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UNIVERSITY OF CALIFORNIA SAN DIEGO

Influences of physical dispersal and environmental selection on mesopelagic and epipelagic
zooplankton distributions at multiple scales across the North Pacific Ocean

A Dissertation submitted in partial satisfaction of the requirements
for the degree Doctor of Philosophy

in

Oceanography

by

Stephanie Alicia Matthews

Committee in charge:

Professor Mark D. Ohman, Chair
Professor Andrew E. Allen
Professor C. Anela Choy
Professor Erica Goetze
Professor Sarah Purkey
Professor Jonathan B. Shurin

2023

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University of California San Diego

2023

DEDICATION

For my parents

Dad, who taught me the joy of discovery

Mom, who taught me to observe the natural world

EPIGRAPH

*You've asked me what the lobster is weaving there with his golden feet?
I reply, the ocean knows this.
You say, what is the ascidia waiting for in its transparent bell? What is it waiting for?
I tell you it is waiting for time, like you.
You ask me whom the Macrocystis alga hugs in its arms?
Study, study it, at a certain hour, in a certain sea I know.
You question me about the wicked tusk of the narwhal, and I reply by describing how the sea
unicorn with the harpoon in it dies.
You enquire about the kingfisher's feathers,
which tremble in the pure springs of the southern tides?
Or you've found in the cards a new question touching on the crystal architecture of the sea
anemone, and you'll deal that to me now?
You want to understand the electric nature of the ocean spines?
The armored stalactite that breaks as it walks?
The hook of the angler fish, the music stretched out in the deep places like a thread in the
water?*

*I want to tell you the ocean knows this, that life in its jewel boxes
is endless as the sand, impossible to count, pure,
and among the blood-colored grapes time has made the petal
hard and shiny, made the jellyfish full of light
and untied its knot, letting its musical threads fall
from a horn of plenty made of infinite mother-of-pearl.*

*I am nothing but the empty net which has gone on ahead
of human eyes, dead in those darknesses,
of fingers accustomed to the triangle, longitudes
on the timid globe of an orange.*

*I walked around as you do, investigating
the endless star,
and in my net, during the night, I woke up naked,
the only thing caught, a fish trapped inside the wind.*

– Pablo Neruda

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LIST OF SUPPLEMENTARY FILES

Matthews_Appendices.zip

- Matthews_AppendixA_TraitDatabase.xlsx
- Matthews_AppendixB_ReferenceSpecimens.xlsx
- Matthews_AppendixC_ExtractionProtocolDNA.docx
- Matthews_AppendixD_MockCommunities.docx
- Matthews_AppendixE_ParticleTrajectories.pptx

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ABSTRACT OF THE DISSERTATION

Influences of physical dispersal and environmental selection on mesopelagic and epipelagic zooplankton distributions at multiple scales across the North Pacific Ocean

by

Stephanie Alicia Matthews

Doctor of Philosophy in Oceanography

University of California San Diego, 2023

Professor Mark D. Ohman, Chair

The processes influencing biogeographic distributions and community composition in the mesopelagic ocean are not well understood. In the pelagic ocean, the magnitude of dispersal potential and environmental variability vary with depth. In this dissertation, I

compare mesopelagic zooplankton communities with epipelagic communities to determine the relative influence of dispersal and selection on midwater zooplankton.

First, I compare DNA metabarcoding-resolved community composition against carbon biomass from digital Zooscan imaging, and find good overall correspondence between methods for more abundant taxa. This chapter provides methodological refinements and validation.

I then examine zooplankton communities across a strong environmental gradient within the California Current Ecosystem. At a regional scale, I find cross-shore changes in community composition and vertical shifts in habitat use. The magnitude of change varies among zooplankton with different traits. In a space-for-time framework, these results suggest community turnover and vertical reorganization within future, more stratified oceans.

I then contrast epipelagic and mesopelagic zooplankton communities across major biogeographic transitions between the Subarctic Pacific, North Pacific West Wind Drift, and North Pacific Subtropical Gyre. I find stronger biogeographic breaks in the epipelagic zone and larger distributional ranges for mesopelagic taxa. Varying widths of environmental and distributional ranges among taxa with different traits suggest trait- and depth-dependent effects of selection and dispersal.

At the basin scale, I compare zooplankton distributions and community similarity of mesopelagic and epipelagic zooplankton at 28 locations spanning all major biogeographic provinces in the North Pacific Ocean. I find no differences in the extent of biogeographic ranges or levels of community dissimilarity between epipelagic and mesopelagic assemblages. I find stronger associations of community dissimilarity with dispersal potential and environmental dissimilarity in the epipelagic zone than in the mesopelagic zone.

This dissertation finds greater similarity between epipelagic and mesopelagic biogeographic distributions at the basin scale than at intermediate or regional scales. Varying extents of environmental and biogeographic ranges among taxa with different vertical habitat use patterns, feeding behaviors, diets, and reproductive strategies suggest trait-dependent effects of selection and dispersal. This dissertation is a first step toward describing the ecological processes influencing the distributional boundaries of mesopelagic zooplankton.

INTRODUCTION

Zooplankton distributions

The biogeographic distributions of plankton in the ocean have been studied since the Challenger expedition (Thomson et al., 1889), and the currently accepted major biogeographic regions of the North Pacific have been described in some recognizable form of their current division for nearly a hundred years (*cf.*, McGowan, 1971). Throughout the intervening century, the spatial distributions of zooplankton in the surface ocean have received significant attention. Distinct zooplankton communities in the subpolar, subtropical and transition zones were described in detail, including by the Marine Life Research Group at Scripps Institution of Oceanography (e.g., Fager & McGowan, 1963; Hayward et al., 1983; McGowan, 1971; McGowan & Walker, 1985; Reid et al., 1978; Venrick, 1979). Distributions of the species within many of the dominant zooplankton taxa were documented with care, either in the form of monographs and dissertations or within the California Current-focused CalCOFI atlases (Alvariño, 1965, 1971, 1971; Bé, 1977; Brinton, 1962; Chen, 1986; Fleminger & Siegel-Causey, 1986; Vinogradov et al., 1996). These descriptive reports often describe similar distributional patterns shared by species from different phyla, and complementary distributions among more closely related species (Fager and McGowan 1963).

The extensive biogeographic analyses of the twentieth century not only described distributional patterns, they also discussed the processes shaping species distributions and controlling community composition. Generally, the ecological processes shaping community composition can be categorized into four types of processes: dispersal (i.e., primarily physical transport of organisms into or out of a region), selection (i.e., differential survival rates between

species), or evolution (appearance of new species), with some unexplained stochasticity (drift) (Vellend, 2016). In this framework, selection refers only to species selection, or unequal rates of survival and reproduction among species leading to changes in community composition, and does not include intraspecific dynamics. The importance of each of these processes varies among communities and between species. Additionally, the dominant process limiting any individual species' range can vary along the perimeter of its distribution (Fredston-Hermann et al., 2020). In the pelagic ocean, biogeographic ranges often co-occur with the boundaries between major water masses (Backus, 1986). Early oceanographers studying the Pacific Ocean noted this association and determined that zooplankton distributions are shaped by the combined effects of physical dispersal and selection due to temperature and related variables (Beklemishev, 1969; Brinton, 1962; McGowan, 1971, 1974).

The process of evolution occurs over longer timescales than dispersal or selection (Vellend, 2016). In recent years, significant efforts have been extended to constrain evolution in the open ocean by mapping genetic structure within species (Goetze, 2003, 2005). The location of genetic breaks within species often co-occur with physical barriers to dispersal and environmental gradients; there are various processes by which this co-occurrence may arise (Burrige et al., 2015; Goetze et al., 2015; Johnson et al., 2022; Wall-Palmer et al., 2018). Observations of spatial patterns in biogeographic ranges and community composition must therefore be interpreted with consideration of the evolutionary and geologic history of the environment (Bradford-Grieve, 2002; Spoel & Heyman, 1983).

In contrast to zooplankton inhabiting the epipelagic zone (0 – 200 m), biogeographic distributions of zooplankton and spatial patterns in community composition in the mesopelagic zone (200 – 1000m, but see Kaartvedt et al. 2019) are not well understood. Oceanographic

expeditions and programs have preferentially sampled the epipelagic zone, due to the cost, time and difficulty of conducting net tows in the deeper depth zones (Koslow & Couture, 2015; Robinson et al., 2010). The collections of mesopelagic taxa that have been analyzed indicate that distributional ranges may be larger in the deep ocean (Reygondeau & Dunn, 2018). These larger ranges suggest that mesopelagic distributions are primarily shaped by a more homogenous midwater environment. Conversely, taxon-specific ecological studies have found significantly smaller environmental niches for midwater taxa, with fine-scale habitat partitioning among closely related species (Laakmann et al., 2012; Matsuura et al., 2010). The evolutionary history of mesopelagic organisms appears to diverge from the evolutionary history of epipelagic taxa, with evidence for recent radiation and increased speciation events in the midwater (Hirai et al., 2020).

In comparison to epipelagic biogeographic ranges, decreased environmental heterogeneity and decreased rates of physical dispersal in the mesopelagic ocean could have opposing effects on the biogeographic ranges of mesopelagic zooplankton. This dissertation investigates the distributional ranges of mesopelagic zooplankton in the North Pacific Ocean, assessing the influence of dispersal potential and environmental selection on biogeographic distributions and community composition.

Study system

The Pacific Basin is the largest continuous environment on planet Earth. In the epipelagic zone, ocean circulation is primarily geostrophic (Talley et al., 2011). The major geostrophic features of the North Pacific Ocean are the North Pacific Subtropical Gyre (NPSG) and the North Pacific Subpolar Gyre (SPG), simplified from Talley et al. (2011) as follows. The NPSG is

an anticyclonic gyre with clockwise flow. The balance of horizontal pressure gradient and Coriolis forces leads to slight doming of the gyre and diffuse downwelling throughout the gyre. The southern portion of the NPSG is formed by the westward-flowing North Equatorial Current, with an average velocity of 10 cm s^{-1} . In the western Pacific, the narrow Kuroshio Current has surface velocities reaching $75 - 250 \text{ cm s}^{-1}$, and velocities of 10 cm s^{-1} can extend to the seafloor. The Kuroshio Current Extension forms a portion of the eastward-flowing North Pacific Current (also called the West Wind Drift), which bifurcates into the southward-flowing California Current (maximum surface velocity $40 - 80 \text{ cm s}^{-1}$, but see also Rudnick et al. 2017) and the Alaska Current to the north. The SPG, a cyclonic, counterclockwise gyre, is comprised of the Alaska Current in the east which follows the Alaskan coastline to turn toward the south where it becomes the Alaska Stream, and eventually contributes to the North Pacific Current either directly or through the Oyashio Current (up to $20 - 50 \text{ cm s}^{-1}$). These two gyres encompass the majority of the broadscale surface ocean currents.

The mesopelagic circulation of the North Pacific Ocean is also primarily shaped by the currents described above. There are only slight influences of deep water masses, which in the Pacific Ocean are primarily Circumpolar Deep Waters and Antarctic Intermediate Water (Kawabe & Fujio, 2010; Xie & Zhu, 2008). However, with increasing depth, the fingerprint of upper ocean circulation decreases. By 1000 m depth, geostrophic current speeds relax to $5 - 20\%$ of their surface speed (Grey and Riser 2014). Slower ocean currents at depth can limit dispersal potential for mesopelagic organisms.

