Zooplankton Species Richness Estimates are Increased with eDNA and Metabarcoding

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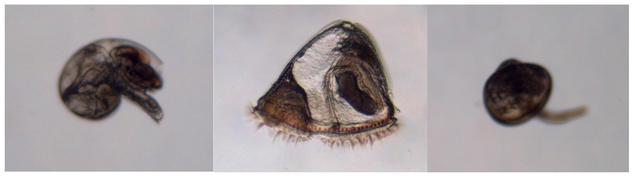
Introduction

Zooplankton communities are understudied by as much as 70-90% (Appletans et al, 2012, Maslakova et al 2022) despite their critical importance in marine food webs, energy transfer and nutrient cycling. We may be able to detect biodiversity changes and build robust conservation responses by knowing which species are present and when in the plankton. In addition to monitoring biodiversity changes, eDNA and metabarcoding may be helpful for monitoring commercially traded species like clams, mussels, and crabs, as well as monitoring for invasive species.

The goal of this study is to examine the use of bulk sampling with environmental DNA (eDNA) and metabarcoding for documenting species richness compared to hand sorting and direct sequencing individuals. Our study focuses on zooplankton collected in front of Friday Harbor Laboratories (FHL), San Juan Island, WA because it has a well-documented invertebrate fauna and a history of invertebrate larval life history and morphology research. We compared our eDNA data to more traditional method of zooplankton biodiversity assessment, which involves hand sorting plankton samples, imaging, and direct DNA sequencing of individuals, a slow process that does not do a good job of accounting for rare, cryptic, or new species. **Our results demonstrate that eDNA is an excellent tool for biodiversity documentation, yet hand sorting and direct sequencing is still required for the most complete inventory of biodiversity.**



Above: Examples of plankton imaged for direct sequencing. From left to right, holoplanktonic *Linacina helacina*, bryozoan cyphonautes *Membranipora sp*, and pediveliger of *Mytilus trossulus*. magnification 400X.

Materials and Methods

Plankton Collection and Preservation

Plankton samples were collected at Friday Harbor Laboratories at noon (day sample) and 11:30 pm (night sample) on June 28 and July 1, 2021, respectively. We used a 153 um mesh plankton net and towed by hand just below the sea surface for five minutes. Both samples were divided in half by volume. One half was passed through a Strivex filter and preserved in 100% ethanol. The other half was sorted for unique morphotypes. The hand sorted plankton fractions were imaged and preserved for direct sequencing. The Smithsonian Institution Laboratory for Analytical Biology used cytochrome oxidase subunit 1 (COI) primers (Bucklin et al. 2011) for next generation sequencing of the filtered material and capillary sequencing for the unique hand-picked morphotypes.

Species Identification

Resulting DNA reads were trimmed and edited using BBDUK in Geneious Prime 2.0. We accepted resulting contigs that had a 95% or higher quality score and were longer than 200 bp. These COI sequences were searched across four databases; CoArbitor, Midori, GenBank Blast nr, and Wells et al 2021 dataset, which includes zooplankton species and operational taxonomic unit (OUT) found at Friday Harbor Laboratories in addition to an extensive database of COI sequences from marine invertebrate macrofauna. We accepted species classifications if there were a 95% or greater match and the sequence length was >200bp.

Works Cited

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Results and Conclusions

We captured 198 unique animal OTUs or morphospecies from both the day and night samples of which **47% percent are not found in any database**. There were several patterns with respect to species discovery in the resulting data (Table 1) :

- eDNA and metabarcoding captured most of the species richness in the plankton (70%) compared to hand sorting and direct sequencing (17%) (Figure 1, table 1).
- While direct sequencing recovered fewer species than eDNA and metabarcoding, this method added many unique species/OTUs that were missed by eDNA.
- Many of the smaller phyla, e.g. Bryozoa, Chaetognatha, Nematoda, Nemertea, and Platyhelminthes, had OTUs that were not in any databases! New OTUs may represent new species, cryptic species, or a lack of a COI DNA sequence accessioned in databases.
- For two phyla, Echinodermata and Mollusca, we were able to identify all OTUs to an actual species, but the larvae of these phyla have morphologically indistinct larvae and would not otherwise be easy to distinguish just by observing morphology (Shanks 2001).

