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Vertical gradients in species richness and community composition across the twilight zone in the North Pacific Subtropical Gyre

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Abstract

Although metazoan animals in the mesopelagic zone play critical roles in deep pelagic food webs and in the attenuation of carbon in midwaters, the diversity of these assemblages is not fully known. A metabarcoding survey of mesozooplankton diversity across the epipelagic, mesopelagic and upper bathypelagic zones (0–1500 m) in the North Pacific Subtropical Gyre revealed far higher estimates of species richness than expected given prior morphology-based studies in the region (4,024 OTUs, 10-fold increase), despite conservative bioinformatic processing. Operational taxonomic unit (OTU) richness of the full assemblage peaked at lower epipelagic–upper mesopelagic depths (100–300 m), with slight shoaling of maximal richness at night due to diel vertical migration, in contrast to expectations of a deep mesopelagic diversity maximum as reported for several plankton groups in early systematic and zoogeographic studies. Four distinct depth-stratified species assemblages were identified, with faunal transitions occurring at 100 m, 300 m and 500 m. Highest diversity occurred in the smallest zooplankton size fractions (0.2–0.5 mm), which had significantly lower % OTUs classified due to poor representation in reference databases, suggesting a deep reservoir of poorly understood diversity in the smallest metazoan animals. A diverse meroplankton assemblage also was detected (350 OTUs), including larvae of both shallow and deep living benthic species. Our results provide some of the first insights into the hidden diversity present in zooplankton assemblages in midwaters, and a molecular reappraisal of vertical gradients in species richness, depth distributions and community composition for the full zooplankton assemblage across the epipelagic, mesopelagic and upper bathypelagic zones.

KEYWORDS
18S rRNA, marine zooplankton, mesopelagic, metabarcoding, station ALOHA

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1 | INTRODUCTION

The deep pelagic ocean contains a vast reservoir of poorly sampled and under-studied marine animals that play important roles in biogeochemical cycling and carbon sequestration in the ocean (Robison, 2004, 2009). Animals and bacteria in the mesopelagic zone (200–1000 m) respire 90% of the organic carbon exported from the surface ocean (Robinson et al., 2010), and mesopelagic food webs support commercially important fisheries (~8 mmt annually, gyres). The biodiversity of the ocean's midwaters is poorly characterized relative to either the surface ocean or the seafloor (Webb, Vanden Berghe, & O'Dor, 2010), despite the critical global ecosystem services that these organisms provide. The smallest of mesopelagic animals, the zooplankton, are important mediators of the flux of organic material into the deep ocean through contributions to both the active and passive carbon flux (the biological pump), and community composition has a significant influence on particle export, repackaging of particulate organic carbon (POC) with depth, and carbon attenuation and export through the mesopelagic zone (e.g., Steinberg, Cope, Wilson, & Kobari, 2008; Steinberg, Van Mooy, et al., 2008; Wilson, Steinberg, & Buesseler, 2008). While much of the research to date on mesopelagic zooplankton communities has focused on size-fractionated bulk measurements (e.g., Hannides, Popp, Choy, & Drazen, 2013) or broad taxonomic categories (Steinberg, Cope, et al., 2008), greater taxonomic resolution is required to predict the response of the mesopelagic zooplankton community, and concomitant effects on carbon flux and food webs, to changing ocean conditions and productivity in overlying waters.

In pelagic systems, strong vertical gradients in light, seawater temperature and nutrient concentration structure plankton communities across depth in the upper ocean resulting in vertical zonation in species composition as well as strong gradients in plankton biomass. Epipelagic (0–200 m), mesopelagic (200–1000 m) and bathypelagic (1000–4000 m) depth zones were found to approximately correspond to distinct zooplankton faunal assemblages in qualitative early descriptions for a range of taxonomic groups (e.g., Angel, 1979; Angel & Fasham, 1975; Vinogradov, 1970), with some variation in the reported depths of faunal transition across taxa and ocean regions (e.g., 500 m, 750 m, 1000 m all reported as the upper horizon for deep sea fauna). Depth gradients in species richness have been resolved for particular taxonomic groups, and maxima often are reported to occur at mesopelagic depths (e.g., 300–900 m, ostracods; Vinogradov, 1970; Angel & Fasham, 1975; Deevey & Brooks, 1977; Angel, 1979, 1991, 2010; Lindsay & Hunt, 2005; Yamaguchi, Matsuno, & Homma, 2015). However, because most experts specialize on a particular taxonomic group and report only on diversity and zoogeography for their taxon of interest, our knowledge of vertical gradients in species richness and species composition for the full community often is fragmentary (*but see* Kosobokova & Hopcroft, 2010; Kosobokova, Hopcroft, & Hirche, 2011).

Here we investigate the diversity and vertical structure of the zooplankton assemblage of the North Pacific Subtropical Gyre (NPSG). Oligotrophic subtropical gyres contain global maxima in pelagic species richness (MacPherson, 2002; Reid, Brinton, Fleminger, Venrick, & McGowan, 1978; Tittensor et al., 2010), holding on the order of ½ of global inventories of species within many plankton taxonomic groups. In these lower dominance ecosystems, the majority of species are rare (McGowan & Walker, 1985, 1993; Williamson & McGowan, 2010). Prior work in the NPSG has reported on the abundance and distribution of primarily crustacean zooplankton (Landry, Al-Mutairi, Selph, Christensen, & Nunnery, 2001; McGowan, 1971; McGowan & Walker, 1979, 1985; Williamson & McGowan, 2010), with a focus on collections made in the upper 600 m of the water column. The CLIMAX studies reported on the distribution, diel vertical migratory (DVM) behaviour and patterns of species dominance for 126 co-occurring copepod species (McGowan & Walker, 1979), while more recent studies of epipelagic assemblages from the Hawaiian Ocean Time-series (HOT) program documented seasonal trends in abundance for 79 taxa, of which 67 were copepods (upper 175 m; Landry et al., 2001). Systematic and biogeographic studies on other zooplankton groups provide information on the occurrence of pelagic molluscs (Be, Gilmer, & Ramsay, 1977; McGowan, 1960; Wall-Palmer et al., 2016), euphausiids (Brinton, 1962), amphipods (Vinogradov, Volkov, & Semenova, 1996), siphonophores (Alvariño, 1971), chaetognaths (Bieri, 1959) and urochordates (Berner, 1957; Godeaux, 1998) as well as other noncopepod species in the NPSG. Numerically, the mesozooplankton is dominated by copepods, which make up 70–75% of the zooplankton community above 1000 m (Steinberg, Cope, et al., 2008), with small-bodied calanoid and cyclopoid species the dominant taxa at epipelagic depths (Landry et al., 2001). Following copepods, ostracods, euphausiids and chaetognaths are the next most abundant groups (Steinberg, Cope, et al., 2008), with several gelatinous and crustacean taxa present at approximately an order of magnitude lower abundance (e.g., salps, doliolids, siphonophores, hydrozoan medusae, amphipods, mysids; summer collections). No comprehensive survey of the diversity of the NPSG assemblage has ever been undertaken that spans across animal phyla and across depth zones, and little information is available at high taxonomic resolution regarding species composition and community structure across depth.

Investigations of biodiversity in complex communities are increasingly turning to metabarcoding methods as a cost-effective and comprehensive approach to surveying species diversity (Creer et al., 2016; Taberlet, Coissac, Pompanon, Brochmann, & Willerslev, 2012). This approach is particularly well suited to studies of subtropical mesozooplankton due to high diversity and the difficulty of and time investment required for accurate identification of morphologically similar species. Early application of metabarcoding methods to marine zooplankton communities revealed substantial hidden diversity (58 species vs. 135 operational taxonomic units, OTUs, Lindeque, Parry, Harmer, Somerfield, & Atkinson, 2013), even in a moderately diverse temperate coastal site. Subsequent work on marine zooplankton has shown that in addition to detecting hidden diversity, metabarcoding methods also capture community shifts across oceanographic features and large-scale changes in ocean habitat (Hirai, Kuriyama, Ichikawa,

Hidaka, & Tsuda, 2015; Hirai & Tsuda, 2015; Pearman, El-Sherbiny, Lanzen, Al-Aidaroos, & Irigoien, 2014). These methods have not yet been widely applied, but may be particularly informative in studies of climate and oceanographic drivers of community change, and in understanding the abundance, distribution and ecological roles of small and cryptic taxa within zooplankton assemblages.

In this study, we investigated the diversity and vertical structure of the zooplankton assemblage in the NPSG across epipelagic, mesopelagic and upper bathypelagic depths (0–1500 m, 9 strata), using a metabarcoding approach to characterize the distribution of all zooplankton taxa. Our study site, station ALOHA (A Long-term Oligotrophic Habitat Assessment, 22°45′N 158°00′W), is the location of an important open ocean time series site with >25 years of observations on the chemistry, physics and biology of the subtropical gyre community (Karl & Lukas, 1996). Our goals were to: (i) assess the potential magnitude of undescribed metazoan diversity in poorly characterized midwater habitats, (ii) detect distinct faunal assemblages and identify important faunal boundaries across depth in the twilight zone, (iii) identify suites of species that maximally distinguish these faunal assemblages and (iv) test the hypothesis of a mesopelagic maximum in species richness for the full zooplankton assemblage using a metabarcoding approach.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Depth-stratified zooplankton samples were collected in June 2014 at station ALOHA with a 1 m^2 Multiple Opening and Closing Nets and Environmental Sampling System (MOCNESS, 200 μm mesh) towed between 1500 m and the sea surface. The bathypelagic (1500–1000 m), mesopelagic (200–1000 m) and epipelagic fauna (0–200 m) were sampled in nine target strata: 1500–1000 m, 1000–700 m, 700–500 m, 500–300 m, 300–200 m, 200–150 m, 150–100 m, 100–50 m and 50 m to the sea surface (exact depths in Table 1). Zooplankton from each depth was quantitatively split using a Folsom plankton splitter, with ½ preserved in formalin and a second quantitative fraction (typically ½) size-fractionated and preserved in RNALater (Ambion) for metabarcoding (Tables 1, S1). The metabarcoding material was wet-sieved into five size classes (0.2–0.5 mm, 0.5–1 mm, 1–2 mm, 2–5 mm and >5 mm) that have been the focus of prior research at station ALOHA (Hannides et al., 2013; Landry et al., 2001; Sheridan & Landry, 2004). All RNALater preserved samples were stored at −80°C prior to analysis. Here we report results from all depths and the three smallest size fractions from one night tow (20:52–04:16, local time) and one day tow (08:45–15:28, local time) (54 total samples).

2.2 | DNA extraction, polymerase chain reaction (PCR) and sequencing

DNA was extracted using the E.Z.N.A. HP Tissue DNA Maxi kit (OMEGA), following the manufacturer's protocol. Each sample was filtered through a 200-μm filter to remove excess RNALater, and filters were retained through initial lysis. Samples with >2 g of biomass were split into two extractions to ensure adequate lysis. The elution step was repeated 5× to maximize DNA yield; the elution with the highest concentration of high molecular weight DNA was used in amplification and sequencing. A ~365-bp fragment of the V1-V2 region of nuclear 18S rRNA was amplified in PCR using primers F04 [5′–GCTTGTCTCAAAGATTAAGCC–3′] and R22 [5′–GCCTGC TGCCTTCCTTGGA–3′] (Fonseca et al., 2010; Lindeque et al., 2013). This marker was one of the first to be used in metabarcoding studies of marine metazoans, and in copepods, the V1-V2 region has a higher proportion of parsimony informative sites than other variable regions of 18S rRNA (Wu, Xiong, & Yu, 2015). We report results based on this marker here. Fragments of the mitochondrial cytochrome *c* oxidase subunit I (mtCOI), 12S rRNA, nuclear 28S rRNA and the V4 region of 18S rRNA genes also were amplified (Hirai & Tsuda, 2015; Hirai, Kuriyam, et al., 2015; Leray et al., 2013; Machida & Knowlton, 2012; Machida, Miya, Nishida, & Nishida, 2004). PCR amplifications were run in duplicate for each sample and pooled to reduce stochastic differences in amplification across reactions. DNA concentration was quantified using the Qubit dsDNA Broad-Range Assay Kit (Life Technologies) and normalized across markers, and all PCR products from each sample were pooled into a single DNA template for library preparation. DNA was prepared for sequencing on the Illumina MiSeq platform using the TruSeq Nano library preparation kit, with the size selection step targeting a 550-bp insert. Prior to sequencing, libraries were assessed for fragment length using the Agilent 2100 Bioanalyzer and library concentration using quantitative PCR (Evolutionary Genetics Core Facility, Hawaii Institute of Marine Biology). Libraries were sequenced on three lanes of an Illumina MiSeq using V3 chemistry (300-bp, paired-end). Additional methods details are reported in Appendix S1.

