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SPECIAL ISSUE ARTICLE

DNA metabarcoding provides insights into the diverse diet of a dominant suspension feeder, the giant plumose anemone *Metridium farcimen*

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Abstract

Benthic suspension feeders have significant impacts on plankton communities by depleting plankton or modifying the composition of the plankton through prey selectivity. Quantifying diets of planktivorous animals can be difficult because plankton are frequently microscopic, may lack diagnostic characters, and are digested at variable rates. With DNA metabarcoding, the identification of gut contents has become faster and more accurate, and the technique allows for higher taxonomic resolution while also identifying rare and highly degraded items that would otherwise not be detected. We used DNA metabarcoding to examine the diet of the giant plumose anemone *Metridium farcimen*, a large, abundant, competitively dominant anemone on subtidal rock surfaces and floating docks in the northeast Pacific Ocean. Gut contents of 12 individuals were compared to 80- and 330-µm filtered plankton samples collected one hour prior between 0.02 and 1.5 km from the anemones. The objectives of this study were to determine if *M. farcimen* has a selective diet and compare our findings with traditional gut content analyses. Metabarcoding demonstrated that *M. farcimen* captured a wider range of prey than previously suspected using traditional visual sampling techniques. Individual gut contents had less richness than the 80-µm filtered plankton samples but had greater richness than the 330-µm filtered plankton samples. The diet of the anemones was 52% arthropods with a surprisingly high relative abundance of an ant (10%) that has mating flights in August when this study was conducted. The gut contents of *M. farcimen* likely include all prey that elicit a predation response and that cannot escape. There were no statistically overrepresented taxa in the gut contents compared to the plankton but there were underrepresented taxa. This study highlights the usefulness of the metabarcoding method in identifying prey within the gut of planktivorous animals and the significant terrestrial input into marine food webs.

KEYWORDS
Anthozoa, benthic-pelagic coupling, Cnidaria, community ecology, DNA barcoding, ecosystem subsidies, historical comparisons, predation

1 | INTRODUCTION

Benthic suspension feeders can have major impacts on the structure of littoral food webs (Gili & Coma, 1998; Kimmerer et al., 1994; Petersen, 2004; Sebens & Koehl, 1984; Whitten et al., 2018; Young & Gotelli, 1988). Dense populations of suspension feeders can filter the immediately overlying water volume several times a day (Davies et al., 1989; Jørgensen, 1980; Petersen, 2004; Petersen & Riisgård, 1992; Riisgård, 1991; Vedel et al., 1994) thereby depleting resources from the surrounding water (Riisgård, Jürgensen et al., 1996; Riisgård, Poulsen et al., 1996; Vedel, 1998). For example, the abundance of the introduced clam *Potamocorbula amurensis* in California has been found to reduce chlorophyll concentration and the abundance of three copepods by 53 to 91% (Kimmerer et al., 1994).

Both passive and active benthic suspension feeders rely on water flow to bring food particles to their vicinity. Yet, all prey do not have equal probability of being captured. Prey species use mechanisms such as escape behaviors, morphological defenses, and toxicity to avoid predation (Browman et al., 1989; Dodson, 1974; Engström et al., 2001; Safi et al., 2007; Suchman & Sullivan, 1998; Viitasalo et al., 1998, 2001). For example, the copepod *Acartia tonsa* can remotely detect the hydromechanical disturbances generated by predators and use this information to avoid predation (Kiørboe et al., 1999). In addition, numerous suspension feeders are selective for certain types of prey. Many bivalves, for example, preferentially select plankton based on size, shape, and motility (Cucci et al., 1985; Defossez & Hawkins, 1997; Safi et al., 2007; Shumway et al., 1985). Prey selectivity can result from the inability of predators to capture certain prey, the preferential capture or consumption of palatable and energetically valuable species, or active rejection of prey. Studies on the dietary selectivity of benthic suspension feeders are key to our understanding of the effects of predators on their ecosystem and of the role of dietary niche partitioning for species coexistence (Costello & Colin, 2002; Leray et al., 2019; Suchman & Sullivan, 1998).

