

Introduction

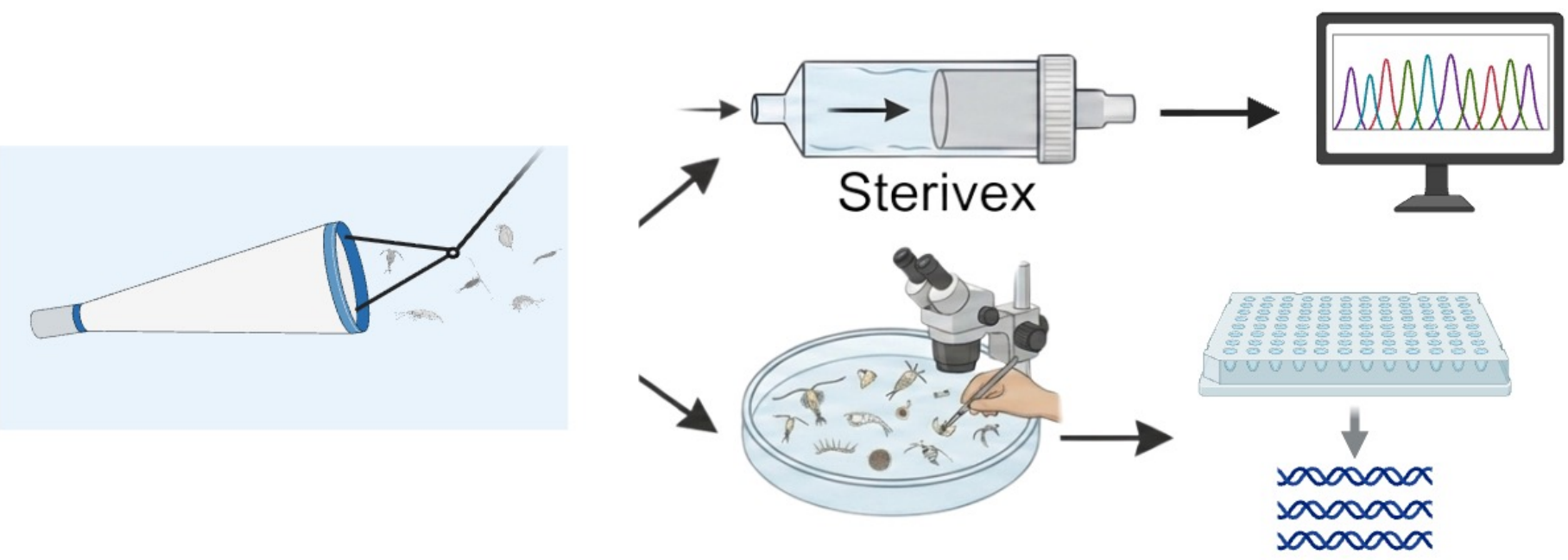
Zooplankton play central roles in marine food webs, energy transfer, and nutrient cycling, yet their biodiversity remains poorly documented. Environmental DNA (eDNA) metabarcoding offers a rapid way to assess species richness, but its performance relative to traditional approaches is still being evaluated. We compared hand-sorted zooplankton samples that were DNA barcoded to bulk eDNA samples that were metabarcoded at Friday Harbor Laboratories, WA, USA collected in 2021. These eDNA samples were re-queried against reference databases updated to 2026.

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, improving reference libraries. Combining these complementary approaches yields a more comprehensive picture of local zooplankton diversity and strengthens future eDNA-based biodiversity assessments.

The re-analysis uncovered previously unidentified species across both day and night samples. However, the newly identified species were subject to taxonomic bias reiterating that marine zooplankton remain understudied in reference databases, despite the long history of zoological research and routine DNA barcoding at FHL.

Materials and Methods

Sampling: Surface plankton tows (153 μ m 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.



Sequencing & Analysis: Filtered samples underwent COI metabarcoding using established primers. Individually sorted specimens were Sanger-sequenced. Reads were quality-filtered in Geneious ($\geq 95\%$ confidence, ≥ 250 bp) and compared across four databases (Midori, GenBank BLAST nr, CoArbitrator, Wells et al. 2021). OTUs were accepted at $\geq 95\%$ identity with sufficient sequence length.