2.3 | Bioinformatics and classification

Sequences from each sample were filtered and trimmed for sequence quality above a PHRED score of 20 using the FASTQ toolkit within BaseSpace (Illumina). Sequences from each marker were demultiplexed using SABRE (https://github.com/najoshi/sabre) based on primer sequence, and R1 and R2 reads were checked for appropriate pairing using FastqPairedEndValidator (http://www.mcdonaldlab.biology.gatech.edu/seqtools_frame.htm). Demultiplexed 18S rRNA sequences were processed in MOTHUR (version 1.36.1) with guidelines as outlined in the MiSeq SOP (Kozich, Westcott, Baxter, Highlander, & Schloss, 2013; Schloss et al., 2009) and in QIIME (version 1.9.1; Edgar, 2010; Edgar, Haas, Clemente, Quince, & Knight, 2011; Caporaso et al., 2010) following the MOTHUR analysis as closely as possible. In the MOTHUR analysis, filtering parameters were as follows: (i) no ambiguous bases; (ii) 25 bp minimum overlap between forward and reverse reads for contig generation; (iii) sequence length between 330 bp and 390 bp; and (iv) a maximum homopolymer length of 10 bp. Unique sequences were filtered, aligned against the SILVA database (release 123) and trimmed to the target region.

TABLE 1 Overview of the sequences, OTUs and OTU classifications per sample, after data processing and analysis in MOTHUR. Final sequences: number of sequences per sample after sequence filtering and removal of OTUs with <15 sequences, but before subsampling to standardize for sequence coverage. OTUs: the number of OTUs present in each sample after subsampling, at 99% similarity clustering. % classified OTUs: % of OTUs with ≥99% identity to a NCBI reference sequence. % holoplankton and % meroplankton: % OTUs in each category

Depth zone	Depth range (m)	Size fraction (mm)	Net	Tow	Final sequences	OTUs	% Classified OTUs	% Holoplankton	% Meroplankton
Epipelagic	0–49.0	0.2–0.5	N9	Day	35,998	449	40.38	78.29	20.84
	0–51.1	0.2–0.5	N9	Night	26,018	467	42.62	74.66	23.49
	0–49.0	0.5–1.0	N9	Day	26,836	470	48.63	87.40	12.60
	0–51.1	0.5–1.0	N9	Night	40,037	512	45.91	83.63	15.67
	0–49.0	1.0–2.0	N9	Day	12,340	408	51.96	85.54	13.73
	0–51.1	1.0–2.0	N9	Night	42,245	444	48.48	86.97	12.27
	49.0–100.0	0.2–0.5	N8	Day	22,559	646	39.12	80.31	18.26
	51.1–101.7	0.2–0.5	N8	Night	25,413	603	32.52	80.49	18.28
	49.0–100.0	0.5–1.0	N8	Day	17,462	594	42.90	86.09	13.17
	51.1–101.7	0.5–1.0	N8	Night	28,808	685	35.47	88.84	10.53
	49.0–100.0	1.0–2.0	N8	Day	15,566	508	47.91	87.11	12.52
	51.1–101.7	1.0–2.0	N8	Night	29,253	641	41.20	89.67	9.99
	100.0–150.3	0.2–0.5	N7	Day	24,462	700	25.61	91.39	8.50
	101.7–152.4	0.2–0.5	N7	Night	36,103	770	27.43	92.49	7.00
	100.0–150.3	0.5–1.0	N7	Day	19,292	616	31.16	93.74	5.99
	101.7–152.4	0.5–1.0	N7	Night	35,821	751	30.80	96.12	3.88
	100.0–150.3	1.0–2.0	N7	Day	17,331	523	37.31	92.61	7.39
	101.7–152.4	1.0–2.0	N7	Night	47,655	667	37.29	94.07	5.66
	150.3–201.5	0.2–0.5	N6	Day	41,923	697	24.60	91.98	7.23
	152.4–201	0.2–0.5	N6	Night	32,908	652	26.65	94.25	4.81
	150.3–201.5	0.5–1.0	N6	Day	16,155	664	26.18	95.00	4.87
	152.4–201	0.5–1.0	N6	Night	23,408	717	28.06	96.94	3.06
	150.3–201.5	1.0–2.0	N6	Day	17,978	493	33.57	92.76	7.07
	152.4–201	1.0–2.0	N6	Night	35,905	571	33.81	97.16	2.73
Mesopelagic	201.5–301.2	0.2–0.5	N5	Day	20,475	704	24.23	93.11	6.08
	201.0–303.0	0.2–0.5	N5	Night	26,421	698	25.82	95.56	4.23
	201.5–301.2	0.5–1.0	N5	Day	22,829	703	26.19	95.13	4.76
	201.0–303.0	0.5–1.0	N5	Night	31,399	622	27.23	96.88	3.01
	201.5–301.2	1.0–2.0	N5	Day	23,370	436	34.15	92.51	7.49
	201.0–303.0	1.0–2.0	N5	Night	28,900	438	33.80	96.11	3.89
	301.2–500.6	0.2–0.5	N4	Day	24,028	619	34.23	96.69	3.31
	303.0–502.3	0.2–0.5	N4	Night	33,390	606	32.23	97.80	2.09
	301.2–500.6	0.5–1.0	N4	Day	22,551	638	31.78	96.47	3.53
	303.0–502.3	0.5–1.0	N4	Night	26,739	595	33.21	98.60	1.40
	301.2–500.6	1.0–2.0	N4	Day	27,184	385	40.15	98.28	1.72
	303.0–502.3	1.0–2.0	N4	Night	25,400	389	38.92	97.69	2.31
	500.6–700.6	0.2–0.5	N3	Day	36,130	629	31.32	95.77	4.02
	502.3–702.5	0.2–0.5	N3	Night	40,359	603	32.33	96.67	3.11
	500.6–700.6	0.5–1.0	N3	Day	21,305	597	36.78	97.85	2.15
	502.3–702.5	0.5–1.0	N3	Night	31,025	627	34.05	96.84	3.16
	500.6–700.6	1.0–2.0	N3	Day	23,155	359	45.01	95.54	4.46
	502.3–702.5	1.0–2.0	N3	Night	40,940	413	41.56	96.88	3.13
	700.6–1001.9	0.2–0.5	N2	Day	41,655	560	27.70	96.82	3.18

(Continues)

TABLE 1 (Continued)

Depth zone	Depth range (m)	Size fraction (mm)	Net	Tow	Final sequences	OTUs	% Classified OTUs	% Holoplankton	% Meroplankton
	702.5–1000.3	0.2–0.5	N2	Night	33,864	615	31.67	97.87	2.13
	700.6–1001.9	0.5–1.0	N2	Day	23,576	610	30.04	98.55	1.45
	702.5–1000.3	0.5–1.0	N2	Night	30,154	643	30.13	98.34	1.66
	700.6–1001.9	1.0–2.0	N2	Day	21,737	527	33.44	98.01	1.99
	702.5–1000.3	1.0–2.0	N2	Night	34,701	424	37.64	98.88	1.12
Bathypelagic	1001.9–1502.0	0.2–0.5	N1	Day	42,512	507	35.73	94.60	5.27
	1000.0–1502.0	0.2–0.5	N1	Night	26,937	467	33.22	96.42	2.77
	1001.9–1502.0	0.5–1.0	N1	Day	19,144	642	30.70	99.35	0.65
	1000.0–1502.0	0.5–1.0	N1	Night	30,736	656	28.01	98.62	1.38
	1001.9–1502.0	1.0–2.0	N1	Day	23,469	462	30.43	98.52	1.48
	1000.0–1502.0	1.0–2.0	N1	Night	37,135	445	37.62	98.71	1.13

Duplicates were removed, and unique sequences were preclustered at both 99% and 97% similarity (4 bp & 10 bp mismatch allowed). Chimeras were identified and removed using de novo UCHIME within MOTHUR. Sequences were assigned taxonomy based on the eukaryotic portion of the SILVA database using the Wang method with taxonomic levels, with <80% bootstrap support discarded (Wang, Garrity, Tiedje, & Cole, 2007); sequences assigned taxonomy within Holozoa were retained for further analyses. Operational taxonomic units were reclustered (cluster.split, taxlevel = 5) at 99% and 97% similarity, and consensus taxonomy for each OTU was assigned using the average-neighbour method. Analyses in QIIME replicated the MOTHUR analysis, as described in Appendix S1. A range of cut-offs were explored for the minimum number of reads per OTU required to retain an OTU study-wide (Table 2; Bokulich et al., 2013; Valverde & Mellado, 2013). Operational taxonomic units with fewer than 15 reads were removed, and samples were rarified to standardize sequencing coverage across samples (e.g., to 12,340 reads/sample for 99% similarity clustering). All further analyses used results from MOTHUR clustered at 99% similarity, with the exception of comparisons between the 99% and 97% MOTHUR results for rarefaction and the total OTUs obtained, as well as comparisons between the 99% MOTHUR and 99% QIIME analyses for rarefaction and community structure. The most common sequence within each OTU was blasted against the National Center for Biotechnology Information (NCBI) nt database with uncultured/environmental sequences excluded. Both the SILVA taxonomy and sequence similarity, including ≥99% sequence identity to the top BLAST hit in the NCBI nt database, were used for taxonomic classification of OTUs (Table 1). Taxonomic inferences for each OTU as reported in Tables 4, 5 and Figure 6 also include consideration of the % identity to NCBI reference sequences, sequence comparisons to congeneric species and knowledge of the biogeography of the TAXON. Given the conserved nature of 18S rRNA (e.g., for copepods, Mohrbeck, Raupach, Martinez Arbizu, Knebelsberger, & Laakmann, 2015; Wu et al., 2015; Albaina, Aguirre, Abad, Santos, & Estonba, 2016), some OTUs may contain multiple, genetically proximate species (e.g., congeners). The majority of OTUs could be placed as holoplankton or meroplankton based on classification to Order; OTUs identified as polychaetes and molluscs required classifications at family level. Seventy-nine OTUs were not placed to

TABLE 2 Effect of a range of OTU cut-offs on the number of sequences and the number of OTUs retained study-wide, for clustering at 99% and 97% similarity (MOTHUR analysis). Counts are reported prior to subsampling to standardize sequence coverage. Bold indicates the cut-off chosen for all downstream analyses (≤14 removed)

	99% similarity	97% similarity
Raw		
Number of OTUs	357,271	106,654
Total sequences	2,038,023	1,523,037
Singletons removed		
Number of OTUs	59,242	31,772
Total sequences	1,739,994	1,448,155
≤2 removed[a]		
Number of OTUs	32,929	19,937
Total sequences	1,687,368	1,424,485
≤5 removed		
Number of OTUs	12,336	10,039
Total sequences	1,612,821	1,387,916
≤10 removed		
Number of OTUs	5,752	5,459
Total sequences	1,563,969	1,353,419
≤14 removed		
Number of OTUs	**4,024**	**3,997**
Total sequences	**1,542,737**	**1,335,469**
3% of sequences removed postsingleton removal		
Number of OTUs	33,142	13,752
Total sequences	1,687,794	1,404,708
OTUs with <0.005% of total sequences removed[b]		
Number of OTUs	765	798
Total sequences	1,439,066	1,240,269

[a]Valverde and Mellado (2013).
[b]Bokulich et al. (2013).

holoplankton or meroplankton due to the lack of reference sequences.