Traditionally, diet selectivity studies included experimental feeding trials (e.g., Baker & Levinton, 2003; Bolam et al., 2019) or autopsies, biopsies, gastric lavages, or feces collections of wild-feeding animals followed by visual identification of gut contents (e.g., Purcell, 1977; Sebens & Koehl, 1984). However, quantifying the diet of planktivorous animals can be difficult because plankton are frequently microscopic, often lack diagnostic characters, and are digested quickly and at variable rates (Fancett, 1988; Larson, 1991; Purcell, 1977; Sebens & Koehl, 1984; Zamer, 1986). Furthermore, resources to support the taxonomic identification of plankton are poorly developed for most regions. Because of this, marine plankton are difficult to identify in gut contents past the class or order level when using traditional visual identification techniques (e.g., Fancett, 1988; Purcell, 1977; Sebens & Koehl, 1984).

With the advent of high-throughput sequencing and powerful molecular techniques such as DNA metabarcoding (Taberlet et al., 2012), identification of specimens within community samples can be rapid, accurate, and relatively cheap (Aylagas et al., 2014; Brandon-Mong et al., 2015; Nielsen et al., 2018). DNA metabarcoding has been used to successfully identify taxa within gut contents of fishes (Albaina et al., 2016; Harms-Tuohy et al., 2016; Leray et al., 2013, 2015), to evaluate biodiversity of insects (Brandon-Mong et al., 2015; Ji et al., 2013; Yu et al., 2012), and to identify the presence of rare taxa using environmental DNA (Deiner et al., 2017; Evans et al., 2016; Valentini et al., 2016). Metabarcoding can be used to analyze diets to reach a higher taxonomic resolution while also identifying rare, and highly degraded items that would otherwise not be detected using visual identification techniques (Nielsen et al., 2018).

FIGURE 1 The giant plumose anemone *Metridium farcimen*

We used DNA metabarcoding to examine the diet of the giant plumose anemone *Metridium farcimen* (Brandt, 1835, Figure 1). *Metridium farcimen* (Cnidaria, Anthozoa, Actiniaria) is a large, abundant sea anemone on subtidal rock surfaces and floating docks in the northeast Pacific Ocean (Fautin et al., 1989; Fautin & Hand, 2000; Hand, 1955; Kozloff, 1973; Ricketts et al., 1968). This anemone, which can extend over a meter into the water column (Fautin et al., 1989), is well-adapted for high-flow environments (Koehl, 1977a, 1977b, 1977c), and is a competitively dominant species on rocky subtidal ledge communities (Nelson & Craig, 2011; Wells, 2019; Wells & Sebens, 2017). Its diet consists primarily of copepods, polychaete larvae, bivalve and gastropod veligers, and arthropod nauplii (Koehl, 1977a; Purcell, 1977; Sebens, 1981; Shick, 1991). While previous studies found that zooplankton were roughly eaten by *M. farcimen* in proportion to their availability, given the challenges associated with visual identification techniques, we wanted to determine if *M. farcimen* has a selective diet by comparing gut contents with (1) available food from plankton tows and (2) previous morphological work.

2 | MATERIALS AND METHODS

2.1 | Sample collection and DNA isolation

Plankton communities were quantified by sampling the plankton at three sites within the San Juan Archipelago: adjacent to the floating docks of the Friday Harbor Laboratories (FHL, 48.5452°N, 123.0124°W); 280 m southeast of the docks (48.5436°N, 123.0100°W); and in the San Juan Channel (48.5490°N,

122.9924°W), 1.5 km northeast of the docks. The docks are in an embayment, while San Juan Channel experiences intense tidal flushing and mixing. Samples were taken during an ebb tide between 1:00 and 2:30 pm on August 4, 2016, a season when plankton are highly diverse. Two simultaneous plankton tows were performed at each site at 1-m depth: one 80-µm mesh size net to capture a broad range of plankton and one 330-µm mesh size net to capture large zooplankton. Tows were kept separate because mesh size was far more influential than location in this study; from this point forward the three sampling locations are treated as replicates of mesh size. Both plankton nets were 50-cm wide, and approximately 98 m^3 (125-m long tow) of water was sampled. Samples were immediately preserved in 50-ml falcon tubes in 95% ethanol in the field and kept at −20°C in the laboratory. Falcon tubes were centrifuged at 2000 × g for eight minutes at room temperature to pelletize planktonic particles and remove ethanol. The pellet was homogenized with a mortar and pestle, and the whole tissue homogenate was used for DNA extraction using the MoBIO PowerMax® DNA Isolation Kit (Qiagen) following manufacturer's instructions.