The basin-scale circulation described above leads to significant spatial heterogeneity in the physical (temperature, mixing), chemical (salinity, nutrient) and biogeochemical (Chl-*a*, phytoplankton community composition) environment within the epipelagic zone (Longhurst,

2007). Longhurst's provinces form the basis for a synthesis of ecosystem-specific research provided by Miller (2004), summarized as follows: The core of the NPSG is warm, depleted in nutrients, and primarily supports small cyanobacteria and nitrogen-fixing phytoplankton. The core of the SPG is characterized by low salinity, limiting vertical mixing, but phytoplankton production tends to be Fe-limited, unlike the N-limited NPSG. On the western margin of the North Pacific, nutrient-rich waters from the Kuroshio and Oyashio Currents form a zone of dynamic mesoscale mixing and eddies, supporting moderate levels of biomass. The California Current is an eastern boundary upwelling current with coastally enhanced production. Further south, in the Eastern Tropical Pacific, primary production is increased relative to the NPSG, and surface waters typically contain higher nutrient concentrations. Trade winds lead to upwelling along the equator, and in western Pacific a halocline results in stratification and nutrient limitation. In addition to these basin-scale patterns summarized from Miller (2004), there is significant mesoscale structure and temporal variability within regions. Seasonal cycles in wind-driven upwelling and light availability further influence primary production, resulting in latitudinal variability in the amplitude of productivity cycles (Longhurst, 1995).

Within the mesopelagic zone there are narrower ranges of temperature and salinity, reflecting the influence of more homogenous thermohaline water masses in the deep ocean as well as reduced influence of effects of thermal heating and evaporation at the surface (Talley et al., 2011). Light limitation within the mesopelagic zone essentially eliminates photoautotrophic production (Miller, 2004). Fixed, bio-available carbon in the mesopelagic zone comes primarily from overlying surface waters (Stukel et al., 2014). Particle sinking and export facilitated by vertically migrating animals occurs on shorter timescales than large-scale ocean currents (Vinogradov, 1970), resulting in spatial patterns of mesopelagic food availability that broadly

mirror epipelagic productivity (Stukel et al., 2018). This export does have seasonality, lagged by the length of export timescales. However, the magnitude of spatial and temporal heterogeneity is less than that observed in the epipelagic (Siegel & Deuser, 1997). Decreased environmental variability at depth could result in larger biogeographic distributions if niche breadths are conserved.

Approach

To assess the effects of dispersal potential and environmental selection on zooplankton biogeographic distributions and community composition in the mesopelagic North Pacific, I compare mesopelagic zooplankton communities against epipelagic zooplankton communities at a range of spatial scales. Although the effects of dispersal and selection are difficult to disentangle, well-studied epipelagic biogeography can serve as a baseline against which to compare mesopelagic distributions. If mesopelagic zooplankton communities are predominantly shaped by dispersal processes, the biogeographic distributions of mesopelagic zooplankton would be expected to be smaller than the biogeographic distributions of epipelagic zooplankton. If selection processes drive zooplankton distributions in the mesopelagic zone, then mesopelagic biogeographic distributions would be expected to be larger than epipelagic distributions.

To characterize community composition, I use DNA metabarcoding. Metabarcoding is an increasingly common method for identifying species within a mixed community (Bucklin et al., 2016). It relies on PCR amplification of specific marker regions, short stretches of DNA that are conserved across taxa but contain variable bases, allowing for differentiation among species. The significant time and expertise required for morphological identification of marine zooplankton are often limiting steps for analyses across the wide range of phyla comprising zooplankton

communities (Agnarsson & Kuntner, 2007). Taxonomic expertise in midwater zooplankton taxa is particularly limited, due to restricted historical interest in these deeper communities.

Metabarcoding allows for comparable resolution across depth zones and biogeographic regions, as well as more efficient processing of the many zooplankton samples included here.

To analyze differences in community composition, I also assign traits to identified zooplankton taxa. Trait-based approaches have only recently been applied to marine zooplankton (Litchman et al., 2013). By considering functional characteristics of species, trait-based analyses allow for comparisons across species and identification of emergent trends that would be lost by focusing exclusively on individual taxa (Ohman & Romagnan, 2016). Trait-dependent distributional patterns indicate that effects of environmental selection can vary among taxa with different traits (Brun et al., 2016).

I examine epipelagic and mesopelagic zooplankton community composition at three spatial scales: the basin scale across the entire North Pacific, the intermediate scale across a major biogeographic transition between the North Pacific Subtropical Gyre and the Subpolar Gyre, and at the regional scale across a strong environmental gradient within the California Current Ecosystem. Spatial and temporal scales of plankton distributions and the processes shaping them are closely related (Haury et al., 1978). At basin scales, I assess climatological patterns in zooplankton distributions, using samples collected over the course of a decade. At smaller spatial scales, samples collected over the course of a few weeks provide insight into the proximate processes influencing mesopelagic zooplankton distributions.

Outline of the dissertation

In this dissertation, I use molecular methods to analyze the distributional patterns of mesopelagic zooplankton and then evaluate the processes influencing these patterns. I consider vertical habitat use, species traits, and the impacts of reduced environmental heterogeneity and decreased dispersal potential in the mesopelagic zone, relative to the epipelagic zone.

In Chapter 1 “Recommendations for interpreting zooplankton metabarcoding and integrating molecular methods with morphological analyses” I validate and refine existing zooplankton metabarcoding methodology. In Chapter 2, “A space-for-time framework for forecasting the effects of ocean stratification on zooplankton vertical habitat use and trait composition” I investigate changes in zooplankton community composition and vertical habitat use along a major environmental gradient in one geographic region, the California Current Ecosystem. Chapters 3 and 4 compare biogeographic distributions and community composition in the mesopelagic zone against the epipelagic zone, testing the effects of depth-dependent differences in dispersal potential and environmental heterogeneity. In Chapter 3, “Divergent patterns of zooplankton connectivity in the epipelagic and mesopelagic zones of the eastern North Pacific”, I focus on the boundaries between three major biogeographic provinces. In Chapter 4 “Distributional ranges of mesopelagic zooplankton across the North Pacific Basin: testing the effects of physical dispersal vs. environmental selection”, I assess basin-scale biogeographic ranges and patterns of community composition.

I conclude the dissertation with a brief summary of my major findings and discussion of their implications. I consider limitations of the work and priorities for future research. I close by noting some emerging research areas of particular potential.

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CHAPTER 1

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Recommendations for interpreting zooplankton metabarcoding and integrating molecular methods with morphological analyses

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Metabarcoding of zooplankton communities is becoming more common, but molecular results must be interpreted carefully and validated with morphology-based analyses, where possible. To evaluate our metabarcoding approach within the *California Current Ecosystem*, we tested whether physical subsampling and PCR replication affects observed community composition; whether community composition resolved by metabarcoding is comparable to morphological analyses by digital imaging; and whether pH neutralization of ethanol with ammonium hydroxide affects molecular diversity. We found that (1) PCR replication was important to accurately resolve alpha diversity and that physical subsampling can decrease sensitivity to rare taxa; (2) there were significant correlations between relative read abundance and proportions of carbon biomass for most taxonomic groups analyzed, but such relationships showed better agreement for the more dominant taxonomic groups; and (3) ammonium hydroxide in ethanol had no effect on molecular diversity. Together, these results indicate that with appropriate replication, paired metabarcoding and morphological analyses can characterize zooplankton community structure and biomass, and that metabarcoding methods are to some extent indicative of relative community composition when absolute measures of abundance or biomass are not available.

Keywords: *California Current Ecosystem*, community structure, metabarcoding, zooplankton, ZooScan

Introduction

The field of zooplankton ecology is beginning to embrace metabarcoding as an approach for describing spatial patterns in diversity and community composition, but there are some notable challenges in data interpretation. It remains difficult to link molecular estimates of diversity and community structure with morphological richness and abundance (Laakmann *et al.*, 2020), and the lack of standardization in metabarcoding methods makes it difficult to compare molecular richness across studies (Santoferrara, 2019). A better understanding of the relationship between metabarcoding and morphological analyses can help optimize methodological choices for studying zooplankton ecology (Bucklin *et al.*, 2019; Brisbin *et al.*, 2020). Here, we provide recommendations for best practices regarding subsampling, replication, and ethanol preserva-

tion. In addition, we test whether metabarcoding read counts are a suitable representation of relative biomass within the zooplankton community.

One of the strengths of metabarcoding is the ability to rapidly characterize community richness. Estimates of zooplankton richness using molecular markers can be up to an order of magnitude greater than morphological species richness (Laakmann *et al.*, 2020). The increased richness captured by metabarcoding may be due to detection of morphologically cryptic species, increased sensitivity to rare species, detection of intraspecific genetic variability (Brown *et al.*, 2015), or presence of pseudogenes or PCR chimeras. In contrast, many zooplankton metabarcoding pipelines require subsampling prior to DNA extraction which can potentially decrease observed richness (Loos and Nijland, 2020). Quantitative subsampling of bulk-fixed samples is a common procedure for

zooplankton analyses (Frolander, 1968; van Guelpen *et al.*, 1982), but the effects of subsampling on molecular diversity have not yet been tested.

A recurring challenge for metabarcoding is characterizing community structure from read counts (Laakmann *et al.*, 2020). While species richness is an important aspect of diversity, abundance and demographic structure are also important for understanding ecosystem dynamics (Elbrecht and Leese, 2015). PCR and sequencing biases can be normalized by including mock communities. This approach has been used for some studies of zooplankton communities (e.g. Hirai *et al.*, 2017) but remains relatively rare, and the effects of body composition, life history stage, cell count, copy number, and primer bias across diverse taxonomic groups are greater in metazoan than in microbial communities (Braukmann *et al.*, 2019). Despite these potential sources of bias, read counts have been positively correlated with morphologically identified abundance or biomass for some zooplankton taxa and in some ecosystems (Lindeque *et al.*, 2013; Hirai *et al.*, 2015; Harvey *et al.*, 2017; Bucklin *et al.*, 2019).

Preservation of field-collected zooplankton samples requires advance selection of a fixation protocol that is optimized for the target organisms and methods. The most common fixative for molecular analyses is 95–100% non-denatured ethanol (Loos and Nijland, 2020). However, ethanol is acidic and long-term storage of zooplankton tissue in ethanol can further increase acidity, resulting in dissolution of calcareous structures (Oakes *et al.*, 2019). This effect is particularly important for long-term preservation of calcifying organisms, when shell dissolution and changes in shell morphology may be interpreted as indicators of ocean acidification (e.g. Bednaršek *et al.*, 2017). Ammonium hydroxide has been used as a neutralizing agent for ethanol-preserved samples, successfully preserving pteropod shells for morphological analysis (Bednaršek and Ohman, 2015; Oakes *et al.*, 2019). However, the effect of ammonium hydroxide neutralization on molecular analyses is unknown.

In this study, we test the effects of subsampling, PCR replication, and preservation method on metabarcoding analysis of zooplankton community richness. We also directly compare paired metabarcoding and imaging analyses to test whether there is a relationship between read counts and morphology-based community composition at coarse taxonomic levels. We expected PCR replication to be a useful method for determining whether rare sequence variants are real or artifacts, and we expected minimal effects of physical subsampling on observed diversity. We also expected to find no relationship between normalized read counts and zooplankton abundance or biomass, given the many methodological steps at which biases could be introduced. We tested these questions using two molecular markers and ZooScan digital imaging of net-collected zooplankton samples from a range of environments across the California Current upwelling biome.

Methods

Zooplankton collection

Samples were collected between 29 April–8 May 2016 on cruise P1604 of the *California Current Ecosystem* LTER program using a depth-stratified 1 m² mouth area, 202 μ m mesh MOCNESS with paired day and night tows (Table S1). Sampling locations (designated “Cycles”; see Ohman *et al.*, 2013) ranged from the high-biomass nearshore upwelling environment to the offshore mesotrophic California Current. Zooplankton were sampled in 25

or 50 m vertical strata between the surface and 400 m. Each net-collected sample was quantitatively split with a Folsom splitter, then 50% fixed in 95% non-denatured ethanol neutralized with 5 mM ammonium hydroxide and 50% fixed in 1.7% formaldehyde buffered with sodium tetraborate. A total of 64 sets of paired ethanol and formaldehyde fixed samples were included in this analysis.