- I. Comparing direct sequencing & eDNA metabarcoding uncovers additional species richness for zooplankton**
- II. Multiple sampling approaches reveal new COI zooplankton diversity at Friday Harbor Labs docks.**
- III. Day versus night zooplankton communities show limited overlap but comparable species diversity.**
- IV. eDNA datasets become more valuable as reference databases are improved and allow previously unidentified sequences to be assigned to species.**

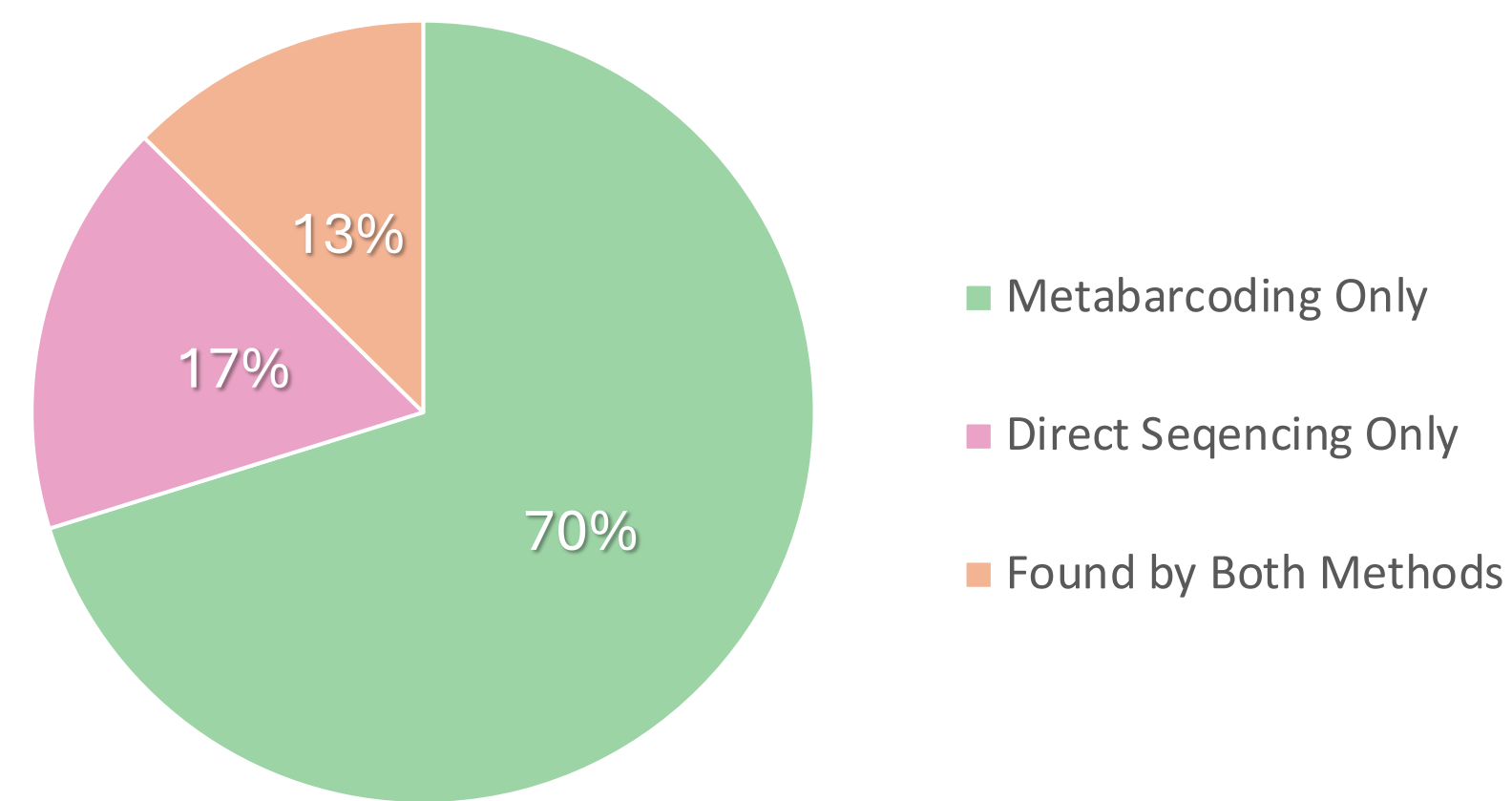


Figure I: eDNA captured 70% of the total resulting OTUs, hand sorting with direct sequencing captured 17%. 13% of resulting OTUs were found by both methods.