Sixteen *M. farcimen* were collected from the floating docks at FHL by hand one hour after the plankton tows. All collected anemones were within 20 m of one another at <1 m depth. Anemones were kept in seawater on ice until gut contents could be extracted (between 0.5 and 3 h following collection). Material attached to the aboral end of the anemones was carefully removed and discarded. In the laboratory, anemones were bisected, allowing efficient extraction of gut contents. Material extracted from the gut consisted of partially digested food, copious amounts of mucus, mesenteries, acontia (tissue for agonistic behaviors), and gonadal tissue in sexually mature individuals. Large food particles (e.g., hydromedusae) were cut up into small pieces to facilitate later grinding. Gut contents were rinsed with 95% ethanol in a 45-µm mesh net to remove excess mucus. Ethanol rinses dissolved anemone mucus more efficiently than seawater. Material within the 45-µm mesh net was further massaged to break up large pieces. During this process, there is a risk of losing partially digested items that lack exoskeletons. The resulting samples were transferred into sterile sample tubes with 95% ethanol and kept at −20°C overnight. As there were still large particles within the sample, samples were centrifuged at 2000 × g for 8 min at 20°C, the supernatant was decanted, and the pellet was ground up within a mortar and pestle into a fine paste. The paste was placed back into another sterile tube with 95% ethanol, centrifuged at 2000 × g for 8 min at 20°C, and the supernatant was decanted. The whole pellet of each sample was used for DNA extraction using the MoBIO PowerSoil® DNA Isolation Kit (Qiagen) following manufacturer's instructions. Genomic DNA for both plankton and anemone samples were quantified with a Invitrogen Qubit fluorimeter (Thermo Fisher Scientific) and diluted to 10 ng/µl.

2.2 | PCR and library preparation

Extracted DNA from the 16 anemones and six plankton samples were amplified using tagged primers targeting a highly variable fragment (~313 bp) of the mitochondrial cytochrome c oxidase subunit I (COI) region with the PCR primers mlCOIintF and jgHCO2198 (Geller et al., 2013; Leray et al., 2013). We used a hierarchical tagging approach with a combination of randomly-assigned tailed PCR primers and single indexed Illumina Y-adapters to sequence all samples in a single Illumina MiSeq run. Three PCR replications were performed per sample. PCR reactions were performed in a total volume of 20.0 µl, containing 13.2 µl of nuclease free water, 2.0 µl of Clontech 10X Advantage 2 PCR buffer (Takara Bio Inc.), 1.0 µl of each primer (10 µM), 1.4 µl of dNTP, 0.4 µl of Clontech 50X Advantage 2 (Takara Bio Inc.), and 1.0 µl (10 ng) of DNA. The reactions were incubated in a Biometra T3 thermocycler (Analytik Jena), starting with 5 min of denaturation at 95°C, followed by 35 cycles of 30 s of denaturing at 95°C, 30 s of annealing at 48°C, and 45 s of extension at 72°C, with a final extension of 72°C. A negative PCR control and extraction control were performed to test whether the reagents were free of contaminants; both were negative for contamination. Purified PCR products were quantified using an Invitrogen Qubit fluorimeter and then diluted to 30 ng/µl. DNA amplification was confirmed on 1.5% gel electrophoresis and then triplicates were pooled. DNA was purified using Solid Phase Reversible Immobilization beads to remove primers, primer dimers, salts, and deoxynucleoside triphosphates (dNTPs). The PCR products of samples amplified with different tailed primers were pooled before library prep as detailed by Leray et al. (2013), and Leray et al. (2016). Samples were prepared for sequencing with the Illumina TruSeq DNA PCR-free LT Library Prep Kit (Illumina, Inc.), which includes end-repair and dA-tailing chemistry, and then ligated with adapters.