ZooScan Imaging

Zooplankton samples preserved in formaldehyde were imaged using a ZooScan (Gorsky *et al.*, 2010; Ohman *et al.*, 2012). Samples were size fractionated into 0.2–1.0-, 1.0–5.0-, and > 5-mm fractions. Quantitative subsamples of each were manually dispersed on the scanning surface, then digitally scanned. Images were segmented into regions of interest (ROIs) in ImageJ, and ROIs classified using machine-learning algorithms trained on manually sorted images of preserved zooplankton from the CCE (Ellen *et al.*, 2015). In addition, 100% of taxonomic assignments of ROIs were validated manually. Carbon biomass for each ROI was calculated from feret diameter using taxon-specific relationships between length and carbon (Lavaniegos and Ohman, 2007). Taxon abundance and carbon biomass were standardized for aliquot volumes and the volume of seawater filtered for each sample.

DNA extraction and amplification

Ethanol-fixed zooplankton samples were quantitatively split to a subsample small enough for DNA extraction with OMEGA EZNA Blood and Tissue Maxi kits (<5 g, typically 1/8–1/16th of the sample). Three to six subsamples were analyzed for four high biomass samples (subsequently termed “subsample replicates”; see Table S1). Subsample replicates were treated independently throughout extraction and amplification. DNA extractions were modified as previously described (Sommer *et al.*, 2017). Extraction negative controls were performed on a blank Nitex filter and included in all PCR steps. Eluent DNA concentrations were normalized to 20 ng μ l⁻¹ prior to PCR amplification.

To more broadly resolve the zooplankton community, two marker regions were amplified and sequenced. A 313-bp region of the mitochondrial cytochrome *c* oxidase subunit I gene (COI) was amplified with jgLCO1490 and jgHCO2198 (Leray *et al.*, 2013), and a 400-bp fragment in the V4 region of 18S was amplified using Uni18S and Uni18SR (Zhan *et al.*, 2013). Each subsample was amplified and sequenced in triplicate (subsequently termed “PCR replicates”). We used two-step PCR amplification and library preparation with duplicate dual indexing to mitigate index hopping, with unique indexes for PCR replicates to test for index biases and stochastic variability (Table S1 and S2; Costello *et al.*, 2018). The first reaction for each PCR replicate was duplicated, then the two reactions pooled and diluted as template for the indexing PCR (Table S2). All PCRs used high-fidelity “MyFi” polymerase (Bioline). Amplified DNA was bead-cleaned and final DNA concentrations quantified by Qubit or PicoGreen. Negative controls were included in sequencing runs if DNA was detected in the bead-cleaned product. Samples were pooled in equimolar concentrations and sequenced on two Illumina MiSeq runs (300bp paired-end V3).

Bioinformatic processing

Sequences were demultiplexed using cutadapt, and only sequences with exact matches to all four indexes retained (Martin, 2011).

Subsequent bioinformatic filtering in QIIME2 v. 2020.6.0 (Bolyen *et al.*, 2018) used marker-specific criteria as follows. For 18S, forward and reverse reads were truncated at 290bp and 275bp. Amplicon sequence variants (ASVs) were denoised independently for each sequencing run using DADA2 within QIIME2 with consensus chimera detection and pseudo-pooling (Callahan *et al.*, 2016). ASVs were not clustered in operational taxonomic units (OTUs) for 18S, as this gene region is highly conserved and even 99% OTUs can fail to resolve species-level differences (Clarke *et al.*, 2017). ASVs were aligned using MAFFT and taxonomy assigned using the 99% SILVA132 database, and by determining the lowest common ancestor (LCA) of up to five top *blastn* hits from GenBank (Pedregosa *et al.*, 2011; Yilmaz *et al.*, 2014; Clark *et al.*, 2016).

Sequences from COI were analyzed similarly. Forward and reverse reads were truncated at 280 and 250 bp. Denoised ASVs from DADA2 from each sequencing run were merged and clustered into *de novo* OTUs at 97% similarity using VSEARCH in QIIME2 (Rognes *et al.*, 2016). The 97% clustering threshold was selected so as not to overestimate alpha diversity, as intraspecific dissimilarity at this region of COI can reach 3–9% (Leray *et al.*, 2013). Representative sequences for each OTU were assigned taxonomy using the MIDORI “unique” database, and by LCA of GenBank *blastn* matches (Pedregosa *et al.*, 2011; Clark *et al.*, 2016; Machida *et al.*, 2017). All subsequent analyses were carried out in R 4.0.0 (R Development Core Team, 2009).

Statistical analyses

Statistical tests in R used “*phyloseq*,” “*vegan*,” and the *tidyverse* (Dixon, 2003; McMurdie and Holmes, 2013; Wickham *et al.*, 2019). Contaminant sequences were identified from negative controls with the combined method in “*decontam*,” with independent detection for each marker and sequencing run (Davis *et al.*, 2018). Contaminants were removed from all samples and negative controls were excluded from subsequent analyses. Sequences identified as non-metazoans, fishes, or mammals also were removed.

Samples were rarefied to 20,000 reads and 25,000 reads for 18S and COI, respectively, with samples falling below these thresholds removed. The relationship between rarefied observed richness, defined as OTU/ASV counts, and sequencing depth was tested with Spearman’s rank-order correlation. Appearance in PCR replicates was tested with a Kruskal-Wallis 1-way ANOVA, including only samples retaining at least three PCR replicates. Observed richness was plotted against the fraction of the plankton sample analyzed, illustrated with the nonparametric LOESS best fit.

To test whether relative read counts were correlated with independent ZooScan-based measures of biomass and abundance, zooplankton sequences were compared to numerical abundance and carbon biomass for taxonomic groups resolved using both digital imaging and at least one metabarcoding marker. Taxonomic groups were combined as necessary so that comparison groups were the same for both methods, including collapsing the fine taxonomic resolution that is present in metabarcoding data. Cnidarians and ctenophores were combined into a single category. Malacostracan sequences were compared against ZooScan-identified euphausiids and shrimp-like decapods. Comparisons were made for all thaliaceans together, and for salps, doliolids, and pyrosomes where identifiable. Separate comparisons were made for eucalanid copepods and for calanoid copepods excluding eucalanids. All data were converted to proportions and arcsine square root transformed, and

Pearson’s product-moment correlations were computed for each comparison, with Bonferroni correction.

Ethanol neutralization

To test the effects of ethanol neutralization with ammonium hydroxide on molecular diversity, three samples were collected near La Jolla Canyon using a 1 m diameter, 333 μ m mesh ring-net surface tow on 18 December 2017 and near the CCE2 mooring (http://mooring.ucsd.edu/dev/cce2/cce2_12/) using a 0.71 m diameter, 202 μ m mesh Bongo net towed obliquely to 180 m on 13–14 March 2018. Each sample was split quantitatively, and 50% preserved in unamended 95% non-denatured ethanol (pH 6.5) and 50% in 95% non-denatured ethanol neutralized with 5-mM ammonium hydroxide (final pH 8.0). These samples were analyzed using metabarcoding as described above, except were sequenced only at the COI marker. These samples were processed in their entirety, using multiple DNA extractions as necessary. Amplified samples were sequenced on two Illumina MiSeq runs, with each sample included in both runs (300bp paired-end V3). Sequences from replicate extractions and MiSeq runs were combined within each sample, and samples were rarefied to the lowest sequencing depth. Observed richness in the paired samples was compared using a Wilcoxon Matched Pairs Signed Rank test. Non-rarefied data were converted to relative abundances, samples clustered using average neighbor clustering based on Bray–Curtis distance and clusters tested for significance with a SIMPROF test. A PERMANOVA was subsequently used to test whether sampling event or preservation method affected observed community composition (Oksanen *et al.*, 2009).

Results

Bioinformatics

On average, 95% of reads demultiplexed by dual 8-mer indexing had the appropriate 6-mer indexes. A total of 12,923,747 sequences were recovered at the 18S marker, 6,882,057 of which passed quality and chimera filtering and were denoised into ASVs. After removal of 16 ASVs identified as contaminants and 280 non-metazoan, mammal, and fish ASVs, 966 ASVs were recovered at 18S. Of the 13,014,170 total COI sequences, 8,751,265 passed quality and chimera filtering. Removal of 19 contaminants and 105 non-metazoan, mammal, and fish OTUs resulted in 1943 OTUs at COI. Observed taxa spanned 17 phyla and included some meroplanktonic and parasitic species (Table 1). OTU rarefaction curves begin to reach saturation at 25,000 reads at COI, and 20,000 reads at 18S (Figure 1a and b). Despite rarefaction curves approaching saturation, richness was positively correlated with sequencing depth at COI (Figure 1c, $p = 2.7 \times 10^{-9}$, $\rho = 0.411$, two-sided Spearman’s rank), and negatively correlated at 18S (Figure 1d, $p = 7.4 \times 10^{-12}$, $\rho = -0.483$, two-sided Spearman’s rank). Analyses with non-rarefied data were similarly significant.

Sub-sampling and PCR replication

Each plankton subsample was analyzed with three independent PCR replicates with unique multiplexing indexes. There was no significant difference in community structure between PCR replicates, ($p \geq 0.05$, SIMPROF analysis of Bray–Curtis distance, not shown). However, there was some variability in the appearance of

Table 1. The number of OTUs or ASVs resolved by each marker for all taxa classified at least to phylum. OTU or ASV counts for each group are reported for both taxonomic assignment methods used and for both marker regions, such that a species can appear in each of the four columns independently. Taxa not classified to phylum are absent from this table.

	COI OTUs		18S ASVs	
	NCBI	MIDORI	NCBI	SILVA
Annelida	47	18	54	49
Arthropoda	995	742	478	502
Brachiopoda	–	–	5	2
Bryozoa	12	8	1	1
Chaetognatha	19	5	–	–
Chordata (Tunicata)	9	1	53	50
Cnidaria	80	64	117	112
Ctenophora	4	–	9	6
Echinodermata	39	25	20	20
Hemichordata	–	–	2	2
Mollusca	142	30	38	35
Nematoda	2	1	–	–
Nemertea	6	2	3	3
Phoronida	1	–	2	–
Platyhelminthes	9	2	2	2
Porifera	–	–	3	–
Sipuncula	1	–	5	5

taxa between PCR replicates. Taxa that did not appear in all three PCR replicates tended to be lower rank order OTUs and ASVs that were less abundant based on sequence counts, but more abundant taxa were also often absent from one or more of the PCR replicates

(Figure 2, $p < 2.2e-16$, Kruskal–Wallis one-way ANOVA). Replicate analyses with non-rarefied data showed similar results.

Each zooplankton sample was subsampled to the maximum biomass possible for a single DNA extraction, resulting in different fractions of the parent sample being analyzed. Observed richness within subsamples increased with the fraction of parent sample analyzed, with richness appearing to saturate around 6.25%, or 1/16th of the initial bulk sample (Figure 3), for both markers. Richness was not correlated with the volume of seawater filtered (Figure 3, colour, Spearman's rank; COI: $p = 0.085$, $\rho = 0.124$; 18S: $p = 0.213$, $\rho = 0.094$). Replicate analyses with non-rarefied data found similar correlations and significance values.