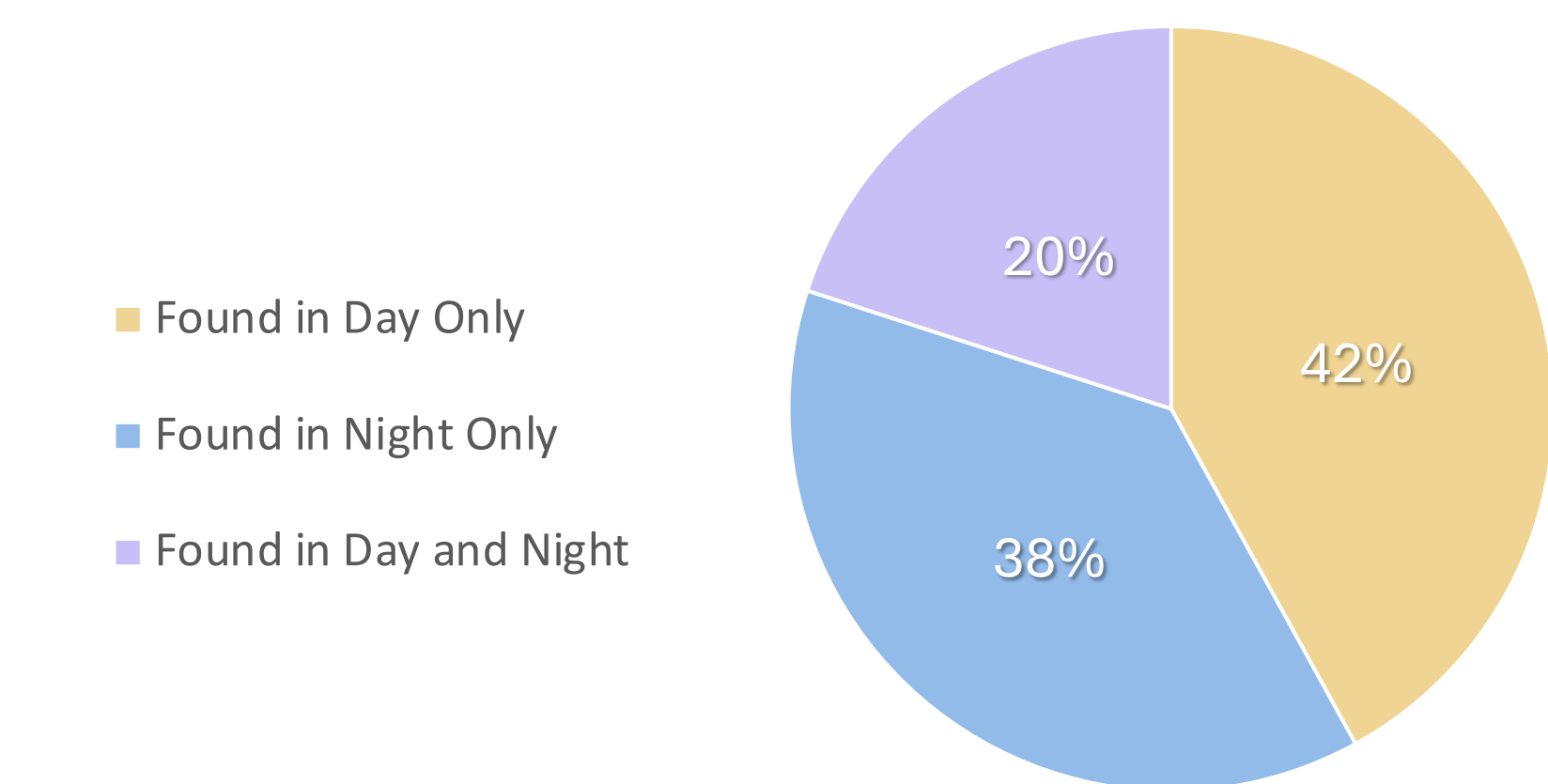


Figure III: Day and night zooplankton OTUs differed by the species present with only 20% of all OTUs found in both day and night samples.