2.3 | Bioinformatics

Analysis of the sequence data followed the same protocol described in Nguyen et al. (2020). Sequences were demultiplexed and Illumina adapters were trimmed using Flexbar (Roehr et al., 2017). DADA2 (Callahan et al., 2016, 2017) was then used to remove primers, discard low-quality sequences, and infer exact Amplicon Sequence Variants (ASVs). DADA2 uses sequence quality scores and abundance information to generate an error model that best fits the data, and subsequently uses the error model to infer ASVs. With DADA2, we removed the first 26 nucleotides from each sequence (trimLeft = 26). Sequences could not have any unidentified bases (maxN = 0), could not have more than two expected errors (maxEE = c[2, 2]), and had to have a quality score higher than 10 (truncQ = 10). ASVs, which can differ by as little as one nucleotide, were clustered into operational taxonomic units (OTUs) at a 97% identity threshold using VSEARCH (Rognes et al., 2016). To further improve estimates of alpha and beta diversity, spurious OTUs were removed using the LULU algorithm with default parameters (Frøslev et al., 2017). This tool, which uses sequence similarity and co-occurrence patterns, was shown to reduce taxonomic redundancy and improve similarity with the true taxonomic composition of test samples (Frøslev et al., 2017).

Taxonomic names were assigned to OTUs using an iterative approach. First, BLASTn searches were used to compare one

representative sequence of each OTU to a database of northeast Pacific DNA barcodes. Many of these species were collected off the floating docks near the source of *M. farcimen*. An OTU was considered to match a local barcode when the level of sequence similarity was higher than 98%. Second, unidentified OTUs were assigned taxonomic information using the Bayesian Least Common Ancestor Taxonomic Classification method (BLCA, Gao et al., 2017) against a curated database of metazoan mitochondrial gene sequences (Midori-Unique v20180221, Machida et al., 2017). Assignments with less than 50% confidence were not taken into account. Third, the numerous OTUs that remained unidentified using BLCA were compared to the whole NCBI NT database (May 2018) using BLAST searches (word size = 7; max e-value = 5e-13) and assigned the taxonomy of the lowest common ancestor of the first 100 hits.

2.4 | Statistical analyses

Gut content samples and plankton samples were rarified to the lowest number of sequences in Microsoft Excel (Microsoft Corporation) using a random number generator. An unequal number of sequences can affect estimates of diversity due to the positive relationship between number of sequences and OTUs (Gotelli & Colwell, 2001). This rarefied dataset was used for all further analyses. Rarefaction curves were built to illustrate the sequencing effort and to determine if sampling effort was exhaustive. All data were analyzed in R version 3.5.2 with the vegan version 2.5–4 package (Oksanen et al., 2019; R Core Team, 2018).

In this study, richness was defined as the number of different OTUs or taxa within a sample or treatment. Abundance was defined as the number of sequences within a sample or treatment. Evenness was defined as the similarity of frequencies of the abundances of OTUs within a treatment. The incidence of an OTU was defined as the fraction of samples or treatments containing that OTU. Mean evenness for each sample type was calculated by using the relative abundances of each OTU within each sample and calculating a Pielou's evenness index (Pielou, 1966).

Matrices of community dissimilarity based on the Bray–Curtis index were created using both the number of sequences and the presence/absence of OTUs (i.e., the Sørensen index). Differences between diet composition of *M. farcimen* and 80- and 330-µm filtered plankton communities were tested using permutational multivariate analyses of variance (PERMANOVA, Anderson, 2001) with 9999 permutations. Patterns of species composition were visualized in two-dimensional space using non-metric multidimensional scaling ordination plots (nMDS) with 9999 permutations. Similarity percentage analyses (SIMPER) were used to determine what OTUs were significantly contributing to the Bray–Curtis dissimilarities calculated between groups of samples (Clarke, 1993).