Comparisons between imaging and sequencing

To test whether relative read abundance reflects community structure, read counts were compared with numerical abundance and carbon biomass from ZooScan analyses. Comparisons were made using proportions, as metabarcoding data are proportional (Lindque *et al.*, 2013; Hirai *et al.*, 2015), and were possible for 16 taxonomic groups of zooplankton, 12 of which were resolved at both metabarcoding markers. Of the 16 taxa analyzed, 15 groups showed a significant correlation ($P \leq 0.05$) between proportion of biomass and proportion of sequences at either COI, 18S, or both (Table 2), although the strength of the correlation was variable. More abundant taxa showed a stronger relationship between carbon biomass and sequences (Table 2, Figure 4). At COI, the strength of the relationship was significantly correlated with average proportion of sequences, but this relationship was not significant at 18S (Figure 4). There was some variability between markers, with oithonids, do-

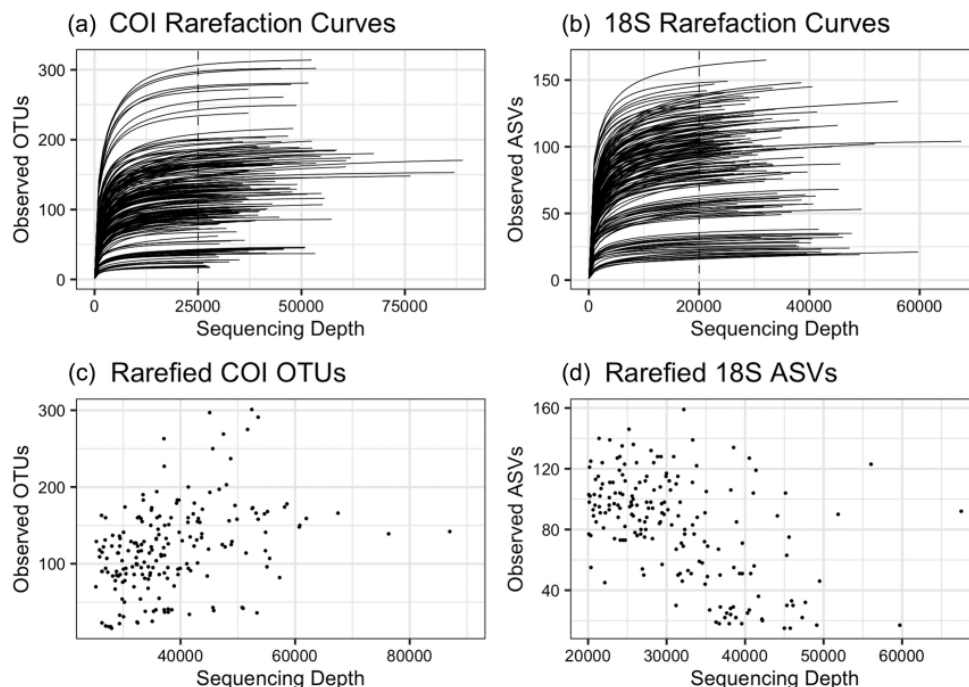


Figure 1. Rarefaction curves for (a) COI OTUs and (b) 18S ASVs, based on sequencing depth. Dashed lines represent the rarefaction depth for each marker. Richness, measured as observed OTUs/ASVs, increased with increasing sequencing depth at (c) COI, but not at (d) 18S.

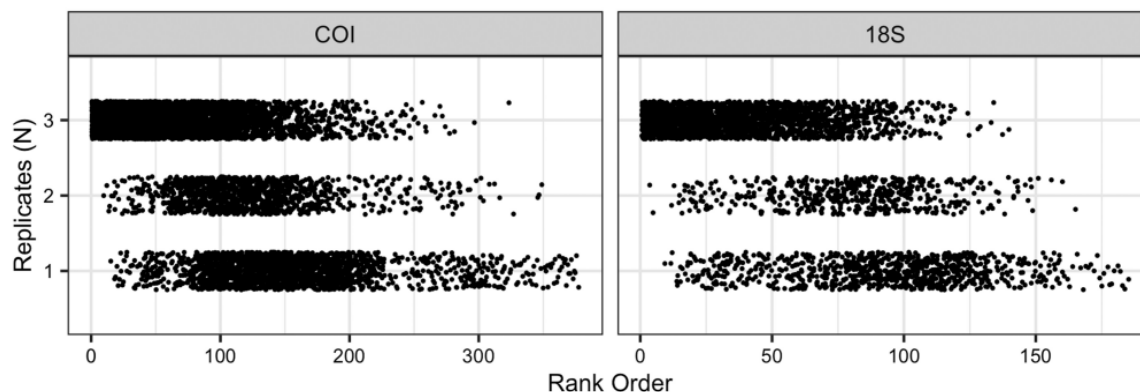


Figure 2. The number of PCR replicates in which each OTU/ASV occurs in relation to rank order of taxa. Rank order is based on summed abundance across PCR replicates. Higher rank order taxa (rarer) were less likely to occur in all three replicates, while low rank order taxa (dominants) tended to occur in more replicates. Data have been rarefied to 25000 and 20000 reads for COI and 18S, respectively.

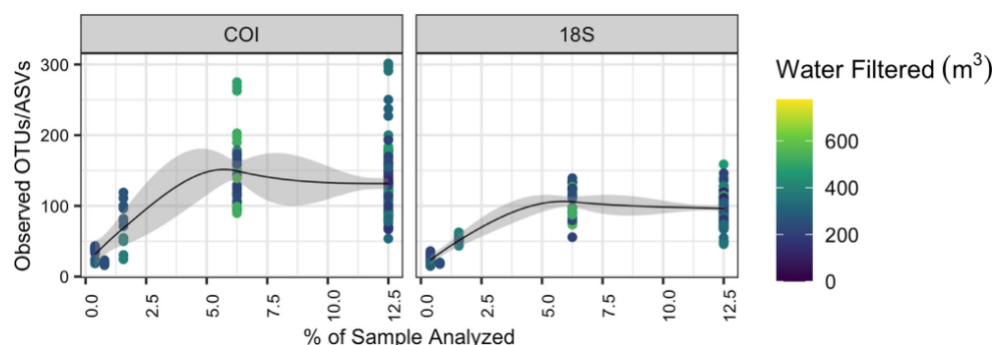


Figure 3. Observed richness at (left-hand panel) COI and (right-hand panel) 18S (in relation to the percentage of the zooplankton sample analyzed). For both markers, richness across all samples approached saturation at $\sim 6.25\%$ analyzed. Colour scale represents the total volume of seawater filtered at collection. Curves show the LOESS fit and shading represents 95% confidence intervals.

lioids, and pyrosomes only resolved at 18S and chaetognaths only resolved by COI. For most groups, the relationship between carbon biomass and sequence counts was equal to or stronger than the relationship between numerical abundance and sequence counts, with the exception of oithonids (Table 2, Figures S1 and S2). Chaetognath sequences did not show a significant relationship against either abundance or carbon biomass. Contrary to our expectation that crustaceans would show a better relationship than gelatinous organisms, there was not a close correspondence between tissue composition and strength of correlation between proportions of sequences and carbon biomass (Table 2).

A closer examination showed that there was wide scatter underlying even strong correlations. Figure 5 illustrates relationships for four representative taxa, showing two abundant copepod groups with stronger relationships and two other taxonomic groups (thaliaceans, ostracods) with weaker relationships and greater variability between markers, while Figures S1 and S2 include all taxa. Notably, there were some data points that were outliers in multiple correlations, such as those from Cycle 3, 250–400 m and Cycle 2, 200–250 and 350–400 m, which were found in the upper left quartile of the eucalanid correlation for both markers, and the lower right quartile for the calanoid correlation at COI (Figure 5). In addition to the taxa absent from one marker, there were taxa found at both markers that

showed stronger relationships at one. Ostracods showed a stronger relationship between COI reads and biomass, while thaliaceans had a strong relationship only at 18S (Figure 5).

Ethanol neutralization

For our test of ammonium hydroxide as a neutralizing agent, 494 COI OTUs were found. Total richness and richness within taxonomic groups were not significantly different between neutralized and untreated samples ($p = 0.22$, Wilcoxon, Figure 6) and there was no taxonomic bias, which would appear as a directional shift within points of a given color in Figure 5. Clustering of samples identified two significant groups separating by collection location (Figure S3, SIMPROF $\alpha = 0.05$). A PERMANOVA revealed that preservation method had no effect on community structure, but that collection event was a significant predictor of observed community structure ($R^2 = 0.79$, $p = 0.002$). Parallel analyses with non-rarefied OTU tables and presence-absence data showed similar results.

Discussion

Our results provide a framework for interpreting metabarcoding results of zooplankton community composition. We found that tech-

Table 2. Pearson's correlation coefficient (R) and the significance level for all comparisons between proportion of sequences and proportion of biomass or numerical abundance (ZooScan-based measures). All proportions have been arcsine square root transformed.

Taxon		Proportion carbon biomass vs. proportion sequences				Proportion abundance vs. proportion sequences			
		COI R		18S R		COI R		18S R	
Crustaceans	Eucalanids	0.77	***	0.72	***	0.67	***	0.66	***
	Calanoids	0.81	***	0.74	***	0.72	***	0.69	***
	Oithonids	–	–	0.46	***	–	–	0.64	***
	Poecilostomatoids	0.37	***	0.11	***	0.11	NS	–0.17	NS
	Malacostracans	0.58	***	0.38	***	0.64	***	0.29	***
	Ostracods	0.57	***	0.19	NS	0.58	***	0.36	***
Tunicates	Doliolids	–	–	0.66	***	–	–	0.65	***
	Pyrosomes	–	–	0.9	***	–	–	0.88	***
	Salps	0.05	NS	0.09	***	0.03	NS	0.09	NS
	Thaliaceans	–0.03	NS	0.88	***	–0.01	NS	0.42	***
	Appendicularians	0.22	NS	0.37	***	0.19	NS	0.27	NS
Other	Cnidarians & Ctenophores	0.48	***	0.36	***	0.48	***	0.18	NS
	Bryozoans	0.87	***	0.72	***	0.77	***	0.62	***
	Polychaetes	0.5	***	0.58	***	0.45	***	0.55	***
	Pteropods	0.39	***	0.46	***	0.62	***	0.049	***
	Chaetognaths	–0.12	NS	–	–	0.03	NS	–	–

NS - not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; Bonferroni corrected significance levels.

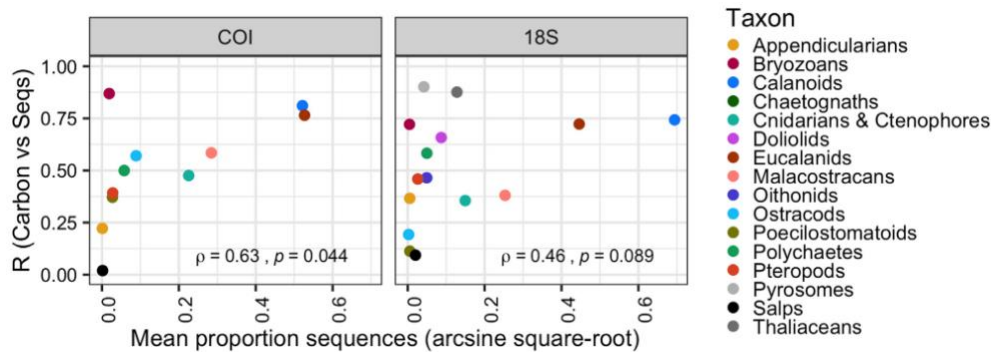


Figure 4. For each taxonomic group, Pearson's correlation coefficient (R) for the relationship between the proportion of sequences and proportion of carbon biomass is plotted against the average proportion of sequences across all samples. The strength of the correlation was stronger for more abundant groups at the COI marker, but the relationship at 18S was not significant ($p < 0.05$, Spearman's rank).

nical replication increases the detection of rare taxa and that sub-sampling has the potential to depress observed richness. In comparisons of relative read counts with morphological analyses, read abundance was positively correlated with the proportion of zooplankton biomass for all but one of the taxonomic groups analyzed, with considerable variability in the strength of the correlation among taxonomic groups. Thus, metabarcoding can be a viable approach for representing relative biomass of several major taxonomic groups but should be interpreted cautiously for less abundant taxa. In an important point for field fixation protocols, we found that neutralized ethanol provides unbiased measures of community composition.