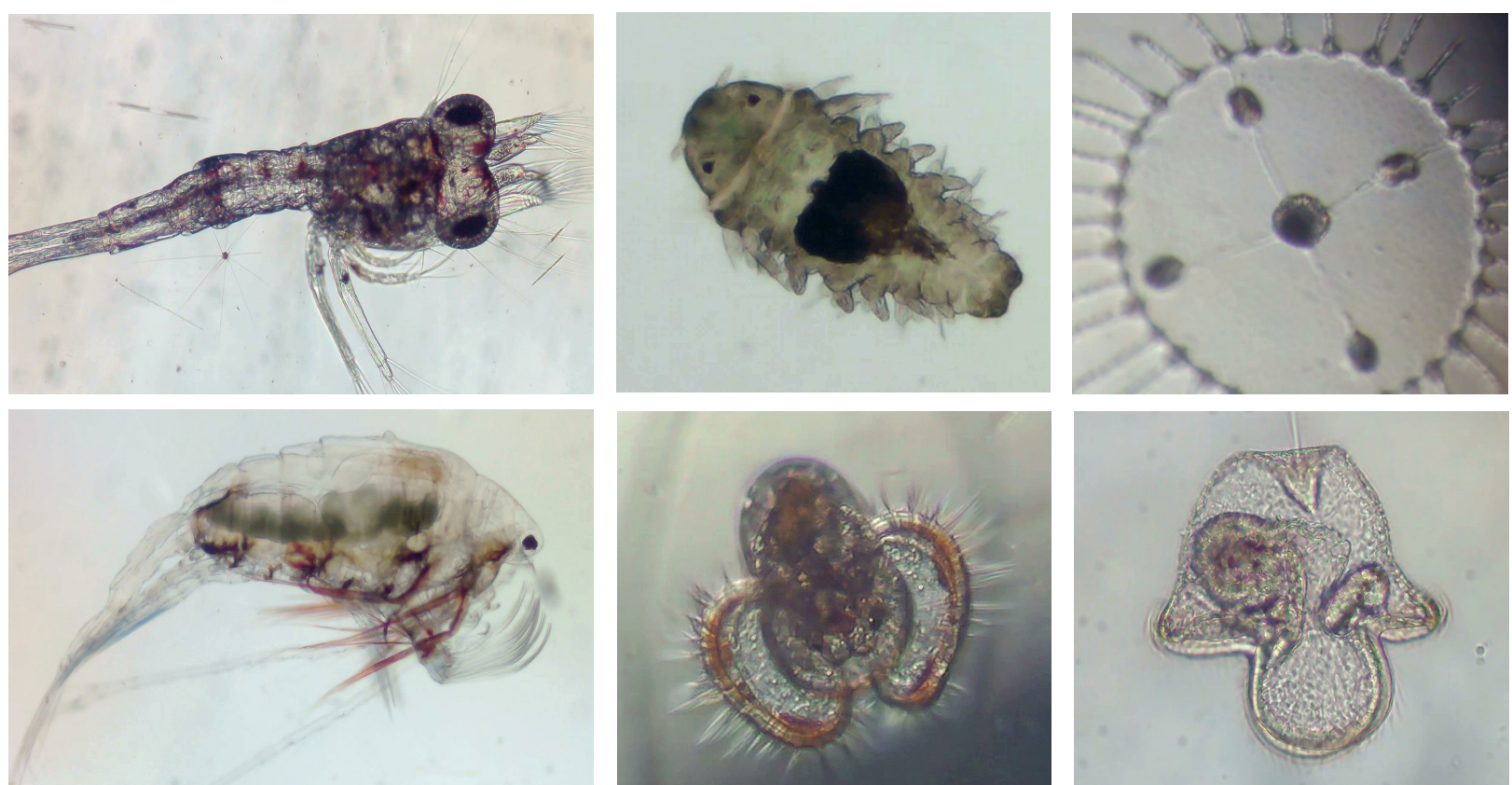


Figure V: Examples of zooplankton imaged for DNA barcoding by students, magnification 400X.