3 | RESULTS

A total of 3,109,361 high-quality metazoan sequences passed the quality controls with an average of 101,000 sequences per plankton sample and an average of 12,500 non-*Metridium* sequences for *M. farcimen* gut content samples (131,000 total sequences per gut content). Four of the 16 gut samples had less than 7000 non-*Metridium* sequences and were thus dropped from the analysis. It was assumed that these individuals were either not feeding at the time of collection or prey sequences were hidden by the abundant co-amplification of *M. farcimen* sequences leading to insufficient data to categorize diet. We identified 438 OTUs in the rarefied dataset. Among these, 126 OTUs were identified to species (29% of all OTUs), 381 OTUs were identified to at least the phylum level (87% of all OTUs), and 57 OTUs were unidentified metazoans (13% of all OTUs). 174 OTUs (40% of total OTUs) were identified using the Pacific northeast barcode database. Rarefaction curves for both plankton and *M. farcimen* plateaued, which indicated that a sufficient number of sequences were obtained to estimate richness and composition of each sample (Figure S1).

Pooled samples indicated that *M. farcimen* gut contents had greater richness than either the 80-µm filtered plankton or the 330-µm filtered plankton (Table 1); however, gut content sample size was four times larger than the plankton tows. On a sample-by-sample basis, gut samples had fewer OTUs on average than the 80-µm filtered plankton samples, but greater richness than the 330-µm filtered plankton samples (Table 1). Greater evenness was found in the 80- and 330-µm filtered plankton samples (0.64 and 0.68, respectively) relative to the gut contents (0.57). Mean incidence was lowest in the gut contents (21%) compared to both the 80- and 330-µm filtered plankton (57% and 54%, respectively).

Twenty-eight classes were detected in the gut contents of *M. farcimen*, with a sample average of 16 classes belonging to 10 animal phyla (Table S1). Gut contents were primarily made up of arthropods (52% of sequences, 28 OTUs), especially crabs (presumably larvae), barnacles (larvae or molts), copepods, and insects (Table S2). Copepoda was the most diverse class for both the gut contents and the 80-filtered plankton samples (11 and 7 OTUs, respectively) whereas Polychaeta was the most diverse class for the 330-µm filtered plankton. Copepoda had the highest proportion of sequences for all three sample types (12%, 34%, and 19%, respectively, Table

TABLE 1 Summary of metabarcoding results. Means and standard errors are reported for per-sample richness and incidence

Sample type	No. of samples	Total richness	Per-sample richness	Evenness	Incidence
Metridium farcimen gut contents	12	356	74 ± 36	0.57	21 ± 18%
80-µm filtered plankton	3	160	91 ± 12	0.64	57 ± 28%
330-µm filtered plankton	3	97	53 ± 12	0.68	54 ± 28%

S1), and had the highest incidence within the gut contents (Table S3). On average, nine metazoan classes were identified in visual identification methods (Purcell, 1977; Sebens, 1981; Sebens & Koehl, 1984, Table 2). Overall, metabarcoding of gut contents detected many more taxonomic groups than previous conventional visual dietary analysis of *Metridium* spp. conducted by Purcell (1977), Sebens (1981), or Sebens and Koehl (1984) . Additionally, OTUs were identified to higher taxonomic resolution.

Communities within both the 80- and 330-μm filtered plankton samples were significantly different from the gut contents when looking at either number of sequences or presence/absence (PERMANOVA, $F_{2,15} > 1.31$, $R^2 > 0.11$, $p \leq 0.03$, Figures 2–4). There were 19 and 14 OTUs which contributed significantly to the difference between gut content and plankton samples (80- and 330-μm respectively, SIMPER, $p < 0.05$, Tables S4 and S5). All OTUs that differed had higher relative abundances in the plankton than in the gut contents, none had lower abundances. The corrugated clam *Humilaria kennerleyi* (Reeve, 1863), the hydrozoan *Clytia hemisphaerica* (Linnaeus, 1767), the brittle star *Ophiopholis kennerlyi* Lyman, 1860, and the peanut worm *Phascolosoma agassizii* Keferstein, 1866 were all more than 25 times less abundant in the gut contents compared to the 80-μm filtered plankton. The speckled sanddab *Citharichthys stigmaeus* Jordan & Gilbert, 1882, the periwinkle *Littorina scutulata* Gould, 1849, the bryozoan *Membranipora membranacea* (Linnaeus, 1767), and the hydrozoan *C. hemisphaerica* were all over 70 times less abundant in the gut contents compared to the 330-μm filtered plankton.