2.4 | Data analysis

Sequence-based rarefaction curves of OTU richness were calculated using the R package VEGAN (Oksanen et al., 2013). Rarefaction curves were calculated by net (with size fractions pooled), by size fraction within major depth assemblages, and for holoplankton and meroplankton communities rarefied separately. Rarefied (or subsampled) data, at 12,340 reads per sample (99% similarity), was used for all subsequent analyses comparing OTUs and reads across samples.

Similarity among samples was quantified and visualized using hierarchical cluster analysis, similarity profile analysis (SIMPROF) and nonmetric multidimensional scaling (nMDS) ordination (PRIMER version 7, Clarke & Gorley, 2015). Differences in community composition across ocean depth and zooplankton size fraction were assessed using OTU presence/absence, as well as read count abundance. Read count abundance data were square root transformed for each OTU, prior to calculations of Bray–Curtis similarity among samples and hierarchical clustering with average group clustering. Four primary faunal assemblages were resolved, and the contribution of each OTU to group similarity was quantified in SIMPER analyses.

Shannon-Wiener (H') and Simpson's ($1 - D$) diversity indices were calculated using read count abundance (in the R package VEGAN). We tested for differences in OTU richness and the % OTUs classified (≥99% sequence identity) across ocean depth and zooplankton size fraction using general linear models (R version 3.3.1, R Core Team 2012). We expected to observe higher % OTUs classified for larger-bodied and shallower-living species. A linear model and ANOVA with % OTUs classified as the response variable and depth assemblage and zooplankton size fraction as predictors were used to test this hypothesis, with post hoc Tukey tests, after evaluating and rejecting logit and arcsine square root transformations of the data. A similar approach was used to test OTU richness as the response variable and depth assemblage and zooplankton size fraction as predictors. Normality and homoscedasticity were evaluated using Shapiro–Wilk and Levene tests, as well as through visual evaluation of residuals. Linear regressions were used to test for an effect of depth separation on OTU sharing across depth strata for three zooplankton size fractions, and to determine whether the size fractions showed similar rates of change in species composition across depth (as evidenced by different slopes).

3 | RESULTS

3.1 | Bioinformatics and classification

A total of 2,694,365 18S rRNA reads were obtained after demultiplexing and quality filtering, 1,366,806 (51%) of which were unique sequences. After preclustering (4-bp mismatch, 99% similarity, MOTHUR), removal of 207,324 chimeric clusters and removal of non-metazoan reads (2.79% of unique sequences), a final clustering step resulted in a total of 2,038,023 sequences clustered into 357,271 OTUs at 99% similarity. In a second analysis, 1,523,037 high-quality, nonchimeric, metazoan reads were clustered into 106,654 final OTUs at 97% similarity. Stringency cut-offs for minimum read counts within each OTU had a significant impact on γ diversity, and a smaller but notable effect on the number of sequences retained study-wide (Table 2), with the overwhelming majority of OTUs in both the 99% and 97% analyses being singletons (Table 2). The final minimum of 15 reads per OTU retained 1.13% of OTUs and 75.70% of sequences in the 99% clustering analysis (4,024 OTUs total), and 3.75% of OTUs and 87.68% of sequences for the 97% clustering (3,997 OTUs total; Table 2). The QIIME analysis at 99% similarity clustering yielded 1,923,778 reads in 5,962 OTUs.

All downstream analyses were based on 99% similarity clustering (in MOTHUR), given results reported above for preliminary analyses and prior work suggesting this threshold as optimal for species delimitation for this gene fragment in similar plankton communities (Mohrbeck et al., 2015). Following removal of low frequency OTUs, all samples were rarefied to 12,340 sequences (666,360 total reads) to standardize sequencing coverage across samples. This step did not affect the total number of OTUs, but it decreased the average number of OTUs within a size fraction by 26%, while on average removing 53% of the sequences in each sample. Between 24% and 52% of OTUs in each sample had ≥ 99% sequence identity to an NCBI reference sequence (Table 1). High stringency in sequence filtering (read lengths 330–390 bp) to prevent inclusion of spurious reads and OTUs resulted in 90% removal of chaetognath sequences, with under-representation of chaetognath OTUs in the final analysis. Re-examination of unfiltered chaetognath reads found an average length of 395 bp (range 346–428 bp) for this gene fragment, with reads clustering into 133 final OTUs (99% similarity, 15 read minimum per OTU).

Results from the general linear model and ANOVA indicated that both depth assemblage and size fraction were significant predictors of the % OTUs classified (≥99% sequence identity to a NCBI reference sequence; $F_{11,42} = 14.34$, $P < 0.001$ both predictors, no significant interaction). Post hoc tests found significantly higher % OTUs classified in the upper epipelagic (0–100 m; mean 43%, $P < 0.05$), significantly lower % classified in the lower epipelagic (100–300 m; mean 30%, $P < 0.05$) and intermediate values in the upper mesopelagic (300–500 m; mean 35%) and deep mesopelagic and bathypelagic (500–1500 m; mean 34%). The largest zooplankton size fraction (1–2 mm; 39%) also had significantly higher mean % OTUs classified than the smaller two size fractions ($P < 0.05$, 0.5–1.0 mm 33%; 0.2–0.5 mm 32%).

3.2 | Sequence-based rarefaction

Rarefaction curves of OTU richness across sequencing coverage by net (size fractions combined) or plankton community (holoplankton/meroplankton) suggest insufficient sequencing depth (Figure 1, MOTHUR). With reads pooled within each size fraction across the four primary species assemblages, rarefaction curves were approaching an

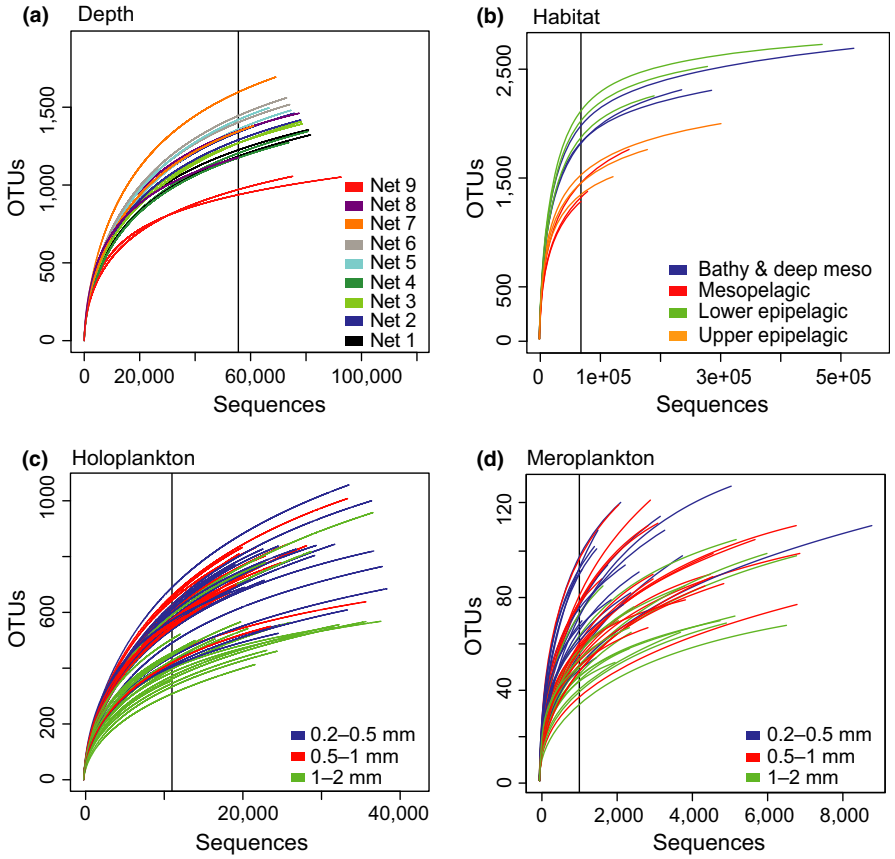

FIGURE 1 Sequence-based rarefaction of operational taxonomic unit (OTU) richness. Samples stratified by (a) depth of sampling (Net, depths as reported in Table 1); (b) habitat, defined as bathypelagic and deep mesopelagic (500–1500 m), mesopelagic (300–500 m), lower epipelagic (100–300 m) and upper epipelagic (0–100 m; after results in Fig. 2); (c) individual samples (Size fraction, Net) for holoplanktonic taxa; and (d) individual samples for meroplanktonic taxa. The vertical line in each plot marks the sample with lowest sequencing coverage; in A for all nets at 55,587 sequence reads, in b for reads from all samples in a habitat at 73,763 reads, in c for holoplanktonic taxa at 11,264 reads and in d for meroplanktonic taxa at 1,063 reads. Analyses at 99% similarity clustering (MOTHUR); *y*-axis reflects OTUs observed (S_{obs} analog)

asymptote at sequencing depths in the range of 300–500 K (Figure 1b). Rarefaction curves with OTUs clustered at 97% similarity exhibited similar patterns as the corresponding 99% results, with no curves reaching an asymptote (Fig. S1). Rarefaction curves for OTUs clustered in QIIME at 99% similarity also suggested insufficient sequencing depth for individual samples as well as when combined by depth habitat (Fig. S1).

3.3 | Community composition

Holoplankton contained the majority of OTU diversity, representing 91% of all OTUs (3,583 holoplankton OTUs; Table 3). Within the holoplankton, the highest OTU richness was observed within crustaceans, with 1,806 copepod (50.40%), 1,155 ostracod (32.24%) and 158 malacostracan OTUs (4.41%, including euphausiids, decapods). In total, crustaceans comprised 87% of the 3,583 holoplankton OTUs. Urochordates (141 OTUs), hydrozoans (118 OTUs) and polychaetes (114 OTUs) were the next most diverse groups, representing 3.94–3.18% of holoplankton OTUs. Pelagic molluscs (63 OTUs), scyphozoans (20 OTUs) and ctenophores (7 OTUs) were the least diverse groups. The majority of reads were from holoplanktonic taxa (619,429 reads, 93.53%), and the distribution of reads across taxonomic groups followed a pattern similar to the distribution of OTUs (Table 3). Community composition inferred from QIIME and MOTHUR analyses were broadly congruent, with higher total OTU richness in the QIIME results, including higher numbers of copepod OTUs (Table S2).

The distribution of reads across depth by taxonomic group showed considerable vertical structure in the holoplankton community (Fig. S2). Polychaete reads occurred predominantly in the 100–300 m depth range. Malacostracan reads (euphausiids, shrimps) demonstrated a diel vertical migratory (DVM) signal with a daytime increase in the 300–500 m depth layer. A higher proportion of ostracod reads occurred in the 100–300 m depth strata (23–30% of reads), coinciding with higher ostracod OTU richness at this depth. Scyphozoans made up 15% of reads in the deep mesopelagic during the night tow (700–1000 m; 19 OTUs) and included several taxa with few prior distributional reports (e.g., *Atorella* sp.).

