Each tow was split evenly by volume. One portion was filtered through a Sterivex cartridge and preserved in ethanol for eDNA analysis; the other was sorted manually to morphotypes and preserved for individual sequencing. Filtered samples underwent COI metabarcoding using established primers. Individually sorted specimens were Sanger-sequenced. Reads were quality-filtered in Geneious (≥95% confidence, ≥250 bp) and compared across four databases (Midori, GenBank BLAST nr, CoArbitrator, Wells et al. 2021). OTUs were accepted at ≥95% identity with sufficient sequence length. Review in 2026 used parameters of ≥97% confidence and ≥200 bp, drawing from local database derived NCBI COI sequence data from relevant animal phyla registered after November 1st 2021.

Areas of Focus

1. **Comparing direct sequencing & eDNA metabarcoding uncovers additional species richness for zooplankton**
2. **Multiple sampling approaches reveal new COI zooplankton diversity at Friday Harbor Labs docks.**
3. **Day versus night zooplankton communities show limited overlap but comparable species diversity.**

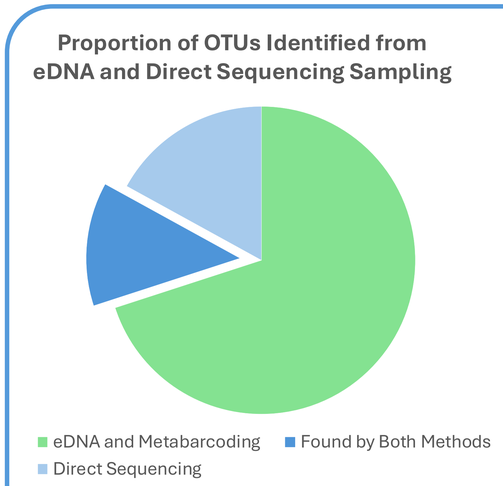

Figure 1: eDNA captured 70% of the total resulting OTUs, hand sorting with direct sequencing captured 17%. 13% of resulting OTUs were found by both methods.

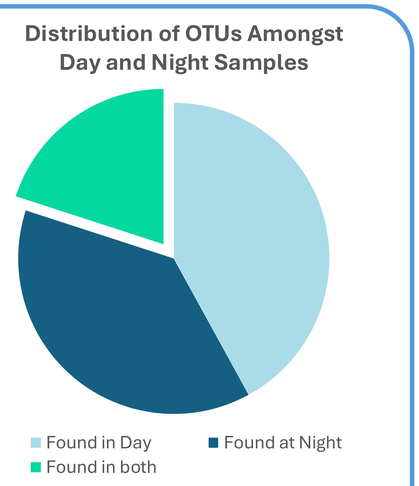

Figure 2: Day and night zooplankton OTUs differed by the species present with only 20% of all OTUs found in both samples.

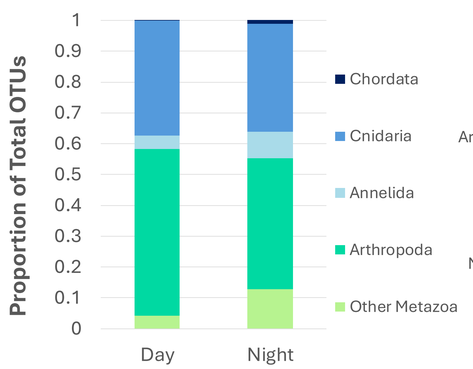

Figure 3: Proportional number of metazoan operational taxonomic units detected by each sampling period that were identified to the phylum level using the five reference sequence databases. Phyla that had <1.0% relative abundance were grouped as "Other Metazoa."

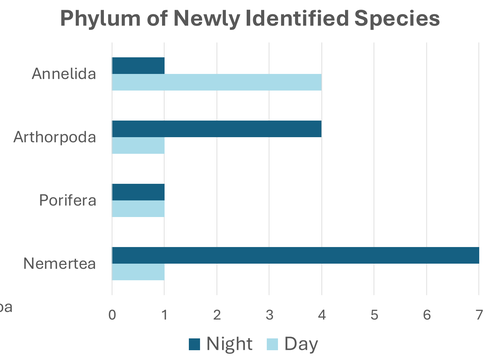

Figure 4: Number of new species found through an updated database, organized by phyla. The amount of Nemertean's found was disproportionate, making up 38% of the total organisms found. Reported species DNA sequences with ≤97% confidence and ≤ 200 bp were discarded.

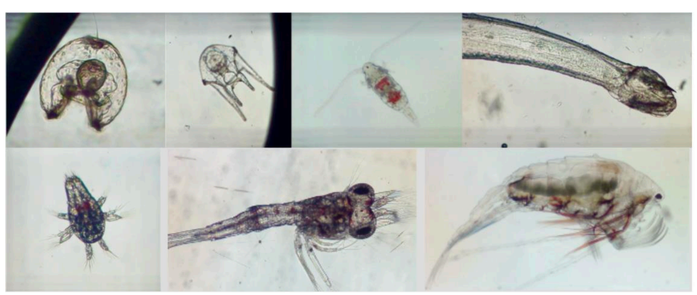

Figure 5: Examples of zooplankton imaged for DNA barcoding, magnification approximately 400X. Top row: Pluteus, Pilidium, Copepod, Chaetognatha. Bottom row: Copepod, Arthopoda, Arthropoda.

Results and Discussion

We recovered 198 animal OTUs, revealing a level of biodiversity not fully represented in current COI databases, as 47% of sequences lacked an identifiable match. eDNA metabarcoding accounted for most detections (~70% of total OTUs), and direct sequencing contributed unique morphotypes (17%) that did not appear in eDNA datasets. Updated analysis identified six new species. Of the 21 newly characterized organisms, 9 were classified as nemerteans. This odd proportion is attributed to DNA sequencing efforts made by a regional specialist, located off of the Oregon coast.

Temporal comparisons of night versus day showed partitioning, with only 1 in 5 OTUs occurred in both day and night samples. Day samples contained 37% unique OTUs, while night collections held even more (40% unique). Several taxa of ecological or economic interest appeared across datasets, along with unexpected invasive forms not previously documented by hand-sorting alone.

eDNA revealed most taxa, and direct COI barcoding contributed additional OTUs and validated identifications. Combined, they expose extraordinary species turnover across a single 24-hr cycle and demonstrate that "well-known" communities remain only partially understood. Future long-term sampling could track phenological shifts, invasions, and climate-driven change.

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References

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Ohnesorge A, John U, Kuczynski L, Neuhaus S, Beng KC, Krock B, Laakmann S. 2023. Roaming the seas—assessing marine invertebrate biodiversity along salinity gradients with zooplankton and eDNA metabarcoding. Evol Appl. 16(8): 1403-1417.