4 | DISCUSSION

Plankton tows and *M. farcimen* gut contents had comparable levels of richness on a per-sample basis (Table 1). Lower evenness and mean incidence (Table 1) as well as larger within-group Bray–Curtis dissimilarities (Figure 4) indicated that there was substantially greater intersample variability in the gut contents than in the plankton samples. Greater intersample variability indicates that there was high variability in feeding among anemones, despite them all being within a 20 m area, highlighting the small–scale spatial and temporal heterogeneity in zooplankton availability to benthic planktivores. Additionally, *M. farcimen* is likely capturing benthic taxa that swim up or are washed off the docks, potentially increasing the diversity of food available.

Based on the 12 anemones sampled, our analyses agree with the results of Sebens and Koehl (1984): *M. farcimen* has a selective diet. Sebens and Koehl (1984) found that *M. senile* preferentially consumed barnacle cyprids, ascidian larvae, and amphipods, and avoided eggs, copepods, and ostracods, compared to availability. In our study, none of the prey were significantly more common in the diet of *M. farcimen*, but several were significantly less common (Tables S4 and S5), suggesting that *M. farcimen* either actively rejected or was unable to detect or capture these potential prey. Sebens et al. (1996) found that dietary selectivity of other anthozoans was dependent on prey escape capabilities rather than predator preferences. While it is unknown how some potential prey species avoid predation, Heidelberg et al. (1997) showed that some zooplankton can detect passive suspension feeders in moving water and subsequently avoid predation. In addition, they showed that relatively small prey such as nauplii were less susceptible to predation. This may be because nematocyst discharge is affected by both chemical cues and mechanical stimulation (Thorington & Hessinger, 1988; Watson & Hessinger, 1988). Larger prey are more likely to impact tentacles at a higher force, increasing capture probability through mechanical or surface chemical detection and consequently eliciting a nematocyst response.

The diet of *M. farcimen* was compositionally different from the diets found by both Purcell (1977) and Sebens (1981). These discrepancies may be partially explained by spatiotemporal differences among these study locations. Purcell (1977) worked in Monterey, CA on *M. farcimen* associated with pilings at 8 m depth, while Sebens (1981) worked in Harper, WA, farther into the same sound as this study, but on subtidal pilings at 3 m below the surface. Alternatively, these differences could be due to amplification biases that can occur in metabarcoding datasets (Elbrecht & Leese, 2015). Relative to previous morphological dietary analysis (Purcell, 1977; Sebens, 1981), our results show a high abundance of demosponge and rotifers. The discrepancy is likely caused by the difficulty in identifying these groups with conventional visual techniques. Additionally, tropical sponges produce large amounts of detrital particulate organic matter which are consumed by neighboring invertebrates (de Goeij et al., 2013) and poriferan particulate matter would be difficult to identify with visual techniques. Impressively, the metabarcoding method found many more classes than traditional techniques, despite the relatively low sample size. Sebens (1981) examined the gut contents of 107 *M. farcimen* and found nine classes of metazoans whereas we examined 12 individuals and found 28 classes.

While Copepoda was the most diverse and abundant group and had the highest richness in the gut contents, likely because of its high diversity, richness, and abundance in the plankton (Table S1), we also found a high relative abundance of insects and ostracods in the gut contents (Table 2; Figures 2 and 3). The most abundant insect prey (98% of insect sequences) was the pale-legged field ant *Lasius pallitarsis* Emery, 1893 (100% similarity match to the reference sequence GenBank Accession JN292076). This ant species has mating flights in August (Nonacs, 1990), the same month this study was conducted. It seems that *M. farcimen*, when associated with floating docks, may be getting a significant portion of their diet from episodic input from the nearby terrestrial environment. Strong tidal currents and mixing, however, could provide this resource to shallow subtidal populations on natural rock surfaces as well. This result highlights the need for further sampling across broader temporal scales, spatial scales, and depth ranges to better understand from where *M. farcimen* and other benthic suspension feeders derive their energy.