Meroplankton represented 8% of OTU richness across the water column (total 350 OTUs; Table 3). Within the meroplankton, molluscs dominated OTU diversity, making up 37.71% of all meroplankton OTUs (132 OTUs), followed by polychaetes (78 OTUs, 22.29%) and fishes (65 chordate OTUs, 18.57%). Moderate diversity was observed in the cnidarians, arthropods and echinoderms (9.71–4.29% of meroplankton OTUs), with low OTU diversity in the hemichordates, nemerteans, brachiopods and bryozoans (1.53–0.29% of OTUs). As in the holoplankton, the distribution of reads across taxa generally followed the same pattern as the distribution of OTU diversity (Table 3). The 10 most abundant (by read count) meroplankton OTUs included bivalves, gastropods, hexacorals, teleosts, polychaetes, echinoids, an octocoral and a shrimp (Table 4). Six of the dominant meroplankton OTUs had 100% sequence identity to an NCBI reference sequence (Table 4),

TABLE 3 Community composition of the zooplankton assemblage at station ALOHA. Distribution of OTU richness and read counts across taxonomic groups and zooplankton size fractions. Data are subsampled for even sequencing coverage across samples, and OTUs were clustered at 99% similarity (MOTHUR analysis)

	Total		OTUs			Reads		
	OTUs	Reads	0.2–0.5 mm	0.5–1 mm	1–2 mm	0.2–0.5 mm	0.5–1 mm	1–2 mm
Holoplankton	**3,583**	**619,429**	**3,010**	**3,243**	**2,635**	**199,104**	**212,707**	**207,618**
Scyphozoans	20	5,871	18	18	18	2,822	1,345	1,704
Ctenophores	7	135	7	6	6	32	26	77
Polychaetes	114	34,107	81	107	92	7,292	15,883	10,932
Copepods	1,806	370,267	1436	1629	1407	114,789	126,818	128,660
Euphausiids/Shrimps	158	46,467	129	145	146	9,023	15,275	22,169
Ostracods	1,155	114,378	1108	1082	687	56,347	43,337	14,694
Chaetognaths	1	5	0	1	1	0	4	1
Urochordates	141	18,078	114	109	116	4,010	3,438	10,630
Hydrozoans	118	21,100	74	95	115	2,465	4,819	13,816
Molluscs	63	9,021	43	51	47	2,324	1,762	4,935
Meroplankton	**350**	**42,851**	**262**	**234**	**207**	**21,163**	**8,713**	**12,975**
Hemichordates	5	559	3	2	5	3	3	553
Echinoderms	15	2,276	12	11	12	298	258	1,720
Brachiopods	1	12	1	0	0	12	0	0
Crustaceans	16	1,173	5	4	15	12	17	1,144
Polychaetes	78	7,511	60	63	39	2,604	3,333	1,574
Cnidarians	34	6,241	23	20	18	2,460	1,844	1,937
Molluscs	132	19,133	126	87	59	15,471	1,949	1,713
Chordates	65	5,912	29	43	59	287	1,291	4,334
Nemerteans	3	23	2	3	0	10	13	0
Bryozoans	1	11	1	1	0	6	5	0

including several shallow-water species, as well as a polychaete (*Phyllochaetopterus* sp. 1) that is only reported to occur in the deep sea.

3.4 | Depth-stratified species assemblages

Community composition differed significantly across both ocean depth and zooplankton size fraction. Hierarchical clustering, SIMPROF analysis and nMDS ordination identified four primary species assemblages that were separated by depth habitat: (i) deep mesopelagic and bathypelagic (500–1500 m), (ii) upper mesopelagic (300–500 m), (iii) lower epipelagic and upper mesopelagic (100–300 m) and (iv) upper epipelagic (0–100 m) species assemblages (Figure 2). The primary separation of assemblages occurred above and below 500 m, with <20% similarity between these groups (Figure 2a). The upper 100 m of the epipelagic was the next most divergent group, with the upper mesopelagic (300–500 m) and lower epipelagic (100–300 m) assemblages most similar of the four primary depth assemblages (Figure 2a). Within these four primary assemblages, distinct clusters of samples by zooplankton size fraction occurred for the deep mesopelagic and bathypelagic (1–2 mm), upper epipelagic (0.2–0.5 mm) and upper mesopelagic (1–2 mm), implying greater faunal similarity within zooplankton size fraction for samples collected within the same depth assemblage. nMDS ordination confirmed separation of samples primarily by depth (Figure 2c) and also indicated some separation among the 0–50 and 50–100 m strata that was not seen in cluster analyses (Nets 8, 9; Figure 2c). Entirely congruent results were obtained from read count abundance and presence/absence-based multivariate analyses, suggesting strong differences in both species composition and read count abundance across both depth and size-based assemblages. QIIME-based analyses were also congruent with MOTHUR results regarding the composition of the four primary assemblages. Only the 1 mm size fraction from Net 3 (500–700 m) was placed differently in the two dendrograms (QIIME: upper mesopelagic, MOTHUR: deep mesopelagic & bathypelagic).

The number of OTUs shared between samples declined as a function of the difference in the median depth of sampling (m) for all three zooplankton size fractions (regressions, $P < 0.001$ all cases, adj-R^2 = 0.43 [0.2 mm], 0.34 [0.5 mm], 0.35 [1.0 mm]), with the rate of decline differing between size fractions (slopes −0.25 [0.2 mm], −0.22 [0.5 mm], −0.18 [1.0 mm]) (Figure 3). Both zooplankton size fraction and the difference in the median depth of sampling had a significant effect on the number of OTUs shared between samples

TABLE 4 Dominant meroplankton OTUs. The percentage of total meroplankton reads for the top ten dominant meroplankton OTUs (by read count) within each zooplankton size fraction and overall. Subsampled data were used to calculated percentages within each category. The top ten OTUs within each category are indicated in bold. For each OTU, the top BLAST hit (NCBI) and % sequence identity (% Id) is listed, as is the SILVA taxonomic classification for each OTU (>80% bootstrap support). Note that for some OTUs that were classified only to Infraclass in SILVA, the top BLAST hit is clearly not the correct species (indicated by *)

OTU	0.2 mm (%)	0.5 mm (%)	1 mm (%)	Overall (%)	NCBI	% Id	SILVA taxonomy
Otu000031	**21.42**	0.95	0.29	**10.73**	*Malleus malleus*	100	Mollusca; Bivalvia; Pteriomorpha; Pterioida; Malleus
Otu000039	**13.65**	**5.50**	0.96	**8.07**	*Abyssochrysos melanioides*	96.4	Mollusca; Gastropoda; Caenogastropoda
Otu000043	**8.50**	1.92	2.59	**5.34**	*Leucozonia cerata*	96.1	Mollusca; Gastropoda; Caenogastropoda
Otu000052	**6.32**	0.00	0.00	**3.09**	*Umbellula* sp.	96.9	Cnidaria; Anthozoa; Octocorallia; Alcyonacea; Umbellula
Otu000058	**1.73**	**7.35**	**4.41**	**3.69**	*Chloeia flava*	99.5	Annelida; Polychaeta; Palpata; Eunicida
Otu000066	1.33	**12.51**	1.46	**3.62**	*Andvakia discipulorum*	100	Cnidaria; Anthozoa; Hexacorallia; Actiniaria; Phellia
Otu000068	1.21	1.07	**7.78**	**3.22**	*Cereus herpetodes*	100	Cnidaria; Anthozoa; Hexacorallia; Actiniaria; Calliactis
Otu000071	0.50	0.61	**7.62**	**2.74**	*Retropinna semoni**	96.8	Gnathostomata; Euteleostomi; Actinopterygii; Neopterygii; Teleostei
Otu000076	**4.68**	0.16	0.01	**2.32**	*Stylocheilus longicauda*	95.8	Mollusca; Gastropoda; Heterobranchia
Otu000077	0.01	**2.24**	**5.90**	**2.29**	*Scortum hillii**	99.5	Gnathostomata; Euteleostomi; Actinopterygii; Neopterygii; Teleostei
Otu000079	0.28	0.50	**5.41**	1.92	*Retropinna semoni**	97.3	Gnathostomata; Euteleostomi; Actinopterygii; Neopterygii; Teleostei
Otu000095	**3.40**	0.11	0.10	1.71	*Alvinichoncha hessleri*	94.9	Mollusca; Gastropoda; Caenogastropoda
Otu000099	0.12	**6.52**	**2.62**	2.18	*Eumida* sp.	96.4	Annelida; Polychaeta; Palpata; Phyllodocida
Otu000111	0.00	**6.07**	0.34	1.33	*Auxis rochei*	100	Gnathostomata; Euteleostomi; Actinopterygii; Neopterygii; Teleostei
Otu000120	0.05	1.35	**5.05**	1.87	*Scolelepsis squamata*	89.7	Annelida; Polychaeta
Otu000128	0.00	0.00	**4.26**	1.32	*Pontophilus gracilis*	99.2	Arthropoda; Crustacea; Malacostraca; Eumalacostraca; Eucarida; Pontophilus
Otu000147	**1.37**	1.69	0.37	1.12	*Rissoa auriscalpium*	96.9	Mollusca; Gastropoda; Caenogastropoda
Otu000153	**1.35**	1.22	0.11	0.94	*Phyllochaetopterus* sp.	100	Annelida; Polychaeta; Scolecida; Spionida; Phyllochaetopterus
Otu000155	0.51	0.16	**2.79**	1.15	*Spatangoida* sp.	99.7	Echinodermata; Echinoidea
Otu000169	0.01	0.45	**3.71**	1.25	*Archaeopneustes hystrix*	99.7	Echinodermata; Echinoidea
Otu000183	**1.54**	0.02	0.01	0.76	*Septifer bilocularis*	100	Mollusca; Bivalvia; Pteriomorpha; Mytiloida; Septifer
Otu000209	0.28	**2.89**	0.12	0.76	*Eumida* sp.	97.2	Annelida; Polychaeta; Palpata; Phyllodocida
Otu000213	0.20	**3.04**	0.14	0.75	*Asetocalamyzas laonicola*	89.4	Annelida; Polychaeta; Scolecida; Spionida
Otu000240	0.00	**2.72**	0.00	0.55	*Phyllochaetopterus* sp.	99.2	Annelida; Polychaeta; Scolecida; Spionida; Phyllochaetopterus
Otu000332	0.08	**2.24**	0.03	0.50	*Ochetostoma erythrogrammon*	97.0	Annelida; Polychaeta; Echiura; Echiuroinea

($F_{3,455}$ = 149.7, P < 0.001). Stronger declines in OTU sharing by depth separation among samples in the smaller zooplankton size fractions likely occurred due to higher OTU richness in smaller-bodied animals and higher numbers of shared OTUs in proximate strata. Depth endemism within each of the four primary assemblages was assessed with OTUs considered present in an assemblage if ≥5% of the total reads were observed within any given depth strata (very rare OTUs excluded). Under this criterion, 41–56% of all OTUs exhibited depth endemism in the bathypelagic and lower mesopelagic, upper mesopelagic or upper epipelagic assemblages, while 89% of OTUs in the upper mesopelagic and lower epipelagic (100–300 m) were unique to that assemblage. There was no apparent faunal transition at 200 m or at 1000 m, with only 15–26% of OTUs restricted to either 100–200 m or 200–300 m and 23–29% of OTUs restricted to either 500–1000 m or 1000–1500 m (an absence of faunal transition at 200, 1000 m also apparent in Figure 2).

Total dissimilarity between depth assemblages derived from low % contribution from each of a large number of OTUs (SIMPER analysis, Table 5); 757, 772 and 779 OTUs were required to account for 70% of the contribution to average group dissimilarity between the four primary species assemblages across depth. For read count abundance-based results, copepods *Spinocalanus abyssalis* [OTU3], *Lucicutia* sp. 1 [OTU15], *Scaphocalanus magnus* [OTU8], *Metridia* sp. 2 [OTU16], *Subeucalanus subtenuis* [OTU19] and an ostracod [OTU20] were among the top 15 OTUs differentiating the deep mesopelagic and bathypelagic assemblage (below 500 m) (Table 5). Upper mesopelagic taxa that contributed to the differentiation from both the deep meso- and bathypelagic assemblage (<500 m) and the lower epipelagic assemblage (100–300 m) included *Pleuromamma xiphias* [OTU1], *P. borealis* [OTU32], *Pleuromamma* sp. [OTU28], *Metridia* sp. 1 [OTU42], *Subeucalanus mucronatus* [OTU27], *Gaetanus tenuispinus* [OTU2], an ostracod [OTU38] and three euphausiid OTUs (cum. 11.2% dissimilarity, Table 5). For the species assemblage in the lower epipelagic/upper mesopelagic (100–300 m), copepods *Haloptilus* sp. [OTU 7], *Mesocalanus tenuicornis* [OTU11], *Lucicutia* sp. 2 [OTU14], polychaetes *Vanadis longissima* [OTU24] and *Rhynchonereella gracilis* [OTU21], and ostracods [OTU4 & OTU6] were important to differentiation of this assemblage from other depths (Table 5). Finally, copepods that are well-known inhabitants of the near surface had the highest % contribution to dissimilarity of the upper epipelagic assemblage, *Acrocalanus monachus* [OTU9], *Pareucalanus attenuatus* [OTU17], *Cosmocalanus darwinii* [OTU22] and *Clausocalanus furcatus* [OTU5], in addition to a meroplanktonic gastropod [OTU43] (cum. 11.6% of dissimilarity, Table 5). Thus, while several copepods were important to differentiation of the depth assemblages, species from other taxonomic groups also contributed.