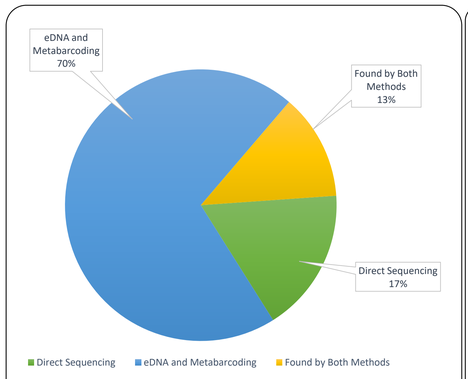

Figure 1: eDNA captured 70% of the resulting species, where as hand sorting and direct sequencing captured only 17%. 13% of species were found in both data sets.

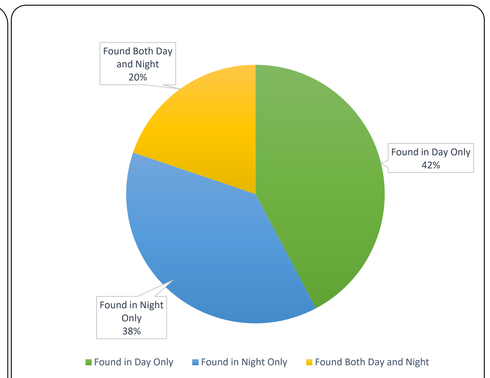

Figure 2: Day and night plankton samples differed by the species present with only 20% of all OTUs overlapping day and night.

In addition to many of the unknown OTUs we documented, we found that night and day plankton **species assemblages are different with only a 36% overlap in species**. Of our total 198 species/OTUs, 37% are found in the day only and 40% are found at night only (table 1, figure 2).

Our work documented a lot more species of zooplankton than expected for a marine laboratory with a well researched fauna! **These results highlight the need for more eDNA and metabarcoding in parallel with hand sorted, imaged, and directly sequenced plankton samples.** We were also able to capture the presence of several important commercial species as well as a couple of invasive species and many unknown species. We hope this preliminary study will serve as a foundation to compare future samples across time and our changing environment.

Acknowledgements

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Table 1: OTUs recovered by all methods, time of day and their frequencies, demonstrating despite extensive knowledge of marine invertebrates, we are undercounting local species richness by at least 47%.