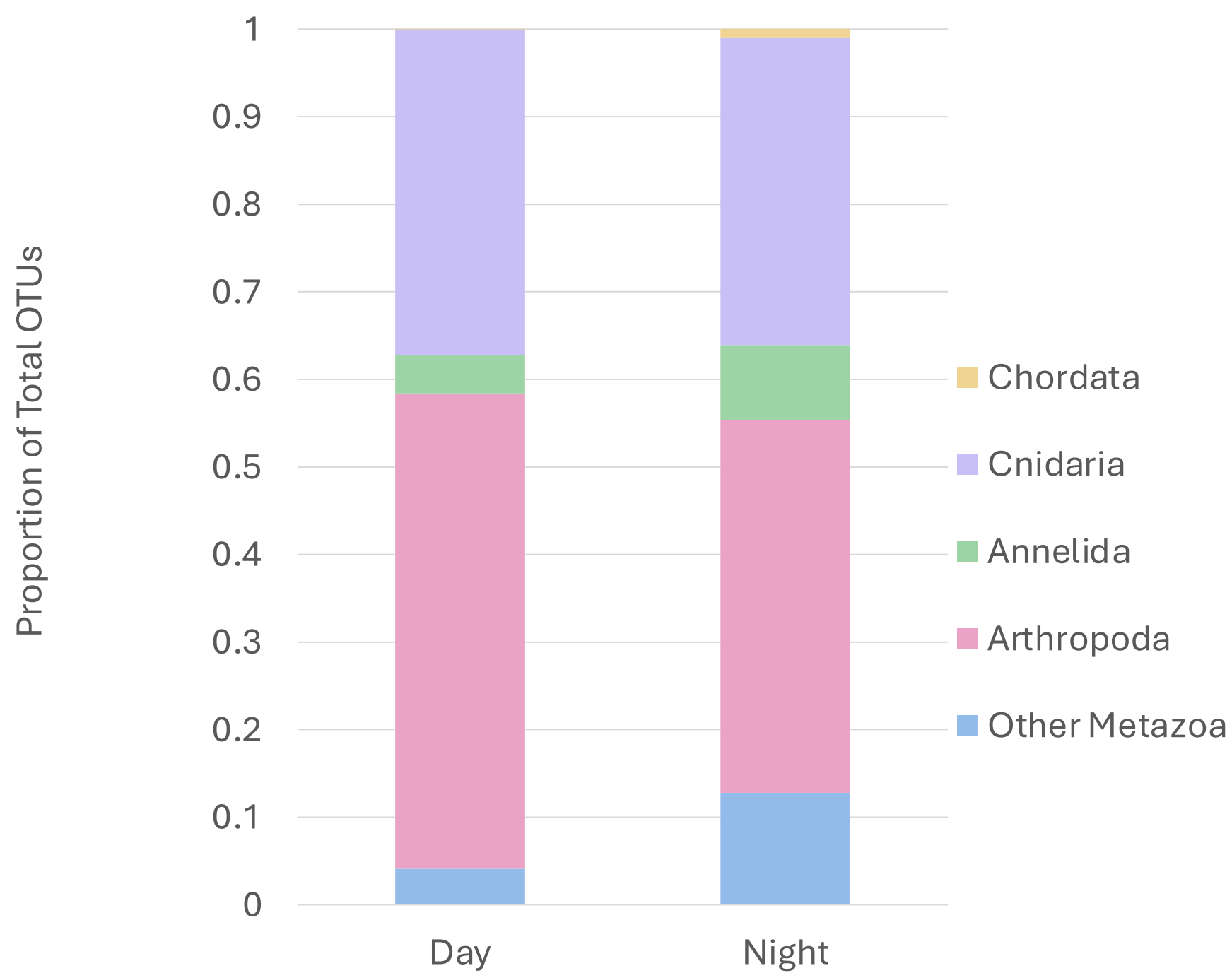


Figure II: Proportional number of metazoan operational taxonomic units detected by each sampling period that were identified to the phylum level using the five reference sequence databases. Phyla that had $<1.0\%$ relative abundance were grouped as “Other Metazoa.”