Using plots of normalized read counts across ocean depth for individual OTUs (Figure 6a), we confirmed that many OTUs were resident at depths that correspond to one of the four primary depth-stratified species assemblages, including *Spinocalanus abyssalis* [OTU3], *Haloptilus* sp. [OTU7], *Acrocalanus monachus* [OTU9], *Mesocalanus tenuicornis* [OTU11], *Pareucalanus attenuatus* [OTU17], *Subeucalanus mucronatus* [OTU27] and *Metridia* sp. [OTU42]. Depth distribution plots of sequence reads for *Pleuromamma xiphias* [OTU1], *Euphausia* sp. 1 [OTU18] and pteropod *Hyalocyalis striata* [OTU55] also confirmed that metabarcoding data can resolve diel changes in the vertical distributions of these mesopelagic migrators (Figure 6b). Finally,

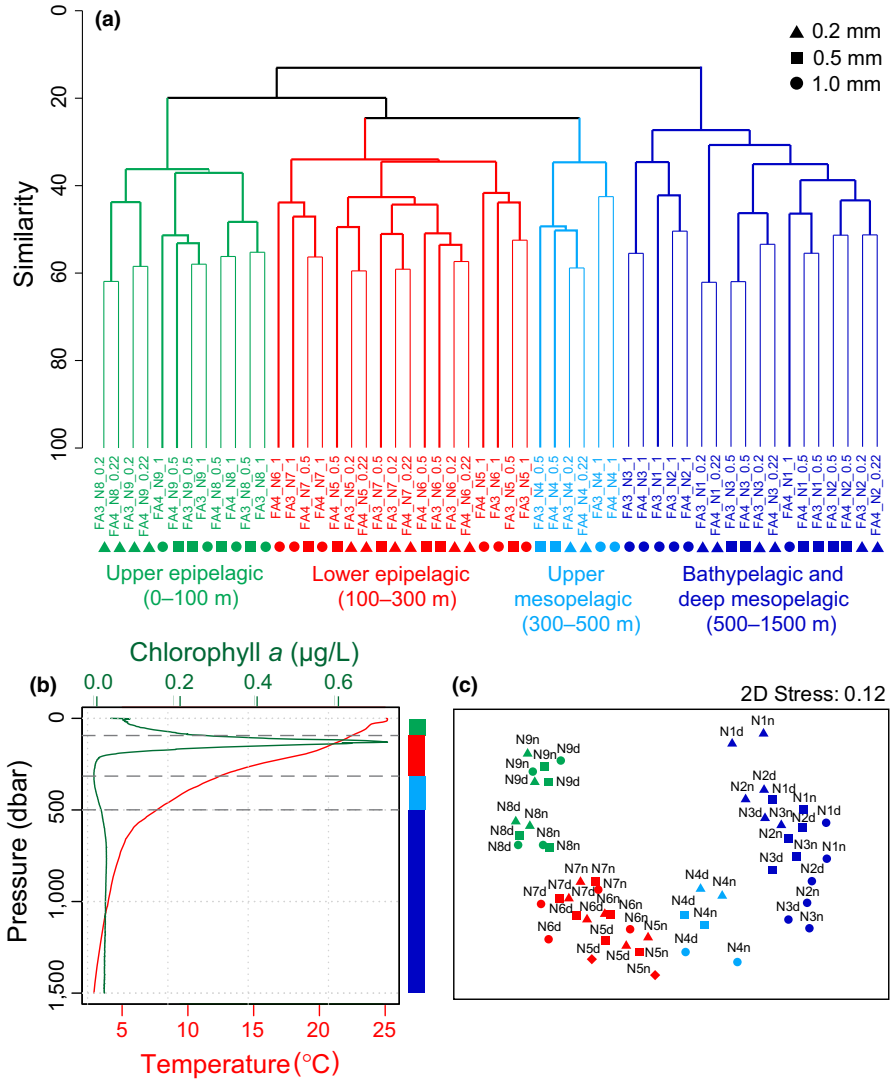

FIGURE 2 Multivariate analyses of zooplankton community structure across depth and size fraction. (a) Hierarchical cluster analysis dendrogram, with four primary clusters highlighted by colour: Upper epipelagic (0–100 m, green), Lower epipelagic (100–300 m, red), Upper mesopelagic (300–500 m, light blue), and Bathypelagic and deep mesopelagic (500–1500 m, blue) assemblages. Significant multivariate structure among samples is indicated by thick lines in the dendrogram (SIMPROF, $P < 0.05$). (b) Vertical profile of chlorophyll *a* [µg/L] and seawater temperature [°C] at station ALOHA in June 2014, with depth strata for each assemblage indicated in colour (at right) and faunal boundaries marked by dotted lines. (c) Nonmetric multidimensional scaling (nMDS) ordination of all nets and size fractions. Labelling in C corresponds to *N#* = MOCNESS net #, and *n* or d indicating night or day tows. Symbols for each plankton size fraction are as shown at upper right. Analyses include read count abundance of each operational taxonomic unit

metabarcoding data enabled resolution of depth distributions for taxa for which limited prior information was available, with examples including a migratory gymnosome *Pneumoderma* sp. (OTU116, Figure 6b), deep mesopelagic scyphozoan *Atorella* sp. [OTU26] and fragile siphonophores *Lensia conoidea* [OTU30], *Rosacea flaccida* [OTU53] and *Bargmannia elongata* [OTU51], as well as many others.

3.5 | Diversity across depth and plankton size fraction

Both depth assemblage and zooplankton size fraction were significant predictors of OTU richness (two-way ANOVA, $F_{11,42} = 8.427$, $P < .001$ both predictors, no significant interaction). Post hoc tests indicated significantly higher OTU richness in the lower epipelagic assemblage (100–300 m, mean 634 OTUs/sample) than in the remainder of the water column (means 535–543 OTUs/sample). Significantly higher OTU richness also occurred in the two smaller zooplankton size fractions (0.2–0.5 & 0.5–1.0 mm = means 611, 630 OTUs, respectively; 1–2 mm = mean 474 OTUs/sample). Shannon and Simpson diversity indices supported the trends observed in OTU richness (Table S1), with highest mean H' and $1 - D$ in the lower epipelagic assemblage (100–300 m; 4.0, 0.95, respectively) and in the intermediate size fraction (0.5–1.0 mm; 3.95, 0.94, respectively).

Depth gradients in diversity also were apparent in the distribution of OTU richness within taxonomic groups across the epipelagic and mesopelagic zones (Figures 4, 5). The total number of OTUs was highest in the epipelagic at night (100–150 m, 1,379 OTUs) and in the upper mesopelagic during the day (200–300 m, 1,207 OTUs). Maximum OTU richness for copepods was observed in the 500–700 m depth range during both day and night (603, 675 OTUs, respectively), with a secondary peak in the epipelagic, between 50 and 100 m during the day (466 OTUs) and 100–150 m at night (568 OTUs; Figure 4). Ostracods had a daytime maximum in OTU richness in the upper mesopelagic at 200–300 m (487 OTUs), with a night-time shoaling to 100–150 m (504 OTUs). MOTHUR and QIIME analyses yielded similar depth gradients in diversity for ostracods and copepods, but with a higher number of copepod OTUs in the QIIME analysis (Fig. S3). Most other taxonomic groups had single depth peaks in OTU richness (Figure 4), although some exhibited bimodal distributions during either day or night. Malacostracans and urochordates peaked in OTU richness in the epipelagic, with a secondary daytime peak appearing in the mesopelagic (300–500 m Malacostraca, 500–700 m Urochordata). Hydrozoans exhibited a primary peak in the epipelagic with a secondary peak appearing in the mesopelagic (500–700 m) at night. Scyphozoans exhibited an unusual peak in the 700–1000 m depth range, occurring only in the night-time tow. Maximum OTU richness for polychaetes, molluscs and ctenophores occurred in the epipelagic. Some aspects of depth trends in OTU richness were distinct across zooplankton size fraction (Figure 5); for example, the number of OTUs in the smallest size fraction decreased notably in the deep mesopelagic and upper bathypelagic zones. Both the 0.2 and 0.5 mm size fractions had clear maxima in OTU richness between 100 and 300 m, and a strong DVM signal was apparent in the largest size fraction (1–2 mm, Figure 5).

Highest meroplankton diversity occurred in the epipelagic during both day and night (Figure 4c,d). In the near surface, 23.5% of all OTUs were meroplanktonic, with strong declines with depth (to ~7% of OTUs at the base of the epipelagic; Table 1). Depth gradients in meroplankton OTU richness were driven by molluscs, the most diverse group (Figure 4). Molluscs, arthropods, echinoderms and hemichordates all exhibited a single peak in OTU richness in the epipelagic. For polychaetes, a secondary peak also appeared between 200 and 300 m in the day tow. Cnidarians and chordates were bimodal in distribution, with a primary peak in the epipelagic and a secondary peak between 500 and 700 m.

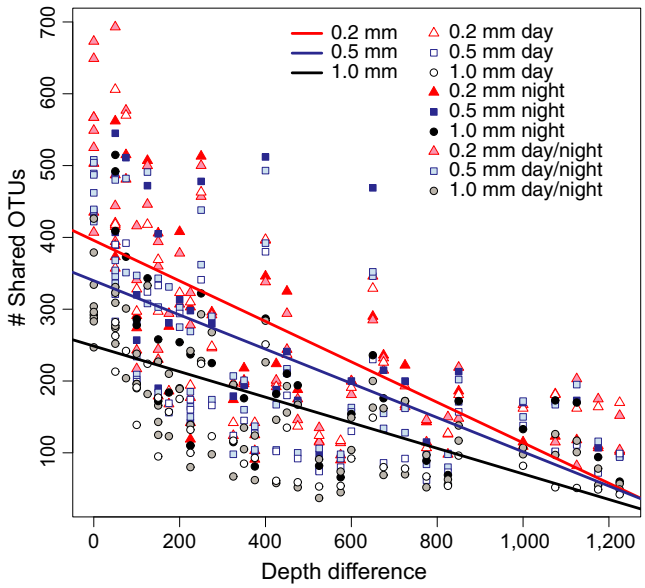

FIGURE 3 Relationship between the number of operational taxonomic units (OTUs) shared between samples and the difference in median depth of collection (MOCNESS nets), for three zooplankton size fractions (0.2 mm: triangle, 0.5 mm: square, 1.0 mm: circle). Open symbols represent comparisons among day tow samples, closed symbols represent night tow samples, and lightly shaded symbols are comparisons between day and night samples. 0.2 mm regression—adjusted R^2: 0.43, 0.5 mm regression—adjusted R^2: 0.34, 1.0 mm regression—adjusted R^2: 0.35 ($P < 0.001$, all cases)

4 | DISCUSSION

4.1 | What is the magnitude of undescribed diversity in the twilight zone?