Sub-sampling and PCR replication

We found a positive relationship between sequencing depth and richness at COI and a negative relationship at 18S. As sequencing depth is determined by sample pooling and library size (Herbold *et al.*, 2015), the observed relationships were primarily an artefact of library pooling. Parallel analyses with non-rarefied data were similar, indicating that the relationship was not an artefact of rarefaction (McMurdie and Holmes, 2014; Willis, 2019).

Good overall correspondence was observed between PCR replicates, although some taxa were absent from one or more PCR replicates. These taxa could be interpreted as spurious sequences arising from PCR errors, and some studies recommend removing OTUs

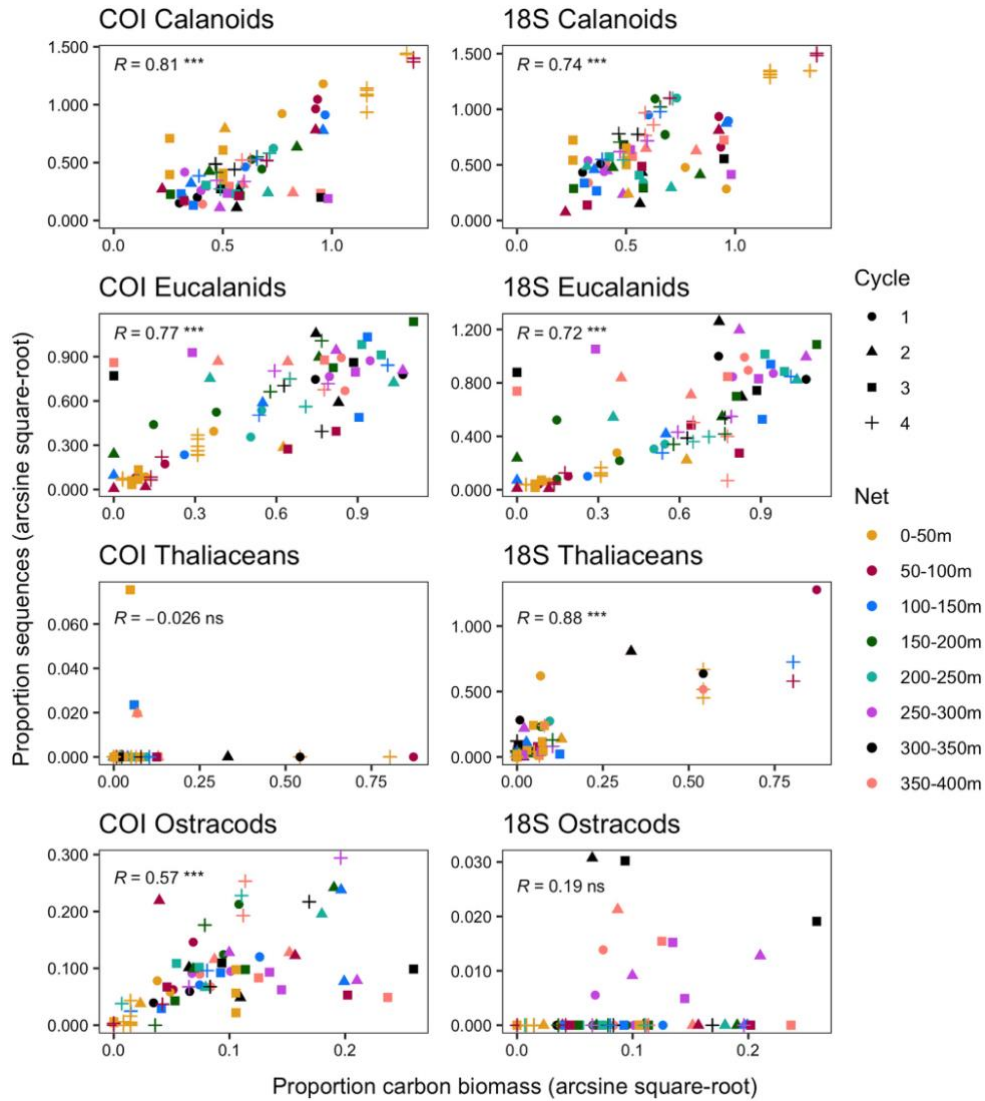


Figure 5. The proportion of metabarcoding sequences classified as calanoid, eucalanid, thaliacean, and ostracod in relation to the proportion of carbon biomass within the full zooplankton community, calculated from ZooScan ROIs. There is a strong positive relationship at both markers for the two copepod groups, and for thaliaceans at 18S and ostracods at COI. Note the different scales for each panel. All data have been arcsine square root transformed. Collection locations (Cycle, Net) are shown to enable comparison between taxa. Additional taxa and all abundance relationships are shown in Figures S1 and S2. (ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

that do not appear in all replicates (Loos and Nijland, 2020, but see also Lahoz-Monfort *et al.*, 2016). However, many of these taxa were found in higher abundance in nearby samples, indicating they represented rare individuals or fragments of animals. Multiple PCR replicates are widely recommended to minimize false negatives in environmental DNA studies (Ficetola *et al.*, 2016; Ruppert *et al.*, 2019). Stochastic variability in the detection of rare species has been observed in tissue-based metabarcoding analyses (Leray and Knowlton, 2017), and our results indicate that in a natural community this stochasticity can affect both rare and more abundant taxa. For our study site, at least three PCR replicates were necessary to

fully capture the diversity of the zooplankton community, and we recommend this for other regions as well.

Richness increased as a function of the fraction of the sample analyzed and appeared to saturate at around 6.25% of the sample. This comparison was made across ocean environments from nearshore upwelling dominated by a few taxa to higher diversity, offshore mesotrophic waters, and at depths ranging from the surface to 400 m. Addition of replicate subsamples resulted in species accumulation curves closely matching that of the full sample set, indicating that the low richness is an artefact of subsampling. Our results indicate that in addition to technical replication, a minimum

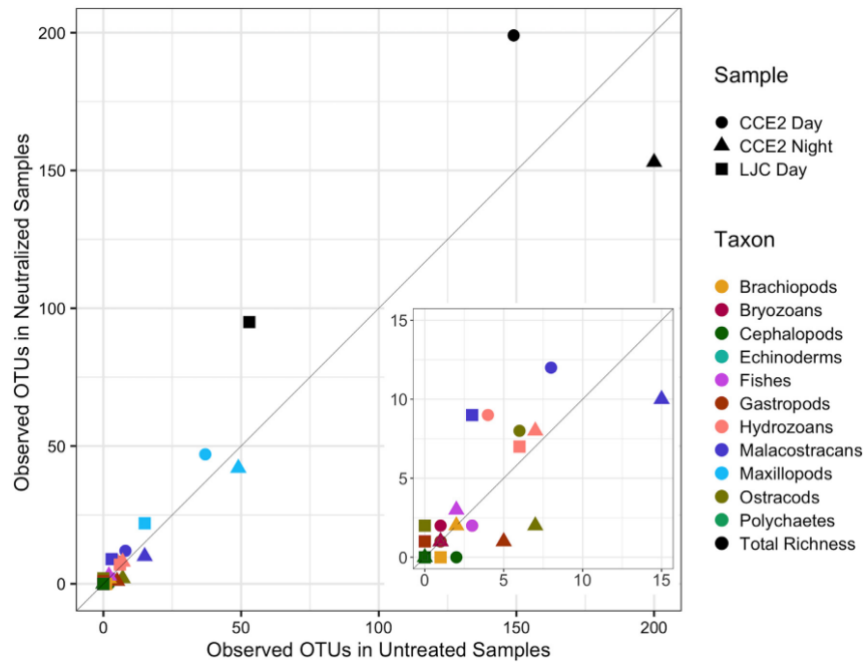


Figure 6. Comparison of number of COI OTUs from paired samples preserved in ethanol neutralized with ammonium hydroxide with those in unamended ethanol. The diagonal is the 1:1 line. Colours indicate zooplankton taxa; symbols indicate the three sets of samples compared. Inset shows OTUs at low counts. There was no difference in overall richness and no taxonomic bias between neutralized and untreated samples.

fraction of the initial bulk sample should be analyzed. For zooplankton assemblages in our region, this threshold was $> 6.25\%$ of the sample, but this value should be estimated for other ocean ecosystems with distinct rank abundance profiles.

The importance of replicate field samples, subsampling, PCR replication, and sequencing depth are likely to vary between ecosystems and study designs, with greater replication at all stages necessary for more diverse systems and increased field sampling effort in systems with high spatial variability. Metabarcoding analyses should be designed with careful consideration of the goals of the study and the types of variability present in the ecosystem of interest (Kelly *et al.*, 2019).

Comparisons between imaging and sequencing

The relationships between carbon biomass and read abundance were stronger for more abundant groups. We found strong relationships at one or both markers for eucalanid and calanoid copepods, malacostracans, ostracods, bryozoans, and polychaetes. Several previous analyses have reported positive relationships between read counts and abundance or biomass within the zooplankton community, for a range of taxonomic groups and marker regions (Harvey *et al.*, 2017; Bucklin *et al.*, 2019; Schroeder *et al.*, 2020). The strong positive relationships we observed for more abundant taxa corroborate these studies, and we similarly found that such relationships are not always significant.

The poor or non-significant relationships observed for some groups are also important, as they help inform the limitations of estimating biomass or abundance from read counts. Because metabarcoding data are proportional, biases introduced by variability in gene copy number, amplification efficiency or primer

bias propagate to all taxa within a sample (McLaren *et al.*, 2019). In our analysis, some of these biases can be seen in the absence of oithonid copepods, doliolids, and salps at COI, of chaetognaths at 18S, and in the better relationships for ostracods and thaliaceans at COI and 18S, respectively. Approaches to account for bias in amplicon data sets, including the use of taxon: taxon proportions or estimation of amplification efficiency from known community composition, often require mock communities or independent data on community composition or primer bias which are less available for zooplankton than for microbial communities (Kelly *et al.*, 2019; McLaren *et al.*, 2019). Our analyses indicate that these normalization procedures could be particularly important for less abundant taxonomic groups.

There was a notable difference between the strengths of relationships observed at different taxonomic levels within a group. For thaliaceans as a whole, there was a strong correlation between proportion biomass and proportion sequences at 18S. However, this relationship was due to good correspondence for doliolids and pyrosomes, while that for salps was quite low. Chaetognath COI sequences and biomass or abundance were not correlated, but chaetognath sequences were only recovered from mesopelagic species while chaetognaths were identifiable from ZooScan images throughout the water column. Similarly, ostracods at 18S were only detected in mesopelagic samples, while ostracods were found in all samples using COI or using ZooScan imaging. These mismatches may be due to poor representation in the NCBI, MIDORI, and SILVA databases used to assign taxonomy, or due to variability in amplification efficiency between species present in different depth zones. Previous work has found that agreement between methods increases as species are grouped at coarser taxonomic levels (Leray and Knowlton, 2015; Harvey *et al.*, 2017). In contrast, our

data indicate that relationships can be either strengthened or weakened at coarser taxonomic resolution, depending on the variance in marker performance between closely related taxa. We recommend that marker biases be accounted for and that estimates of relative abundance and comparisons between survey methods be limited to taxa that are well resolved.