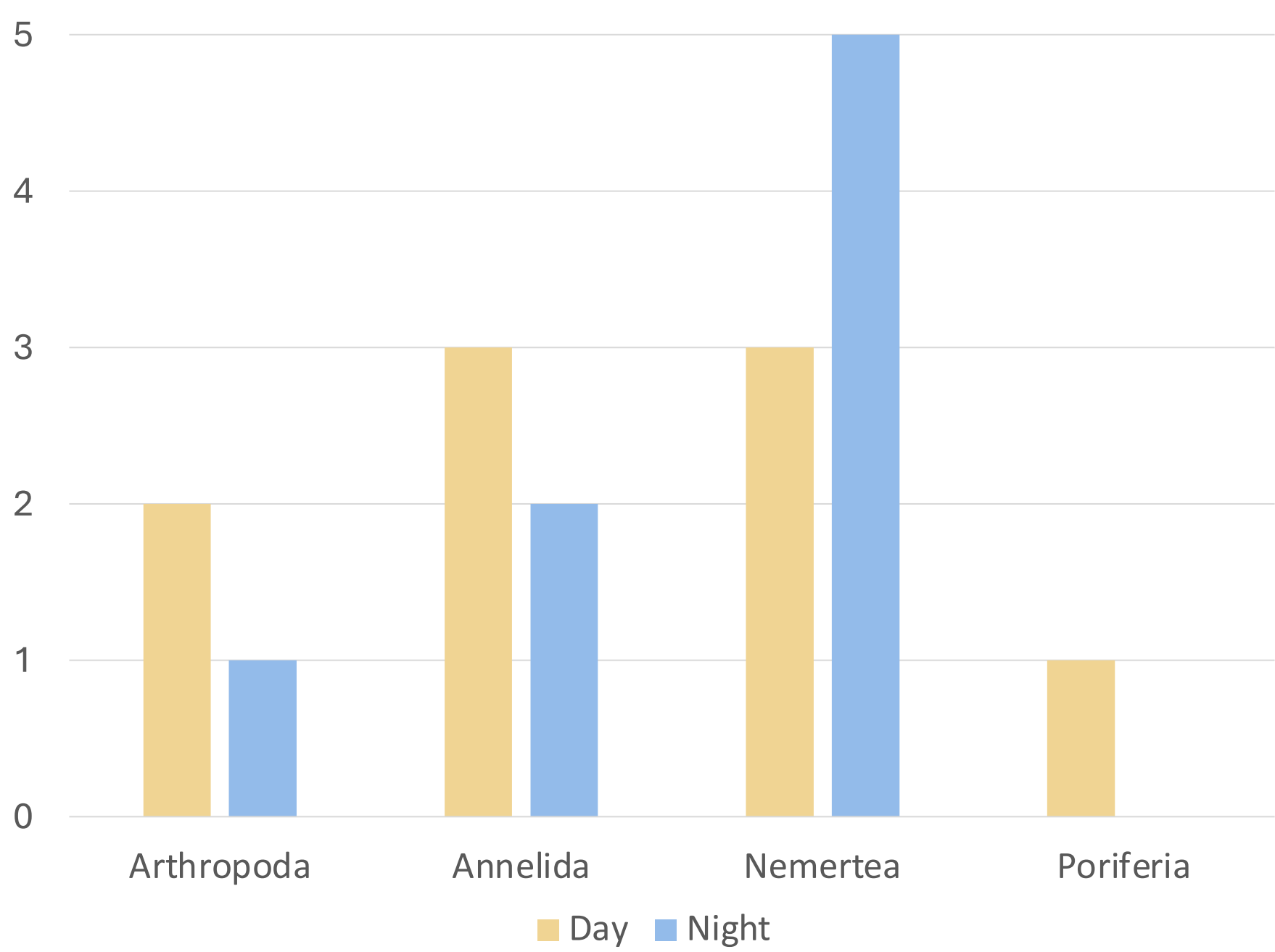


Figure IV: Plankton species richness identified on reanalysis, organized by taxonomic group and compared between day and night sampling. OTUs were accepted at a confidence level of $\geq 97\%$ and a sequence length of ≥ 300 bp.

Results and Discussions

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 ($\sim 70\%$ of total OTUs), and direct sequencing contributed unique morphotypes (17%) that did not appear in eDNA datasets.

Temporal comparisons of night versus day showed partitioning, 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.

Reanalysis of the eDNA dataset revealed seven new previously unidentified species. Predominantly consisting of ribbon worms, a previously underrepresented phyla. This localized expansion of the database is secondary to targeted research efforts focused on nemerteans. Which highlights how taxonomic bias is introduced into reference databases. Nonetheless, eDNA databases serve as a valuable tool which gain value as global reference databases expand, providing valuable insight into historic and future biodiversity assessments.

Acknowledgements

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References

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