DNA metabarcoding is an efficient method for identifying even partially digested gut contents of animals. However, the results can only be ecologically interpreted if sequences can be matched to taxonomic groups. Building libraries of reference DNA barcodes is

TABLE 2 Comparison of diet composition for *Metridium* species from previous studies and this effort

Phylum	Class	Purcell, 1977 *M. senile*	Purcell, 1977 *M. farcimen*	Sebens, 1981 *M. farcimen*	Sebens & Koehl, 1984 *M. senile*	This Study *M. farcimen*
Annelida	Polychaeta	15%	15%	1%		14%
Arthropoda	Arachnida				Present	Present
	Branchiopoda				Present	4%
	Copepoda	35%	42%	11%	7%	18%
	Insecta					14%
	Malacostraca	Present	Present	4%	65%	11%
	Thecostraca	10%	15%	76%	7%	14%
	Ostracoda	Present			1%	7%
Bryozoa	Gymnolaemata	Present	1%	1%	Present	3%
Chaetognatha	Sagittoidea					Present
Chordata	Actinoptergyii					Present
	Ascidiacea				6%	Present
Cnidaria	Anthozoa					Present
	Hydrozoa				12%	2%
	Scyphozoa					Present
	Staurozoa					Present
Ctenophora	Tentaculata					1%
Echinodermata	Asteroidea			1%		
	Echinoidea					Present
	Holothuroidea					1%
	Ophiuroidea					Present
Mollusca	Bivalvia	34%	30%	1%		2%
	Gastropoda	6%	2%	1%		2%
	Polyplacophora					Present
Nematoda	Chromadorea	Present	Present		Present	Present
Nemertea	Hoplonemertea					Present
	Pilidiophora					Present
Platyhelminthes	Rhabditophora					Present
Porifera	Demospongiae				Present	4%
Rotifera	Monogononta					3%

Note: Non-metazoan and unidentified categories (e.g., eggs and embryos) were excluded. Purcell (1977) and Sebens (1981) used relative abundance, Sebens and Koehl (1984) used relative biomass, and we used relative number of sequences. Values less than 0.5% are denoted as "Present".

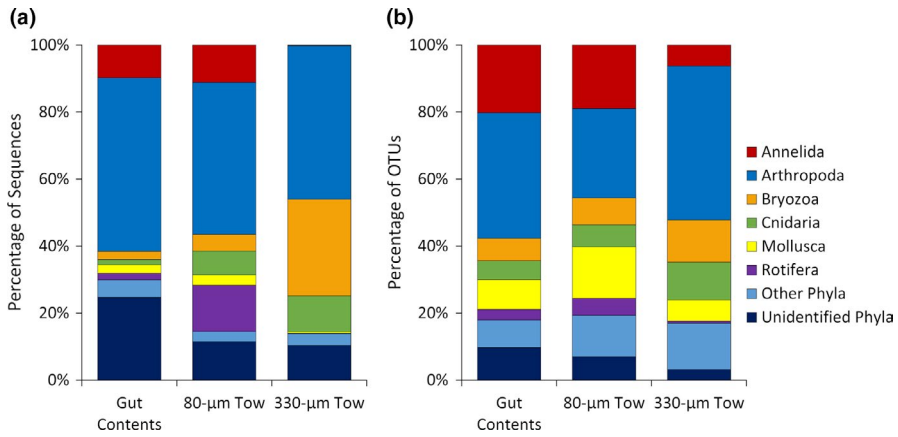

FIGURE 2 Percentage of (a) sequences and (b) OTUs of major phyla in *Metridium farcimen* gut contents (n = 12) and nearby 80- and 330-µm filtered plankton samples (n = 3). Phyla within the other phyla category include Chaetognatha, Chordata, Ctenophora, Entoprocta, Echinodermata, Nematoda, Nemertea, Phoronida, Platyhelminthes, and Porifera