Some outlying samples were clearly visible in the scatterplots of eucalanid and calanoid relationships (Figure 5). These samples were outliers for both marker regions, and incorrect classification with independent reference databases is unlikely. Further verifications of image classifications did not detect misidentifications. It is most likely that these samples reflect a true mismatch in the detection capabilities of the molecular and imaging methods compared. The outliers in question were collected from the upper mesopelagic (200–400 m) and likely included eucalanid copepodites that were not easily identifiable to genus by digital imaging (hence only assigned to calanoid copepods) but were classifiable as eucalanids by metabarcoding. Digital imaging lacks the morphological detail necessary to identify most species, especially for young developmental stages, hence comparisons must be made at more aggregated taxonomic levels. However, ZooScanning accurately reflects body sizes (Gorsky *et al.*, 2010) and numerical abundances (Whitmore *et al.*, 2019) when compared to other methods. Conversions from body length to carbon biomass introduce another source of uncertainty, but were optimized for the zooplankton taxa of our study region (Lavanigos and Ohman, 2007) and are unlikely to bias the biomass proportions calculated here. These outlier samples illustrate the strengths of combining multiple observation methods. While metabarcoding lacks information such as body size, sex, or life history stage, it can increase detection sensitivity to rarer species or taxa that are undetected by visual surveys or other collection methods (Stat *et al.*, 2019). Because metabarcoding does not yield measurements of absolute abundance, we recommend that metabarcoding and morphological or imaging methods be used in combination.

Ethanol neutralization

For our comparison of paired samples preserved in either unamended ethanol or ethanol neutralized with ammonium hydroxide, we recovered fewer OTUs than were found in the full data set, likely due to limited sampling. However, the observed OTUs included a similar taxonomic range. We did not detect any effect of ammonium hydroxide on community structure, composition, or richness between the two sets of samples. These results indicate that ammonium hydroxide addition to ethanol (Bednaršek and Ohman, 2015) permits a single fixative to be used for both molecular and morphological analyses, including calcareous zooplankton. This result bears confirmation with other DNA extraction protocols.

Supplementary data

Supplementary material is available at the ICESJMS online version of the manuscript.

Data availability

OTU tables, fasta files, and sample metadata are available through CCE-LTER DataZoo. Raw sequence are under NCBI SRA PRJNA679794. Code is available at < https://github.com/sammatthews/Matthews_et_al_IJMS_2021 >

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Supplemental figures

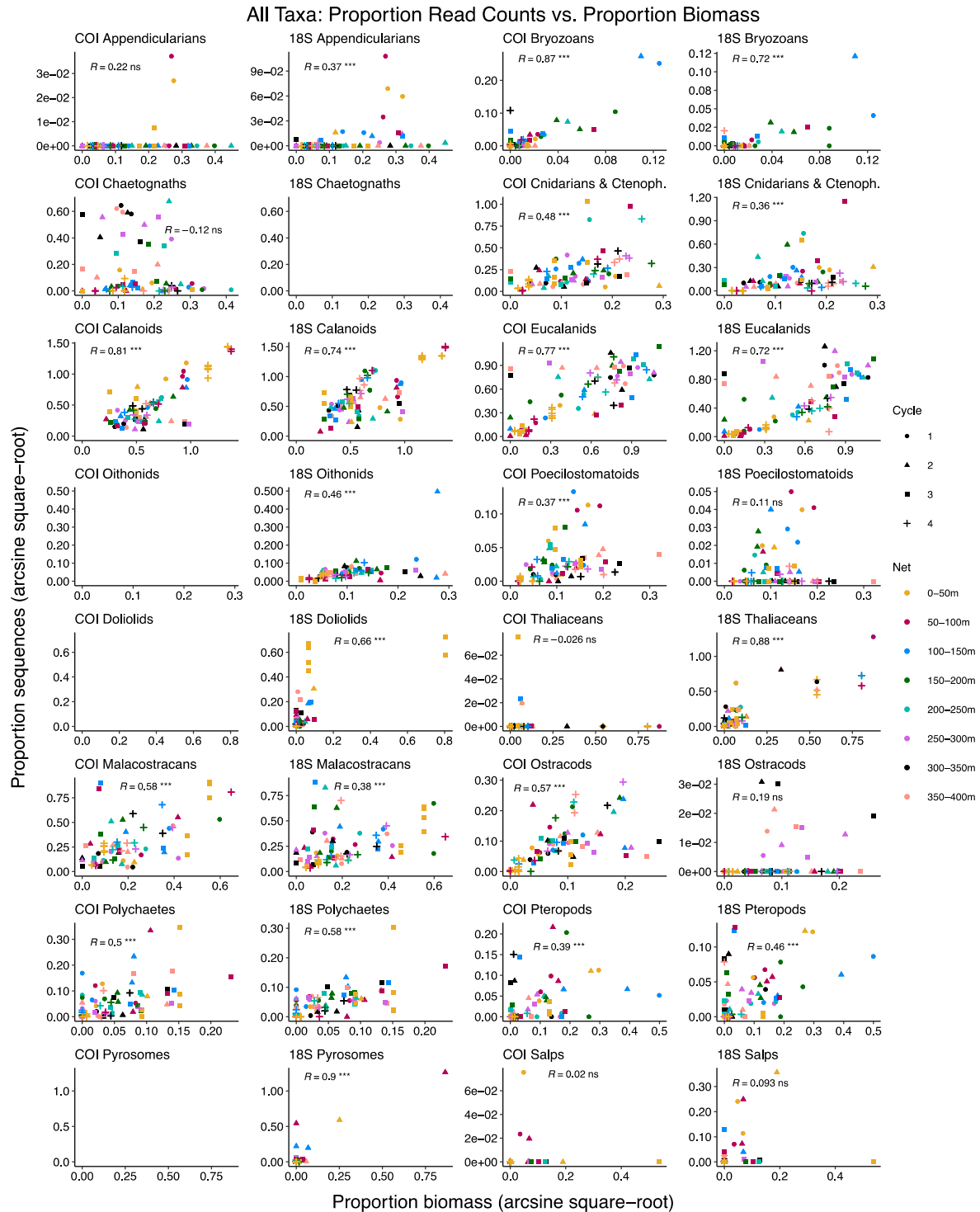


Figure S1. The proportion of metabarcoding sequences for each taxonomic group is plotted against the proportion of carbon biomass of the group within the full zooplankton community, calculated from Zooscan ROIs. Collection location (Cycle, Net) is shown to enable comparison between taxa. Blank panels are due to the absence of data at that marker. Note the different scales for each panel. (ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

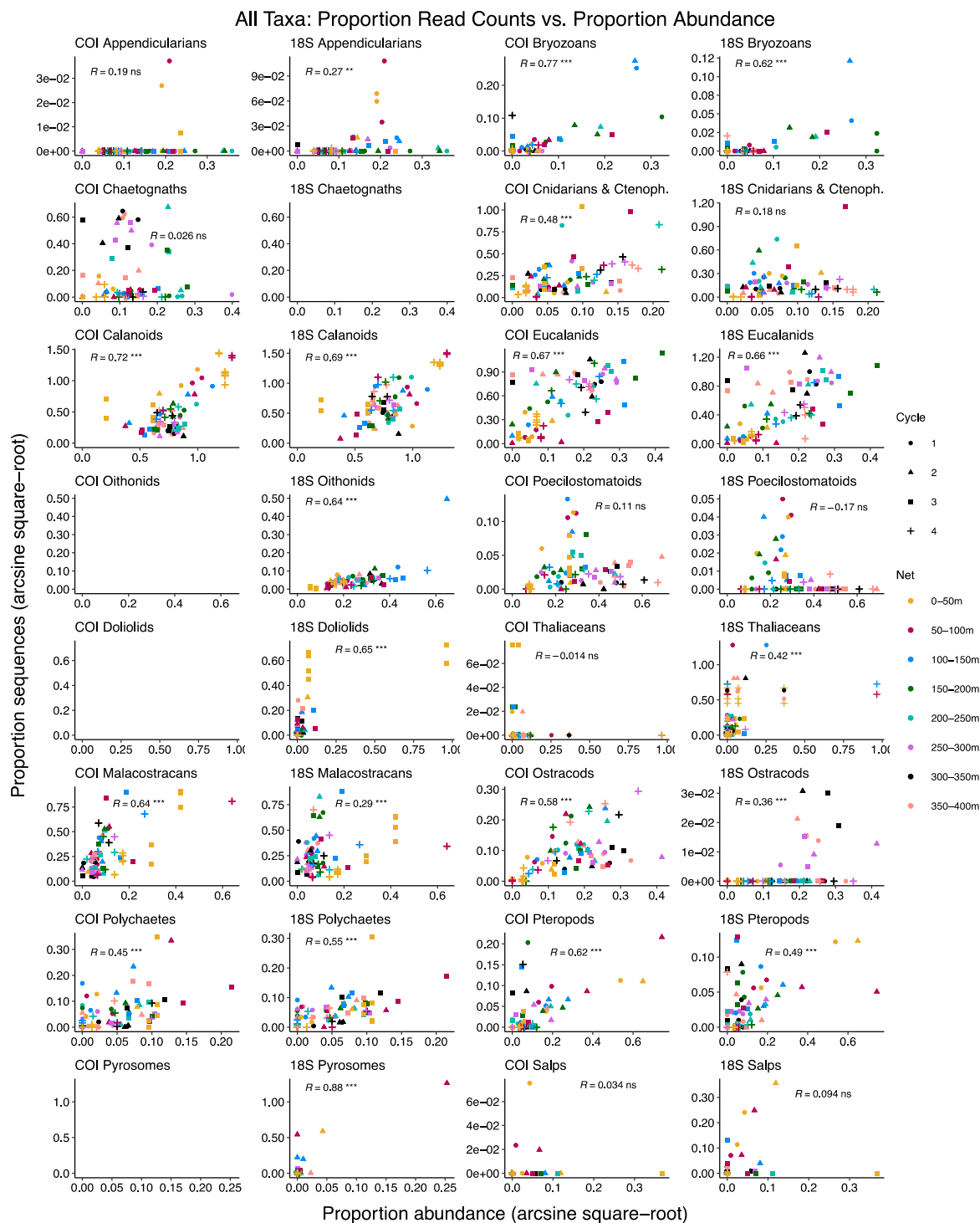


Figure S2. The proportion of metabarcoding sequences for each taxonomic group is plotted against the proportion of numerical abundance of the group within the full zooplankton community, calculated from Zooscan ROIs. Collection location (Cycle, Net) is shown to enable comparison between taxa. Blank panels are due to the absence of data at that marker. Note the different scales for each panel. (ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

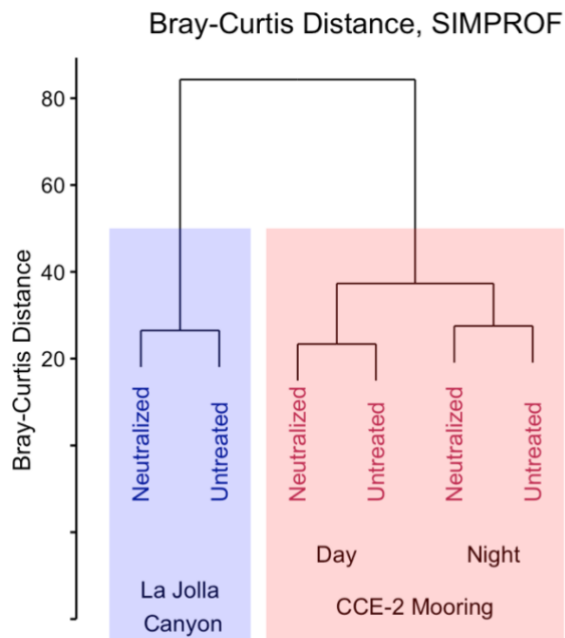


Figure S3. A SIMPROF test with average-neighbor clustering based on Bray Curtis distances. Clusters with non-significant structure are highlighted in blue and red boxes, respectively. Samples collected from two different locations were significantly different, but there was no difference between paired samples preserved in either neutralized or untreated ethanol.