Higher Taxon	Species or OTU	Frequency Meta Day	Frequency Meta Night	Day Direct	Night Direct	New to DB	Found in Day Only	Found in Night Only	Found Both Day and Night	Day Only and New	Night Only and New
Annelida	Please see our other posters for Annelida and Arthropoda data.										
Arthropoda											
Bryozoa											
	Gymnolaemata	11				1	1			1	0
	Membranipora	8	3	2	1	1			1	0	0
	Membranipora membranacea_1	7	3			1			1	0	0
	Membranipora membranacea_2	15	1		1	1			1	0	0
	Membranipora membranacea_3	1	1			1			1	0	0
	Membranipora membranacea_4	1	1		4	1			1	0	0
	Membranipora membranacea_6		1			1		1		0	1
	Membraniporidae	3				1	1			1	0
	Vesiculariidae_2	1				1	1			1	0
Chaetognatha											
	Parasagitta elegans_1	1				1	1			1	0
	Parasagitta elegans_10	1				1	1			1	0
	Parasagitta elegans_5	2				1	1			1	0
	Sagittidae_10	32	3			1			1	0	0
	Sagittidae_4	1				1	1			1	0
	Sagittidae_7	1				1	1			1	0
	Sagittidae_8	6				1	1			1	0
	Sagittidae_9	1				1	1			1	0
	Sagittoidea	12	50			1			1	0	0
	Sagittidae X	1				1				0	0
Chordata											
	Apogonidae		20			1		1		0	1
	Boltenia villosa		3					1		0	0
	Citharichthys stigmaeus		1					1		0	0
	Styela gibbsii		4					1		0	0
Cnidaria	Aequorea X	33	3			1			1	0	0
	Aurelia aurita	708	4						1	0	0
	Bougainvillia muscus_1	14				1	1			1	0
	Campanulariidae_1	2	1	1		1			1	0	0
	Campanulariidae_4		2			1		1		0	1
	Campanulariidae X	44	4			1			1	0	0
	Clytia gregaria	142	840	1					1	0	0
	Clytia hemisphaerica	38	105						1	0	0
	Coryne eximia	3					1			0	0
	Cyanea capillata		1					1		0	0
	Hydrozoa_1	1				1	1			1	0
	Hydrozoa_4	2				1	1			1	0
	Muggiaea atlantica	12					1			0	0
	Nanomia bijuga	3					1			0	0
	Obelia_2	3				1	1			1	0
	Obelia longissima	1109	58	3	1				1	0	0
	Phacellophora camtschatica	1				1	1			1	0
	Phialella quadrata	11	1			1			1	0	0
	Plumularia lagenifera	6				1	1			1	0
Ctenophora											
	Pleurobrachia bachei_1	12	21						1	0	0
	Pleurobrachia bachei_2	10	8						1	0	0
	Tentaculata	12				1	1			1	0
Echinodermata											
	Amphipholis pugetana	12	3						1	0	0
	Dendraster excentricus	48	30	1	1				1	0	0
	Evasterias troschelii		1					1		0	0
	Mesocentrotus franciscanus	1	1						1	0	0
	Ophiopholis kennerlyi	2	11						1	0	0
	Parastichopus californicus	5	7						1	0	0
Mollusca											
Bivalvia	*Crassodoma gigantea*	1					1			0	0
	Humilaria kennerleyi		20					1		0	0
	Leukoma staminea		2					1		0	0
	Kellia suborbicularis		1					1		0	0
	Kurtiella tumida	1	1						1	0	0
	Macoma lipara	1	17						1	0	0
	Macoma nasuta	1	31						1	0	0
	Mya truncata		2					1		0	0
	Mytilus trossulus	3	35	1	1				1	0	0
	Pectinidae 1		4			1		1		0	1
	Saxidomus gigantea		16					1		0	0
	Saxidomus nuttalli		1					1		0	0
Gastropoda	*Alia carinata*				1			1		0	0
	Alvania compacta				1			1		0	0
	Crepipatella lingulata		1				1			0	0
	Eubranchus olivaceus		1					1		0	0
	Haminoea vesicula	1				1				0	0
	Hermea vancouverensis	1				1				0	0
	Hermissenda crassicornis	1					1			0	0
	Lacuna vincta		1				1			0	0
	Limacina helicina		1		1				1	0	0
	Lottia scutum		14					1		0	0
	Margarites pupillus		11					1		0	0
	Nassarius mendicus				1			1		0	0
Polyplacophora	*Cyanoplax dentiens*		1					1		0	0
	Mopalia lignosa				1			1		0	0
Nemertea											
	Cephalothrix		1			1		1		0	1
	Cerebratulus cf. marginatus TCH-2015		3			1		1		0	1
	Lineus sp. 1 TCH-2015	4				1	1			1	0
	Maculaura alaskensis	6	17						1	0	0
	Ototyphalonemertes		1			1		1		0	1
	Tubulanus sp. OR055_22VIII16		4			1		1		0	1
Nematoda											
	Monhysterida_1	1				1				0	0
Phoronida											
	Phoronopsis harmeri	8	2						1	0	0
Platyhelminthes											
	Microstomidae_1	2	14			1			1	0	0
Porifera											
	Porifera_6	1	1			1			1	0	0
Rotifera											
	Ploima_1	1	12	2		1			1	0	0
	Ploima_14	2				1	1			1	0
	Ploima_3	10	19			1			1	0	0
	Ploima_4		4			1		1		0	1
	Ploima_6		1			1		1		0	1
	Synchaetidae	2	2			1			1	0	0
Species Richness	198	122	116	37	25	93	74	79	71	37	35