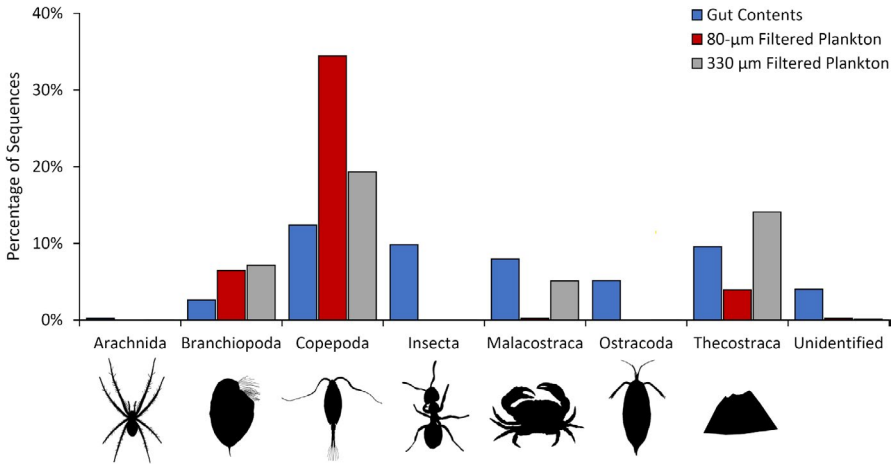

FIGURE 3 Percentage of sequences within each class of the phylum Arthropoda found in the *Metridium farcimen* gut contents and nearby 80-µm and 330-µm filtered plankton samples

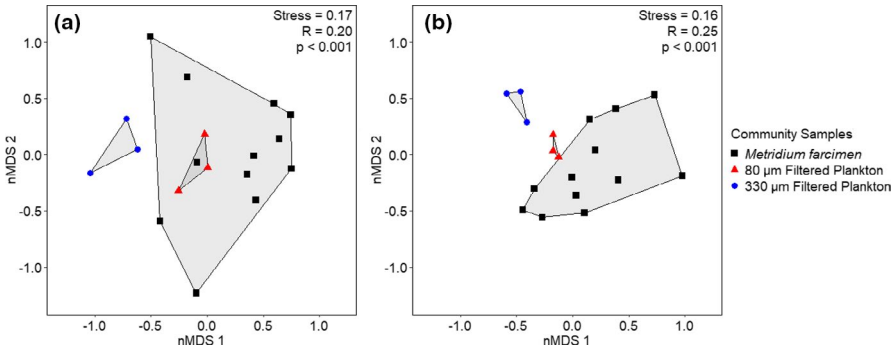

FIGURE 4 Non-metric multidimensional scaling ordination (nMDS) of (a) number of sequences and (b) the presence/absence of OTUs for gut contents of *Metridium farcimen* and nearby plankton samples. Each point represents one sample (plankton tow or gut content). Permutational analysis of variance significance levels are indicated.

time consuming but essential. Metabarcoding has its own limitations that have been reviewed before (Deagle et al., 2019; de Sousa et al., 2019). For example, it does not indicate the life stage of prey organisms, while traditional techniques can. It is also semi-quantitative, whereas visual methods can provide absolute counts or biomass. Therefore, we recommend pairing traditional techniques to identify major patterns and metabarcoding to identify the microscopic and partially digested prey items for future intensive studies. We also recommend either using fasted animals or sampling the available plankton over periods of time that correspond with the focal animal's prey digestion time so samples are more temporally comparable. Partially-digested prey captured hours before sampling can be detected within the gut, but that extended sampling is lost with plankton tows which are a snapshot of the community.

This work provides important insight into the diet of a competitively dominant sea anemone. Even with a smaller sample size than other dietary studies, metabarcoding showed that these animals capture a wider range of prey than previously suspected based on conventional visual analysis. The surprising terrestrial input into the diet of *M. farcimen* highlights the need to consider land–sea interactions in trophic models.

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CONFLICT OF INTEREST

We have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

CDW, GP and ML conceived and designed the study and collected the data. CDW and BNN analyzed the sequence dataset. Visualizations, statistical analysis, and interpretation were done by CDW and ML. CDW wrote the manuscript.

DATA AVAILABILITY STATEMENT

The demultiplexed and adapter trimmed FASTQ files can be accessed on the NCBI SRA under the BioProject accession number PRJNA682207.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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