Table S2. Primer sequences and PCR protocols for both PCR steps for 18S and COI. Red Xs represent variable indexing region, with per-sample indexes found in Table S1.

18S PCR 1			
Primers	Primer name	Primer Sequence	
	Uni18S	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGXXXXXXXXAGGGCAAKYCTGGTGCCAGC	
	Uni18SR	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGXXXXXXXXGRCGGTATCTRATCGYCTT	
Reaction Volumes	Reagent	Volume	
	MiFi mix	10 µl	
	BSA	0.4 µl	
	dH2O	6.6 µl	
	DNA template	1 µl	
	Primer 1	1 µl	
	Primer 2	1 µl	
PCR Cycling	Temperature (°)	Time (MM:SS)	Cycles
	95	3:00	28x
	95	0:15	
	52	0:30	
	72	0:15	
	72	5:00	

18S PCR 2			
Primers	Primer name	Primer Sequence	
	Tag_F	AATGATACGGCGACCACCGAGATCTACACXXXXXXXXTCGTCGGCAGCGTCAGATGTG	
	Tag_R	CAAGCAGAAGACGGCATACGAGATXXXXXXXXGTCTCGTGGGCTCGGAGATGTGTA	
Reaction Volumes	Reagent	Volume	
	MiFi mix	7.5 µl	
	BSA	0.3 µl	
	dH2O	4.2 µl	
	DNA template	1 µl	
	Primer 1	1 µl	
	Primer 2	1 µl	
PCR Cycling	Temperature (°)	Time (MM:SS)	Cycles
	95	3:00	10x
	95	0:30	
	55	0:30	
	72	0:45	
	72	5:00	

COI PCR 1			
Primers	Primer name	Primer Sequence	
	jgLCO1490	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGXXXXXXXXGGWACWGGWTGAACWGTWTAYCCYCC	
	jgHCO2198	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGXXXXXXXXTAIACYTCIGGRTGICRAARAAYCA	
Reaction Volumes	Reagent	Volume	
	MiFi mix	10 µl	
	BSA	0.4 µl	
	dH2O	6.6 µl	
	DNA template	1 µl	
	Primer 1	1 µl	
	Primer 2	1 µl	
PCR Cycling	Temperature (°)	Time (MM:SS)	Cycles
	95	3:00	28x
	95	0:15	
	48	0:30	
	72	0:15	
	72	5:00	

COI PCR2			
Primers	Primer name	Primer Sequence	
	Tag_F	AATGATACGGCGACCACCGAGATCTACACXXXXXXXXTCGTCGGCAGCGTCAGATGTG	
	Tag_R	CAAGCAGAAGACGGCATACGAGATXXXXXXXXGTCTCGTGGGCTCGGAGATGTGTA	
Reaction Volumes	Reagent	Volume	
	MiFi mix	7.5 µl	
	BSA	0.3 µl	
	dH2O	4.2 µl	
	DNA template	1 µl	
	Primer 1	1 µl	
	Primer 2	1 µl	
PCR Cycling	Temperature (°)	Time (MM:SS)	Cycles
	95	3:00	10x
	95	0:30	
	55	0:30	
	72	0:45	
	72	5:00	

CHAPTER 2

A space-for-time framework for forecasting the effects of ocean stratification on zooplankton vertical habitat use and trait composition

Abstract

The effects of environmental change on zooplankton communities and pelagic ecosystems more broadly are difficult to predict due to high diversity of ecological strategies within the zooplankton and their complex interspecific interactions. Trait-based approaches can define zooplankton functional groups with distinct responses to environmental change, and analyses across multiple mesozooplankton groups can help identify key organizing traits. Here, we used the pronounced cross-shore environmental gradient within the California Current Ecosystem as a space-for-time substitution to test potential effects of ocean warming and increased stratification on zooplankton communities, using DNA metabarcoding (18S and COI) and digital ZooScan imaging. We hypothesized that mesozooplankton vertical distributions covary with cross-shore gradients, and that vertical habitat shifts vary among functional groups. Vertical distributions of many mesozooplankton taxa did shift in the cross-shore direction, and light penetration was the strongest predictor of magnitude of change. Changes in vertical distribution were most pronounced among carnivorous taxa, while herbivorous taxa tended to associate with the chlorophyll maximum, especially in clear offshore waters. Our results indicate that increased stratification of future oceans will drive some components of epipelagic mesozooplankton communities deeper, and result in zooplankton communities with more specialized feeding strategies, increased egg brooding, and more asexual reproduction.

2.1. Introduction

Zooplankton are a central component of marine pelagic foodwebs, and variability in species diversity, community composition, and community structure can significantly influence ecosystem function. Within the California Current Ecosystem, zooplankton communities link upwelling-driven primary productivity to economically important fisheries and protected mammal and bird species (Ruzicka et al. 2012). As zooplankton have broad dispersal and relatively short generation times, the effects of environmental variability on zooplankton communities can appear relatively rapidly (Peijnenburg and Goetze 2013). However, predicting the impact of anthropogenic climate change on zooplankton communities and ultimately on pelagic ecosystems requires understanding of community dynamics and species-specific habitat preferences.

The California Current Ecosystem (CCE) is located in a highly productive eastern boundary current upwelling system and is the site of significant cross-shore environmental gradients. Nearshore, nutrient rich waters are upwelled then advected offshore, where they encounter the cool, fresh waters of the southward flowing California Current (Bograd et al. 2019; Chabert et al. 2021). The California Current, centered about 150-300km offshore, is bounded on the west by the warmer waters of the North Pacific Subtropical Gyre (NPSG) (Lynn and Simpson 1987; Thomson and Krassovski 2010). This transition from coastal to offshore waters results in spatial changes in primary production rates, biomass, and community composition at multiple trophic levels (Kahru et al. 2015; Morrow et al. 2018). Along the cross-shore gradient in nutrient availability and primary production rates, zooplankton abundance and biomass are positively correlated with primary production rates and negatively correlated with ocean temperature (Valencia et al. 2018). Using a space-for-time substitution framework, which

assumes that spatial variability in ecosystems can be used as a proxy for temporal trends within one subregion, the cross-shore gradient from active upwelling to more stratified mesotrophic habitats can be used to test the potential effects of future warming and more stratified oceans on zooplankton habitats (Pickett 1989; Ohman et al. 2013).

The trait-based approach uses traits to define groups of species with similar ecological characteristics, as a framework for examining functional groups and tradeoffs between ecological strategies (Litchman et al. 2013; Heneghan et al. 2020; Martini et al. 2021). Variable effects of changing pelagic environments have been observed between zooplankton groups with different ecological constraints, for multiple traits and at various scales. Mesoscale changes in the dominant diet and feeding mode within the zooplankton community have been observed across ocean fronts within the CCE, with a shift from particle-grazing communities to carnivory and omnivory (Ohman et al. 2012). Within copepod communities, functional groups defined by size, trophic group, feeding mode, myelination, and spawning strategy exhibit complementary biogeographic distributions on global latitudinal scales (Benedetti et al.). Body size within copepods is strongly correlated with temperature and productivity, but environmental variability only explains approximately 50% of spatial patterns in marine copepods, indicating that additional structuring variables such as phylogenetic history may play a role (Brun et al. 2016). In addition to horizontal changes among zooplankton functional groups, traits can shape vertical habitat use, which can be actively selected even by planktonic species that cannot swim against horizontal ocean currents. For most zooplankton species, the optimal vertical habitat is a tradeoff between food availability and predator avoidance (Aksnes and Giske 1993). The optimal vertical habitat varies between zooplankton species with different metabolic needs, feeding niches, and predation risk, which depend on body size and morphology (Litchman et al. 2013; Brun et al.

2017). For example, intermediate-sized copepods exhibit cross-shore changes in behavior, with stronger Diel Vertical Migration (DVM) amplitude in clearer, offshore waters where predation risk is higher, but small and large copepods do not exhibit DVM behavior (Ohman and Romagnan 2016).

There is a tradeoff between trait specificity and the phylogenetic resolution at which traits are analyzed, due to variability in the level of trait expression among taxa. For example, the morphology of shelled thecosome pteropods makes the entire clade particularly vulnerable to long-term changes in pH due to ocean acidification (Bednaršek et al. 2019) but the strength of shifts in vertical distributions due to decreasing aragonite saturation state varies among species (Bednaršek and Ohman 2015), as does sensitivity to short-term changes in dissolved oxygen and pH (Maas et al. 2016). Trait-based analyses across multiple zooplankton taxa comprising the metazoan community allow for comparisons of a wide diversity of trait modes. These comparisons across taxonomic groups can lead to novel insights of broad ecological patterns that are not apparent within a group (Villarino et al. 2018). Metabarcoding of zooplankton communities can resolve community composition at species resolution across multiple major zooplankton taxa, making metabarcoding datasets an ideal target for trait-based analyses that have both high species resolution and broad taxonomic range.

Here, we use trait-based analyses of a paired metabarcoding and digital imaging dataset to detect high resolution changes in species distribution, community composition, and shifts among zooplankton functional groups. We use cross-shore environmental gradient in upwelling, stratification, and optical environment in the CCE to test whether mesozooplankton community composition and habitat use changes predictably with environmental structure, and whether there are particular traits that increase species sensitivity. Specifically, we test 1) whether there are

changes in zooplankton community composition across a major environmental gradient; 2) whether particular zooplankton species alter their vertical distributions; 3) whether vertical shifts in distribution are correlated with food availability, predation risk, or water column hydrographic structure, and 4) whether vertical habitat changes vary among species traits. These analyses provide insight into the differential effects of environmental changes on different zooplankton functional groups, and help predict how zooplankton communities will respond to increased stratification in the future ocean.

2.2. Methods

2.2.1. Study area

The California Current Ecosystem is a dynamic coastal region characterized by multiple ocean currents, including the California Current, the California Undercurrent, and the Inshore Countercurrent, comprised of an admixture of Pacific Subarctic, Eastern North Pacific, and Pacific Equatorial water masses (Bograd et al. 2019). The present study targeted four distinct water masses, including two nutrient-limited sites, one in the offshore stratified region (Cycle 1) and one in the core California Current (Cycle 2), one transition site characterized by moderate productivity and a phytoplankton community dominated by small ($<1\mu\text{m}$) phytoplankton cells (Cycle 3), and one coastal upwelling site with high salinity surface waters, high productivity, and larger phytoplankton (Cycle 4) (Fig. 2.1) (Morrow et al. 2018). Vertical profiles for the four Cycles (Fig. S1) show stratification offshore, decreased oxygen nearshore due to upwelling, and chlorophyll maximum depth increasing offshore while the magnitude decreases. All sampling was Lagrangian, conducted in repeated daily ‘Cycles’ of measurement while following a water parcel with a satellite-tracked drifter with a holey sock drogue at 15m depth.