Over the epipelagic, mesopelagic and upper bathypelagic zones in the NPSG, we observed an approximately 10-fold higher number of zooplankton OTUs than expected, given the number of morphologically described species reported for the region. The observation of far higher diversity than expected is concordant with results of other metabarcoding studies (Lindeque et al., 2013; de Vargas et al., 2015), and with reports of the cryptic diversity present within particular zooplankton genera or families (Cornils & Held, 2014; Goetze,

TABLE 5 Results of SIMPER analyses to identify OTUs that contribute most to differentiation of depth-stratified faunal assemblages. The top 15 OTUs for each of three group comparisons are listed: (A) Deep mesopelagic and bathypelagic vs. upper mesopelagic, (B) Upper mesopelagic vs. lower epipelagic and (C) Lower epipelagic versus upper epipelagic assemblages. Columns "Meso/Bathy," "Epi/Meso" and "L Epi/U Epi" designate the dominant faunal group for each OTU. Each OTU's % contribution to average dissimilarity is given (Contrib %), as is the Cumulative % (Cum %). The top NCBI hit for each OTU is reported (NCBI scientific name), with % identity (BLAST % Id). Analyses included read count abundance. Taxonomic inference (at right) for each OTU includes consideration of % identity, sequence comparisons to congeneric species, and knowledge of the biogeography of each taxon. Note that given the conserved nature of 18S rRNA, some OTUs may contain multiple, genetically proximate species

A. Bathypelagic and deep mesopelagic vs. upper mesopelagic [757 OTUs, 70% of contributions]						
Taxon	**Meso/Bathy**	**Contrib %**	**Cum. %**	**BLAST % Id**	**NCBI scientific name**	**Taxon inference**
Otu000001	M	1.32	1.32	100	*Pleuromamma xiphias*	*P. xiphias*
Otu000003	B	0.99	2.31	100	*Spinocalanus abyssalis*	*S. abyssalis*
Otu000015	B	0.83	3.14	99.71	*Lucicutia ovaliformis*	*Lucicutia* sp. 1
Otu000032	M	0.82	3.95	99.43	*Pleuromamma xiphias*	*P. borealis*
Otu000042	M	0.78	4.73	98.56	*Metridia asymmetrica*	*Metridia* sp. 1
Otu000012	M	0.77	5.5	99.74	*Euphausia pacifica*	*Euphausia* sp.
Otu000027	M	0.75	6.25	100	*Subeucalanus mucronatus*	*S. mucronatus*
Otu000008	B	0.71	6.97	100	*Scaphocalanus magnus*	*S. magnus*
Otu000020	B	0.7	7.67	90.13	*Conchoecia* sp.	Ostracod sp. 1
Otu000018	M	0.69	8.36	99.47	*Euphausia pacifica*	*Euphausiid* sp. 1
Otu000038	M	0.69	9.05	85.75	*Conchoecia* sp.	Ostracod sp. 2
Otu000016	B	0.62	9.67	99.71	*Metridia asymmetrica*	*Metridia* sp. 2
Otu000019	B	0.6	10.27	100	*Subeucalanus subtenuis*	*S. subtenuis*
Otu000028	M	0.58	10.85	99.15	*Pleuromamma xiphias*	*Pleuromamma* sp.
Otu000034	M	0.58	11.42	99.47	*Thysanopoda aequalis*	*T. aequalis*
B. Upper mesopelagic vs. lower epipelagic [772 OTUs, 70% of contributions]						
Taxon	**Epi/Meso**	**Contrib %**	**Cum. %**	**BLAST % Id**	**NCBI scientific name**	**Taxon inference**
Otu000001	M	0.87	0.87	100	*Pleuromamma xiphias*	*P. xiphias*
Otu000011	E	0.87	1.74	99.72	*Mesocalanus tenuicornis*	*M. tenuinornis (or lighti)*
Otu000042	M	0.85	2.59	98.56	*Metridia asymmetrica*	*Metridia* sp. 1
Otu000024	E	0.8	3.39	99.44	*Vanadis longissima*	*V. longissima*
Otu000006	E	0.77	4.16	88.59	*Conchoecia* sp.	Ostracod sp. 7
Otu000021	E	0.76	4.92	99.72	*Rhynchonereella gracilis*	*R. gracilis*
Otu000028	M	0.76	5.69	99.15	*Pleuromamma xiphias*	*Pleuromamma* sp.
Otu000007	E	0.76	6.45	100	*Haloptilus ocellatus*	*Haloptilus* sp.
Otu000012	M	0.73	7.18	99.74	*Euphausia pacifica*	*Euphausia* sp.
Otu000027	M	0.71	7.88	100	*Subeucalanus mucronatus*	*S. mucronatus*
Otu000032	M	0.69	8.57	99.43	*Pleuromamma xiphias*	*P. borealis*
Otu000018	M	0.68	9.25	99.47	*Euphausia pacifica*	*Euphausiid* sp. 1
Otu000034	M	0.67	9.92	99.47	*Thysanopoda aequalis*	*T. aequalis*
Otu000002	M	0.62	10.53	100	*Gaetanus tenuispinus*	*G. tenuispinus*
Otu000014	E	0.61	11.15	99.71	*Lucicutia flavicornis*	*Lucuicutia* sp. 2
C. Lower epipelagic vs. upper epipelagic [779 OTUs, 70% of contributions]						
Taxon	**L Epi/U Epi**	**Contrib %**	**Cum. %**	**BLAST % Id**	**NCBI scientific name**	**Taxon inference**
Otu000009	U Epi	1.26	1.26	100	*Acrocalanus monachus*	*A. monachus*
Otu000017	U Epi	1.02	2.28	100	*Pareucalanus attenuatus*	*P. attenuatus*
Otu000007	L Epi	0.9	3.19	100	*Haloptilus ocellatus*	*Haloptilus* sp.
Otu000006	L Epi	0.84	4.02	88.59	*Conchoecia* sp.	Ostracod sp. 7
Otu000001	L Epi	0.84	4.86	100	*Pleuromamma xiphias*	*P. xiphias*

(Continues)

TABLE 5 (Continued)

C. Lower epipelagic vs. upper epipelagic [779 OTUs, 70% of contributions]						
Taxon	**L Epi/U Epi**	**Contrib %**	**Cum. %**	**BLAST % Id**	**NCBI scientific name**	**Taxon inference**
Otu000022	U Epi	0.82	5.68	100	*Cosmocalanus darwinii*	*C. darwinii*
Otu000004	L Epi	0.81	6.49	91.42	*Conchoecia* sp.	Ostracod sp. 8
Otu000012	U Epi	0.81	7.3	99.74	*Euphausia pacifica*	*Euphausia* sp.
Otu000025	U Epi	0.72	8.02	99.44	*Cosmocalanus darwinii*	Calanid
Otu000024	L Epi	0.68	8.7	99.44	*Vanadis longissima*	*V. longissima*
Otu000011	L Epi	0.68	9.38	99.72	*Mesocalanus tenuicornis*	*M. tenuinornis* (or *lighti*)
Otu000005	U Epi	0.57	9.95	100	*Clausocalanus furcatus*	*C. furcatus*
Otu000018	U Epi	0.57	10.53	99.47	*Euphausia pacifica*	*Euphausiid* sp. 1
Otu000021	L Epi	0.57	11.09	99.72	*Rhynchonereella gracilis*	*R. gracilis*
Otu000043	U Epi	0.46	11.56	96.12	*Leucozonia cerata*	*Leucozonia* sp.

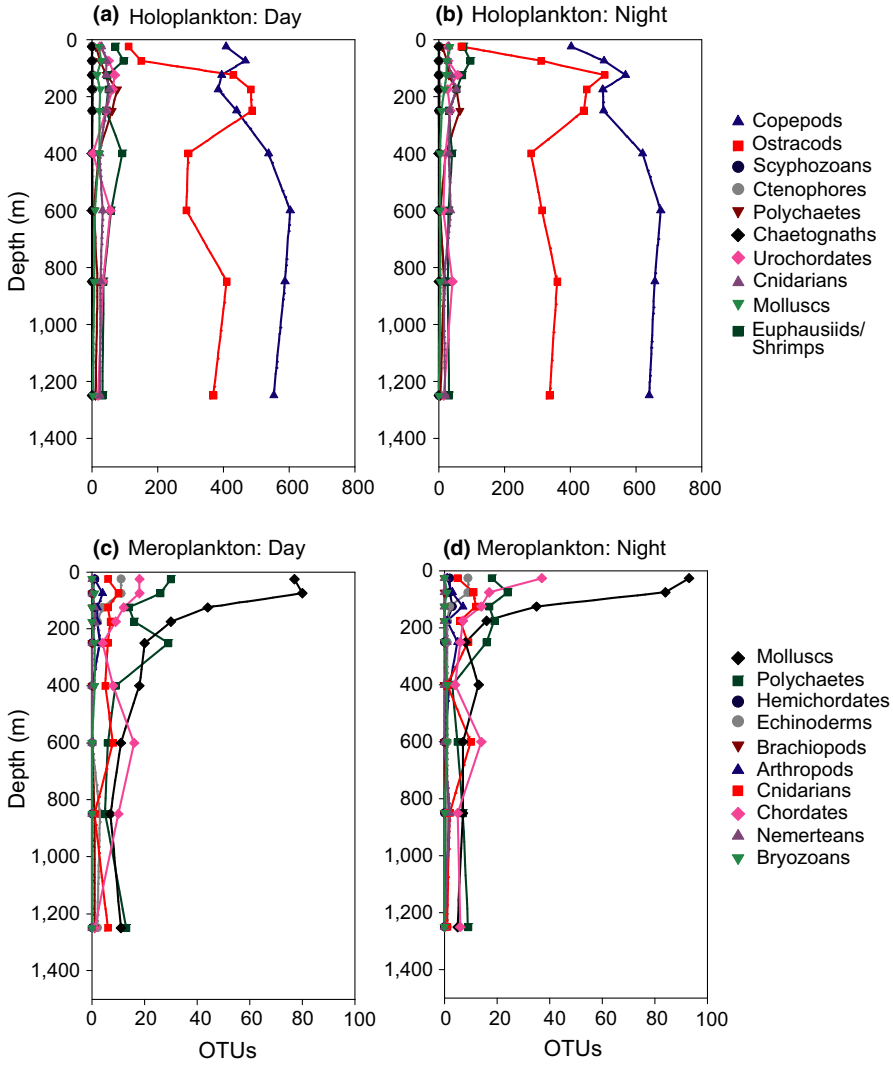

FIGURE 4 Operational taxonomic unit (OTU) richness across depth at station ALOHA for holoplanktonic (a, b) and meroplanktonic (c, d) taxonomic groups, shown for both day and night tows. Data are subsampled for even sequencing coverage across samples (size, net). Symbols for each depth-stratified net are plotted at the median depth of collection (depth ranges listed in Table 1). Analyses at 99% similarity clustering (MOTHUR)

2003, 2010; Halbert, Goetze, & Carlon, 2013; Hirai, Tsuda, & Goetze, 2015; Nigro, Angel, Blachowiak-Samolyk, Hopcroft, & Bucklin, 2016). However, because sequence processing and clustering steps affect the diversity recovered in metabarcoding studies (Coissac, Riaz, & Puillandre, 2012; Flynn, Brown, Chain, MacIsaac, & Cristescu, 2015), it is important to consider whether these OTU richness estimates are artificially inflated. Both the sequence filtering parameters and the read abundance cut-off used in this study (≥15 reads per OTU) were highly stringent in comparison with similar studies or methods suggested by comparative analyses (e.g., Bokulich et al., 2013; Flynn et al., 2015; Hirai & Tsuda, 2015; Pearman & Irigoien, 2015; Valverde & Mellado, 2013), and our methods would be

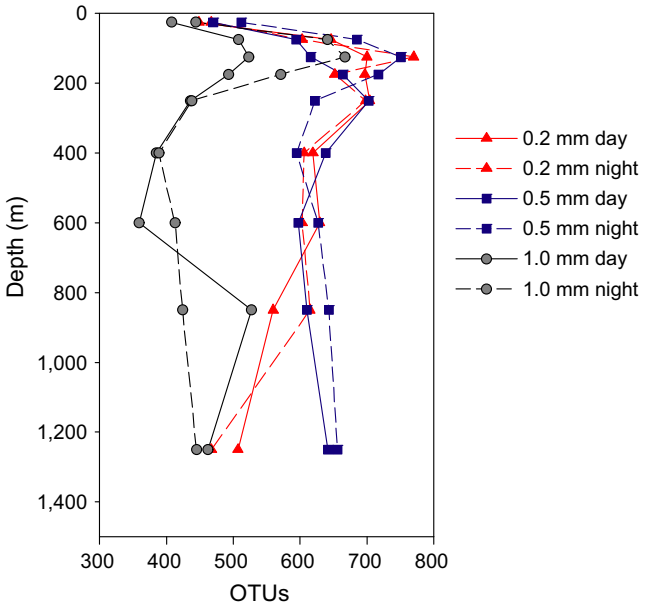

FIGURE 5 Operational taxonomic unit (OTU) richness across depth at station ALOHA for three zooplankton size fractions (0.2–0.5 mm, 0.5–1.0 mm, 1–2 mm), shown for both day and night tows. Data are subsampled for even sequence coverage across samples (size, net). Symbols for each depth strata are plotted at median depth of collection (depth ranges listed in Table 1)

expected to err on the side of removing true diversity of rare organisms in addition to potentially spurious low read count OTUs. For example, many metabarcoding studies remove only singleton or doubleton OTUs (Hirai & Tsuda, 2015; Pearman et al., 2014), an approach that in our case would yield estimates of upwards of 32K OTUs for the full water column (Table 2). In addition, the V1-V2 region of 18S rRNA is a conservative marker that is known to have fairly low resolution for species-level discrimination of crustaceans in particular (Mohrbeck et al., 2015; Wu et al., 2015), but it performs well for PCR amplification across phylogenetically divergent groups, enabling recovery of diversity across the full zooplankton assemblage (Clarke, Beard, Swadling, & Deagle, 2017; Fonseca et al., 2010; Lejzerowicz et al., 2015; Lindeque et al., 2013). The 99% similarity used in clustering in this study was chosen to optimize separation of crustacean OTUs at as close to species-level resolution as possible, but we recognize that many closely related species will remain indistinguishable at this marker due to 100% sequence identity (Albaina et al., 2016; Goetze, 2003), resulting in lower than true estimates of diversity. Parallel analyses with OTUs clustered at 97% resulted in OTU counts of approximately the same magnitude (within 15%) for both copepods and ostracods, indicating that the pattern of high species richness observed in these groups does not result from oversplitting of OTUs at 99% similarity clustering. The average-neighbour clustering algorithm implemented in MOTHUR is known to be robust, yields reproducible OTU demarcation in comparison with several other methods (e.g., Schloss & Westcott, 2011; Schmidt, Matias Rodrigues, & von Mering, 2015) and is widely used in microbial ecology (e.g., Ghiglione et al., 2012; Purahong et al., 2016). Analyses in QIIME (using UCLUST) also yielded a > 10-fold increase in the number of OTUs (5,962 OTUs), further supporting the inference of significant undescribed diversity in the region. In sum, our methodological choices would be expected to err on the side of overconservative estimates of diversity, and the 10-fold increase in OTU richness observed here is therefore unlikely to be artificially inflated. In addition, a few modestly diverse zooplankton groups were absent or poorly recovered following bioinformatic processing (chaetognaths, hyperiid amphipods), and the described diversity in these groups was not accounted for in this study.

Higher than expected OTU richness was observed across the majority of taxa, but a few zooplankton groups stand out as important reservoirs of undescribed diversity. A 10-fold increase occurred in the number of copepod OTUs, which make up 60–80% of zooplankton biomass in the 0.2–2 mm size range (Landry et al., 2001). A total of 221 planktonic copepod species are reported to occur in the NPSG in waters near Hawaii (Landry et al., 2001; McGowan & Walker, 1979; Wilson, 1950). Based on HOT collections, Landry et al. (2001) enumerated 67 copepod species and 12 multispecies groups within >0.5 mm zooplankton, and 89 taxa (species/genera) across all size fractions collected from the upper 155 m of the water column. In comparison, we observed 893 copepod OTUs within the two larger size fractions (0.5–1.0, 1–2 mm) in the upper 150 m, and 959 copepod OTUs within all three size fractions. McGowan and Walker's (1979) records of copepod community structure in the upper 600 m at the CLIMAX site (28°N 155°W) included data on 126 copepod species that were consistently present (>505 μm). Our metabarcoding data reveal high copepod richness at mesopelagic depths, resulting in a total of 1580 copepod OTUs observed in the upper 700 m, a > 10-fold increase from the McGowan and Walker study. These increases in diversity likely occur primarily due to greater capacity for metabarcoding methods to detect rare species in the assemblage, but may also occur partly due to greater taxonomic resolution in small-bodied genera that are difficult to discriminate by morphological means (e.g., *Clausocalanus*, *Paracalanus*, often combined during enumeration). A recent metabarcoding study of the copepod assemblage in the Pacific (NPSG, SPSG, ETP, Kuroshio Current) resolved 404 copepod OTUs in the epipelagic (200 m), approximately ½ the number we observed in the same depth range (Hirai & Tsuda, 2015). However, several important methodological aspects differed between the two studies, including choice of marker (18S rRNA vs. 28S rRNA), % similarity used for clustering (99% vs. 97%), sequencing depth (12,340 vs. 6,229 reads per sample), the cut-off chosen for elimination of potentially spurious, rare OTUs (<15 reads vs. <3 reads) and the number of total samples (54 vs. 20). The higher sequencing coverage of this study, the higher volume of seawater filtered by our nets and the larger number of total samples analysed likely resulted in greater detection of a long tail of rare copepod species. Studies that report on several markers would be informative to assess the taxonomic resolution of all loci that have been used or proposed for metabarcoding studies in metazoan plankton (e.g., Clarke et al., 2017), and would facilitate cross-study comparisons.

Surprisingly high OTU richness also was observed within the ostracods. Comprehensive studies of the subtropical ostracod fauna

of the Pacific basin have not been conducted (*but see* temperate/boreal studies; Deevey, 1983; Kaeriyama & Ikeda, 2002; Kaplun, Mazdygan, Chavtur, Gorbatenko, & Bashmanov, 2015); however, the subtropical assemblage in the North Atlantic is reported to include 118 species (Angel, 1979, 2010; Angel, Blachowiak-Samolyk, Drapun, & Castillo, 2007; Angel & Fasham, 1975). Comparable magnitude of species richness is expected in the subtropical North Pacific, but our OTU counts are approximately 10-fold these estimates (1,155 OTUs). Together, copepods and ostracods represent ~ two-thirds of the OTUs observed in this study, and an order of magnitude increase in their diversity implies far higher richness for the full assemblage than is currently described. The diversity observed in several other holoplanktonic groups also was higher than expected based on described morphospecies (e.g., polychaetes, malacostracans), but these OTU counts only modestly increased the total diversity within the NPSG.

Finally, the metabarcoding data provide unique insights into the diversity of the meroplankton assemblage at this offshore time series site, about which nearly nothing is known from prior work. In the vicinity of the Hawaiian archipelago, a range of modelling and population genetic studies have documented or inferred dispersal of larvae both to and away from the Islands (e.g., Eble, Rocha, Craig, & Bowen, 2011; Iacchei, Gaither, Bowen, & Toonen, 2016; Kobayashi, 2006), but few meroplankton or ichthyoplankton surveys have been published from the region (*but see* Scheltema, 1986; Wren & Kobayashi, 2016 and references therein). This study provides baseline sequence data of invertebrate larvae present offshore of the Hawaiian Islands (Table 4). Of the six dominant OTUs with 100% sequence identity to a NCBI reference sequence, several correspond to shallow-water species that inhabit muddy or sandy beaches (*Malleus malleus*, *Andvakia discipulorum*—described from Hawaii; Rossi, 1982; Temkin, 2010; Tsubaki, Kanmeda, & Kato, 2011), with some taxa cryptic due to small-size and/or burrowing habits (Daly & Goodwill, 2009). Several of these six OTUs require hard substrate (*Cereus* sp.; Haussermann & Forsterra, 2003), including the possibly invasive species *Septifer bilocularis* (bivalve, ship bottom Pearl Harbor; Paulay, 1996). Finally, OTUs with 99.7–100% sequence similarity to two deep sea species were recovered, implying that we may have collected pelagic larvae of *Heterobrissus hystrix*, a widespread bathyal echinoid known to produce pelagic larvae (Young, Ekaratne, & Cameron, 1998); and *Phyllochaetopterus* sp. 1, an undescribed polychaete present at whale falls in the deep ocean off the California margin (Osborn, Rouse, Goffredi, & Robison, 2007).

4.2 | Vertical gradients in species richness and community composition

Community composition was strongly depth-stratified, with four distinct species assemblages identified across a depth gradient from the surface ocean to the upper bathypelagic. The most pronounced transition between communities occurred at 500 m (Figure 2), rather than at the base of the epipelagic (200 m) or mesopelagic zones (1000 m) (Sutton, 2013; Vinogradov, 1970), with secondary faunal transitions at 100 and 300 m. Vinogradov's (1970) synthesis of early qualitative efforts to describe biological zonation across depth resulted in a scheme with faunal layers in the surface (or epipelagic) zone (0–150 m), a transitional (or mesopelagic) zone between 150 and 700 m and a deep-sea zone below 750 m. The location of these faunal boundaries varied across several earlier proposed zonation schemes (e.g., Bogorov, 1948; Birshtein, Vinogradov, & Chindonova, 1954; Hedgpeth, 1957; summarized in Vinogradov, 1970), with boundaries variably described at 100 m, 200 m and several authors suggesting 500 m as the upper boundary of the deep-sea assemblage. Several planktonic groups, for example, copepods, chaetognaths, siphonophores, hydromedusae and scyphomedusae, are well described to have species, genera or exclusively deep-sea families that appear below 500 m (e.g., gelatinous examples—*Halicreas* spp, *Crossota* sp, *Pantachongon* sp., *Atolla* sp., *Periphylla* sp.; Deevey & Brooks, 1977; Osborn, Silver, Castro, Bros, & Chavez, 2007; Matsuura, Nishida, & Nishikawa, 2010; Mapstone, 2016). Our sequence-based depth distributions for individual OTUs provide confirmation that many species appear immediately below the 500 m depth horizon, including examples from copepods, appendicularians, siphonophores, polychaetes and scyphomedusae (Figure 6 and results not shown). In some cases, these taxa extend into the bathypelagic, and in others, they inhabit a discrete depth layer in the deep mesopelagic zone (500–700 m or 700–1000 m, e.g., *Eucalanus spinifer* [OTU62], *Calanus* sp. [OTU85], Figure 6 and results not shown). A few species that occur in the upper water column in other ecosystems appear to be restricted to the deep mesopelagic at station ALOHA (e.g., *Subeucalanus subtenuis* [OTU19], epipelagic in ETP). In sum, our metabarcoding results are concordant with early inference of a primary faunal transition at 500 m, and refute suggestions that an important zooplankton faunal boundary occurs deeper in the water column (e.g., 750 m, 1000 m). In particular, there was a notable absence of structure in multivariate analyses and in patterns of OTU depth endemism that would suggest a faunal transition at the base of the mesopelagic (1000 m).

Faunal transitions observed in the metabarcoding data at 100 and 300 m were also partially concordant with earlier observations; while 100 m has been identified as a faunal boundary, few authors have proposed 300 m as a depth of important transition (Vinogradov, 1970). Here we document the presence of a distinct faunal assemblage in the lower epipelagic and upper mesopelagic (100–300 m) that spans across the base of the epipelagic zone (200 m). Significantly, we find no evidence to support the inference that high diversity in this assemblage occurs due to faunal overlap of distinct epipelagic (0–200 m) and mesopelagic (200–1000 m) fauna: 89% of OTUs were restricted to this depth horizon (100–300 m). The upper water column at this subtropical study site is persistently stratified (Karl & Church, 2014; Karl & Lukas, 1996), and the deep chlorophyll maximum (DCM) occurred at 130 m during our collections (0.75 μg/l; Figure 2b). This diverse zooplankton assemblage (100–300 m) therefore extends vertically across the DCM and the base of the euphotic zone, with zooplankton biomass also declining across the

100–300 m depth horizon (Hannides et al., 2013; Steinberg, Cope, et al., 2008). The upper epipelagic assemblage (0–100 m) occurs in a region of high primary productivity in the well-illuminated portion of the water column and supports maximum zooplankton biomass (Steinberg, Cope, et al., 2008). Extension of this work to a larger collection of material will provide important sample replication and increasing confidence in observed vertical gradients in community composition.

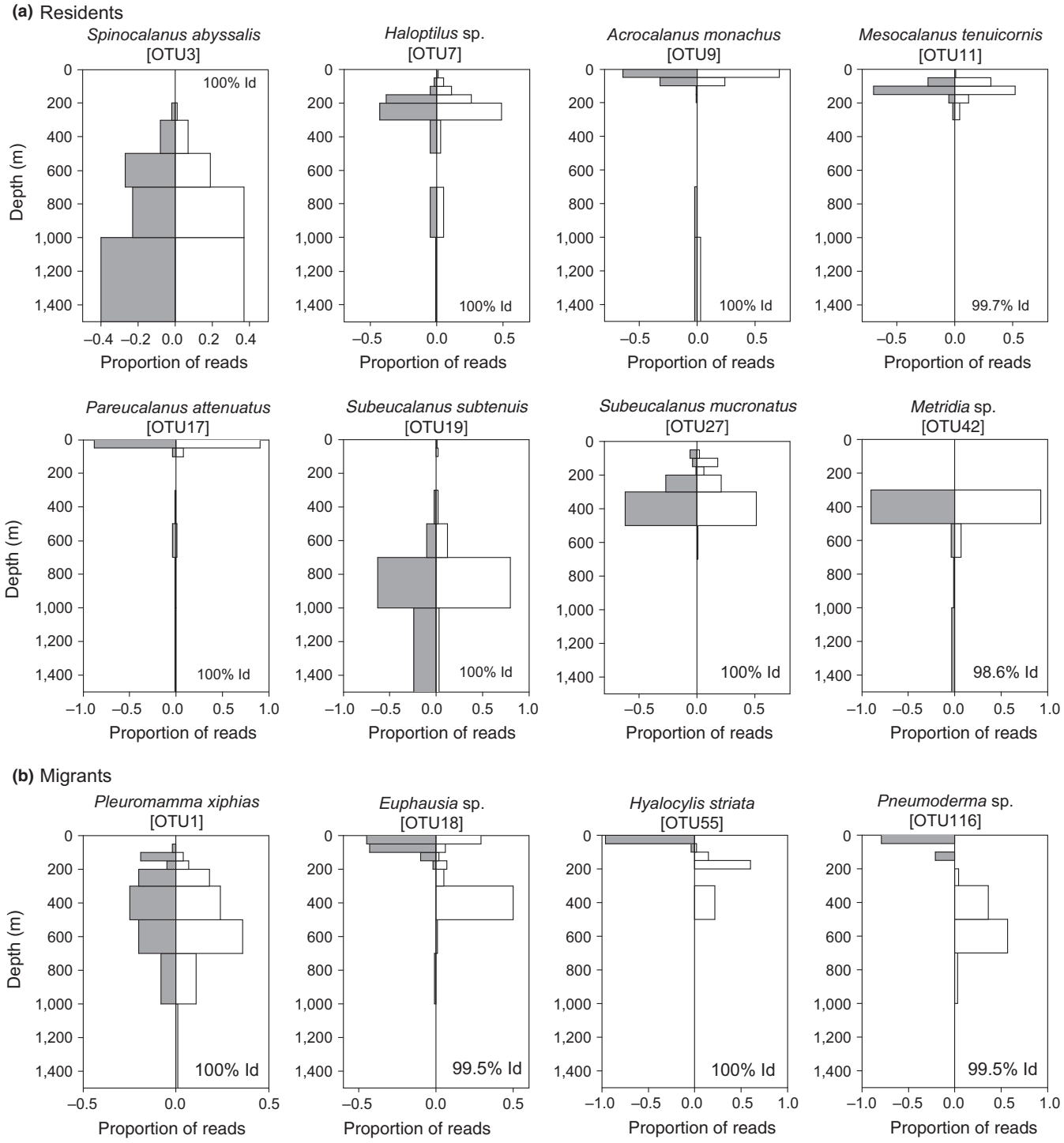

FIGURE 6 Depth distributions of individual operational taxonomic units (OTUs) shown in day–night plots, as a proportion of reads sampled in each of the day (white) and night (grey) tows (right, left sides of the plot = 1.0; sequencing coverage standardized for each tow). (a) Eight common copepod taxa at station ALOHA that contributed to differentiation of species assemblages (by depth). (b) Examples of depth distributions for migrant OTUs (copepod, euphausiid, pteropods). Vertical width of the histogram bars indicates the sampled depth stratum for each net (listed in Table 1). % identity to a NCBI reference sequence (% Id) is listed as an inset in each panel

Both depth and zooplankton size fraction were significant predictors of diversity in the assemblage, with maximum OTU richness observed in the lower epipelagic and upper mesopelagic (100–300 m) depth strata and in the smaller zooplankton size fractions (0.2–0.5, 0.5–1 mm; Figure 5). Diel vertical migration influences overall richness of the assemblage, with slightly shallower and greater maxima in OTU richness at night. Meroplankton diversity is highest at epipelagic depths, but does not significantly impact diversity of the full assemblage, which is driven by the much higher OTU richness of the holoplankton. Finally, the two hyperdiverse groups, the copepods and ostracods, have complementary patterns of diversity across depth, with maximum ostracod diversity in the lower epipelagic and upper mesopelagic (100–300 m) and maximum copepod diversity in the deep mesopelagic and bathypelagic (>500 m). Our results are in notable contrast to earlier reports on vertical gradients in species richness for particular, but not all, taxonomic groups. Angel and collaborators (Angel & Fasham, 1975; Angel, 1979; Angel et al., 2007; Angel, 2010) reported increasing ostracod species richness with depth to approximately 1000 m, with a broad depth range of high diversity between ~400 and 1000 m, even at 18°N—latitudinally close to station ALOHA in the Pacific basin. The differences between Angel's and our vertical profiles for ostracods may result from true and distinct patterns between Pacific and Atlantic assemblages, or may result from the comparison of molecular and morphological descriptions of species, which would imply more cryptic taxa in the lower epipelagic/upper mesopelagic than elsewhere in the water column. Maximum species richness for planktonic copepods occurs in the deep mesopelagic in the oceanic subtropical North Atlantic (Deevey & Brooks, 1977), subtropical North Pacific (Yamaguchi et al., 2015) and in the Arctic (Kosobokova & Hopcroft, 2010; Kosobokova et al., 2011), as observed here, although less oceanic sites may exhibit an epipelagic maximum (Dias, Araujo, Paranhos, & Bonecker, 2010). Regional studies of chaetognaths report vertical gradients in species richness that are similar to our results for the full assemblage at ALOHA, with highest diversity in the 100–300 m depth layer (Atlantic, Pierrot-Bults & Nair, 2010; Andaman Sea, Nair & Gireesh, 2010). Finally, the only other metabarcoding study to examine depth gradients in diversity for zooplankton assemblages was conducted in the oceanographically unique Red Sea, and the declines in diversity across depth observed there are unlikely to be generalizable to other ocean regions (Pearman & Irigoien, 2015).

4.3 | Metabarcoding: new insights and new limitations

An increasing number of studies are using metabarcoding methods to characterize metazoan plankton communities (Bucklin, Lindeque, Rodriguez-Ezpeleta, Albaina, & Lehtiniemi, 2016; Clarke et al., 2017; Hirai & Tsuda, 2015; Lindeque et al., 2013; Pearman & Irigoien, 2015). Metabarcoding has the potential to reveal large-scale patterns in zooplankton diversity and community structure that were largely invisible in morphological analyses, due to large numbers of undescribed species and the difficulty in characterizing the species present in the full zooplankton community. Metabarcoding may also better capture rare species within the community in comparison with analysis by microscopy, enabling studies of the ecological role of a deep reservoir of low abundance taxa. However, there are several limitations of the metabarcoding approach. The demarkation of molecular OTUs is a key but difficult analysis step, with clustering algorithms and filtering protocols yielding variable results (e.g., Coissac et al., 2012; Flynn et al., 2015). Current sequence databases also are limited, making it difficult to assign accurate species identifications to molecular OTUs. In this study, only 29% of all OTUs had ≥99% sequence identity to a previously published 18S rRNA sequence in NCBI, implying that the majority of species in our region are absent from current databases. Finally, no single marker can fully resolve the diversity of metazoan communities due to the inherent trade-off in resolution between taxonomic breadth and depth, and the research community has not yet converged on any given suite of markers for metazoans (Clarke et al., 2017; Deagle et al., 2014). However, with standardization of molecular markers and development of more complete reference databases for the relevant gene regions, metabarcoding will be a very powerful approach for comparison of plankton communities across ocean basins and over time, in order to detect ecological responses to climate change, and test a number of higher-order hypotheses regarding plankton diversity and ecosystem function.

5 | CONCLUSIONS

Although metazoan animals in the mesopelagic zone play critical roles in deep pelagic food webs and in the attenuation of carbon in midwaters, the diversity of these assemblages is incompletely known. Our results provide some of the first insights into the hidden diversity present in zooplankton assemblages in midwaters, and a molecular reappraisal of vertical gradients in species richness, depth distributions and community composition for the full zooplankton assemblage across the epipelagic, mesopelagic and upper bathypelagic zones in the North Pacific Subtropical Gyre. Primary observations from this study are that (i) extant diversity in this high diversity ecosystem is likely an order of magnitude higher than current estimates based on morphological descriptions of species, with high diversity present in copepods and ostracods; (ii) the community is depth-stratified into four primary assemblages with faunal transitions at 100 m, 300 m and 500 m; and (iii) the maximum in species richness for the full assemblage occurs in the lower epipelagic and upper mesopelagic (100–300 m), rather than the deep mesopelagic zone. This study was conducted in a region with global maxima in pelagic diversity, and at one of the most important open ocean time series sites (station ALOHA, Karl & Lukas, 1996). Ongoing and future work aims to establish standard markers for metabarcoding studies of marine zooplankton, and then use these markers to assess community change across time, depth and environmental gradients.

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DATA ACCESSIBILITY

Data reported on in this publication are available under https://doi.org/10.1575/1912/9040. Files include OTU tables with taxonomic identification for each OTU, FASTA files with representative sequences for each OTU, as well as the NCBI Sequence Read Archive (SRA) accession numbers for demultiplexed sequence data (individual data citations below).

Goetze, E. (2017) Metabarcoding zooplankton at station ALOHA: Operational taxonomic unit (OTU) tables and FASTA files for representative sequences from each OTU (Plankton Population Genetics project). Biological and Chemical Oceanography Data Management Office (BCO-DMO). Data set version 2017-05-25. https://doi.org/10.1575/1912/bco-dmo.704664.

Goetze, E. (2017) NCBI Sequence Read Archive (SRA) accession numbers for fastq sequence files for each zooplankton community sample (Plankton Population Genetics project). Biological and Chemical Oceanography Data Management Office (BCO-DMO). Data set version 2017-05-25. https://doi.org/10.1575/1912/bco-dmo.704665.

AUTHOR CONTRIBUTIONS

S.A.S., P.L. and E.G. designed the study; L.V.W., G.C. and E.G. collected the samples; and S.A.S., L.V.W., G.C. and E.G. conducted laboratory work and generated the data. Data analyses were conducted primarily by S.A.S., with assistance from E.G. and P.L. S.A.S. and E.G. wrote the manuscript, with all authors contributing to the final draft. S.A.S., P.L. and E.G. provided funding for the work.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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