2.2.2 Mesozooplankton collection and analysis

Samples were analyzed by DNA sequencing and digital ZooScan imaging (Gorsky et al. 2010; Matthews et al. 2021). Briefly, paired day and night depth-stratified 202 μm MOCNESS tows were conducted between 0-400m, or 0-200m over the continental shelf. Samples were quantitatively split and preserved in either 1.8% sodium borate-buffered formaldehyde or 95% NH_4OH -buffered ethanol. Formalin-preserved samples were imaged on a ZooScan and sorted into 23 morphologically identifiable groups with a Convolutional Neural Network model, with all vignettes validated manually (Table S2.1) (Ellen et al. 2019). Carbon biomass was determined from length-carbon conversions, as in (Lavaniegos and Ohman 2007). Ethanol-preserved samples were split to 1/8th, DNA extracted in bulk, and each sample amplified in triplicate at the 18S-V4 and COI regions (Leray et al. 2013; Zhan et al. 2013). Amplified sequences were processed in QIIME2 with *dada2* amplicon sequence variant (ASV) delimitation, followed by clustering into operational taxonomic units (OTUs) at 97% similarity at the COI marker (Callahan et al. 2016; Bolyen et al. 2019). COI amplicons were further filtered to remove sequences containing stop codons, in order to remove sequences resulting from pseudogenes, PCR errors, or sequencing error (Nugent et al. 2020). Resulting ASVs and OTUs were given taxonomic assignments where possible based on the global MetaZooGene database for each marker, trimmed to the target region (Bucklin et al. 2021). Non-zooplankton taxa, including laboratory contaminants, all vertebrate sequences, and sequences unidentified at phylum level were removed, resulting in a dataset that included both holozooplankton and invertebrate meroplankton taxa.

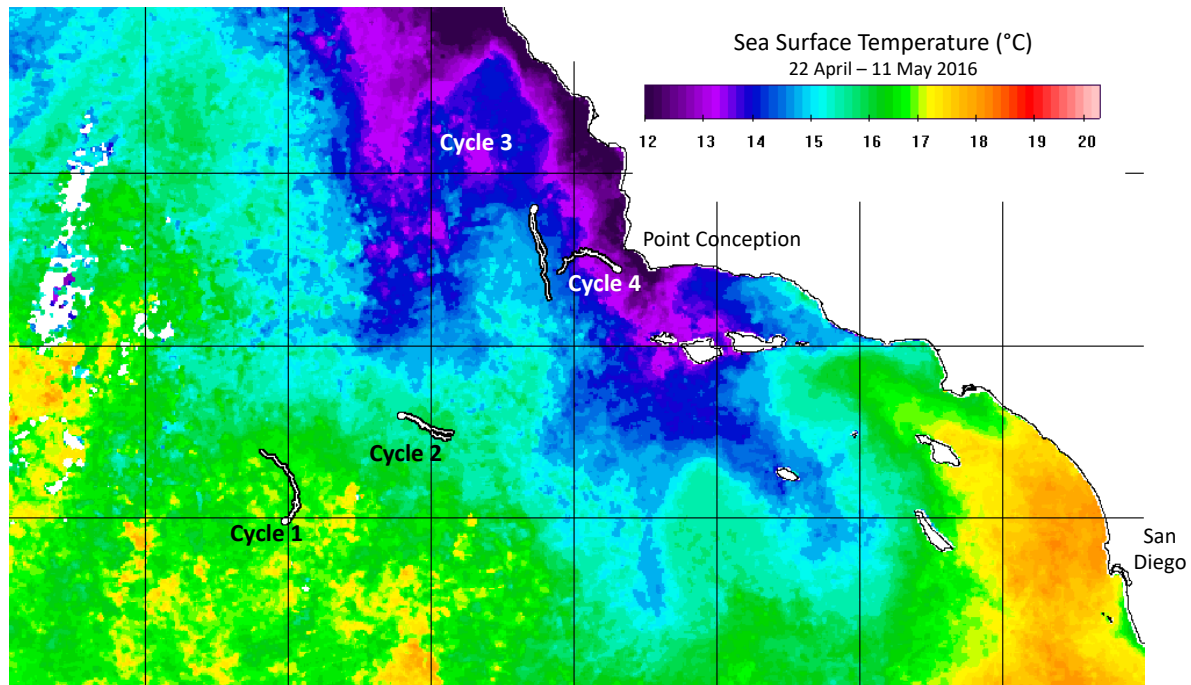


Figure 2.1. Sampling locations of 4 quasi-Lagrangian Cycles within the California Current Ecosystem overlain on composite satellite sea surface temperature for the region. Each Cycle shows the 3-day trajectory of the holey-sock drift array, around which day and night MOCNESS tows were centered. Zooplankton sampling was conducted between 0-400m at Cycles 1-3, and 0-200m at Cycle 4. Image courtesy of M. Kahru, Scripps Institution of Oceanography

2.2.3 Trait assignments

Based on assigned taxonomy, each ASV or OTU was classified for the following traits: size, body composition, transparency, diet, feeding behavior, spawning strategy, presence of asexual reproduction, and Diel Vertical Migration (DVM). The eight traits were selected to identify tradeoffs in ecological strategy, while targeting traits with sufficient data available for multiple taxonomic groups. Traits were assigned to taxa based on published trait databases (for copepods) (Benedetti et al. 2016; Brun et al. 2017) and species-specific observations (Appendix A).

Body size was selected as it is a ‘master trait’ with significant impacts on predation risk and prey field, as well as metabolic rates and feeding needs. Taxon-specific measurements were collected as follows: for siphonophores: total colony length; for copepods: female prosome

length; for euphausiids and amphipods: total length; for medusae: bell height when given, or bell width (not including tentacles). Carbon content, expressed as percent of wet weight, was included as a measure of gelatinous vs non-gelatinous tissue composition, which is typically a tradeoff with size (Kiørboe 2013). Transparency was assigned as a qualitative category for each major taxonomic group, ranging from 1 (opaque) to 5 (transparent), based on mean transparency detected with transmitted red light through the *Zooglider* in situ imaging system (Ohman et al. 2019).

Two feeding variables were analyzed: trophic level (carnivore, omnivore, or herbivore) and feeding behavior (ambush, cruise, current, or parasitoid). Trophic level and feeding behavior followed previously published trait databases where available (Kiørboe 2011; Benedetti et al. 2016; Brun et al. 2016), supplemented with taxon-specific literature. Ambush feeding is characterized by lack of feeding movement prior to prey detection, including passive ambush (interception, as by pteropod mucous nets and ctenophore tentacles) and active ambush (pursuit of a prey item following detection, as displayed by chaetognaths and *Oithona* copepods). Cruise feeding refers to active movement of the predator through the water column with the intent to detect prey, such as is observed by visually feeding fish larvae, visually predatory heteropods, and mechanoreception by *Clausocalanus* and *Metridia* copepods. Current feeding includes all taxa that set up feeding currents across a portion of their body, such as feeding currents across mouthparts or across tentacles. These currents can be for filter feeding (in ciliated taxa such as thaliaceans or pumping taxa such as appendicularians), direct interception (copepods), or scanning for individually handled prey (copepods). Finally, parasitoid feeding includes taxa identified as parasites or parasitoids, primarily amphipods. While these two traits and the modes we used for them do not fully capture the range of feeding behaviors, mechanisms, and

preferences found among zooplankton, they are important measures of the target prey field, likelihood of direct or indirect effects of phytoplankton prey, metabolic rate, and feeding effort, and are broadly comparable among zooplankton groups.

Spawning behavior (brooding or broadcast spawner) and evidence for asexual reproduction (whether obligate or alternating) were included as proxies for population turnover and the capacity of the population to exhibit bloom behavior. These reproductive traits are also related to life history strategy and reflect the importance of parental investment in offspring.

Finally, DVM behavior can have significant effects on food availability and predation risk, as well as the metabolic rate of the organism due to the costs of migration and temperature variability across depth. DVM behavior was based on observed day and night vertical distributions from sequence read abundance in our data. Taxa were classified as migratory if the modal depth of relative abundance shifted by at least two nets in at least one sampling location. DVM was only calculated if the taxon was observed in at least two nets of each tow, if total daytime and nighttime reads were no more than twofold different between the day and night, and if the taxon comprised at least 0.01% of the total read abundance within all samples. These criteria are conservative and there may be additional migratory taxa we did not detect, including taxa that have been previously described as migratory in our sampling region or other regions. However, the width of our sampling bins limits detection of small-amplitude vertical migrations, and we refrain from assigning a behavior without strong evidence for it in the population we sampled, as our reads may represent any life history or developmental stage of the species and DVM behavior typically varies through the life history and may vary spatially.

2.2.4 Statistical analyses

Data analysis, statistical testing, and all visualizations were conducted in R version 4.2.1 (2022-06-23, Funny-looking Kid), primarily with the packages *phyloseq*, *vegan*, and *tidyR*. Sample clustering was performed on the combined 18S and COI datasets after rarefaction, using the complete linkage of *chao* distance, and clusters were identified with the R *stats* function *cutree*. Mean depth of occurrence (MDO) was calculated from the presence or absence in each depth stratum after filtering out occurrences that were less than 5% of the total reads for the OTU or ASV. Cross-shore shifts in vertical distribution were defined as a change in MDO of at least 50m, with vertical shifts tested between Cycles independently for daytime and nighttime distributions, and only tested for the three Cycles where sampling extending to 400m. To account for taxa that were found only at two Cycles, taxa were defined as exhibiting shifts if their MDO shifted deeper in the offshore direction between any two Cycles. The linear relationship between OTU-specific MDO and environmental variables was tested independently for MDO calculated from 0-400m for Cycles 1-3, and for MDO calculated from 0-200m for all four Cycles. Cross-shore shifts in single ASVs/OTUs or binary traits were not tested for statistical significance, due to low statistical power and absence of sampling replicates. All data processing and analysis scripts are available at <https://github.com/samathews/VerticalHabitatShiftsCCE>.

2.3. Results

ZooScanning of zooplankton samples from four locations ('Cycles') along a cross-shore gradient, sampled between the surface and 400m, showed that non-eucalanid calanoids (hereafter, 'calanoids') and eucalanid copepods dominated the overall zooplankton community, comprising 69% and 14% of the total zooplankton C biomass, respectively. At the 18S marker,

8.9 million reads were denoised into 1,667 ASVs, and 8.3 million COI reads were clustered into 1,282 OTUs at 97% similarity. Eighty percent of 18S ASVs were identified at least to order, and 40% of COI OTUs, while only 34% and 26% were identified to species (Table 2.1). Trait assignment based on the best taxonomic identifications led to 27% and 16% of taxa being assigned trait values for size, 58% and 32% for diet, 65% and 33% for spawning behavior, 75% and 43% for asexual reproduction, 91% and 57% for carbon content, and 83% and 46% for transparency, for 18S and COI respectively. DVM behavior was detectable in 4.8% and 7.7% of 18S ASVs and COI OTUs.

Table 2.1. Number of ASVs (right, 18S) and OTUs (left, COI) assigned taxonomy at each taxonomic rank, and the number assigned a characteristic for each trait. DVM counts reflect the number of taxa exhibiting detectable DVM.

	18S	COI
Assigned Taxonomy		
Kingdom	1,665	1,282
Phylum	1,534	741
Class	1,485	596
Order	1,334	519
Family	1,031	402
Genus	802	352
Species	571	332
Assigned Traits		
Maximum Size	443	210
Diet	965	407
Feeding Behavior	899	316
Spawning	1,090	423
Asexual Reproduction	1,255	545
Carbon	1,520	732
Transparency	1,381	590
Exhibiting DVM	80	99
Total	1,667	1,282

In the three Cycles sampled to 400m, total taxon richness decreased with depth both during the day and night, with the maximum in richness observed within the epipelagic zone at

all locations (Fig. 2.2). Within the epipelagic zone (0-200m), the minimum in richness was observed near shore (Cycle 4, Figs. 2.2a and 2.1c), while in the mesopelagic zone (200-400m) richness was comparable among Cycles and the minimum was observed in the offshore (Cycle 1, Figs. 2.2a and 2.2c). Copepods (Hexanauplia) had the highest richness detected by both marker regions, with high richness also observed within Malacostraca and Hydrozoa (both markers), as well as Polychaeta, Anthozoa, and Thaliacea (18S) (Figs. 2.2b and 2.2d). Unidentified taxa were more common at COI than at 18S.

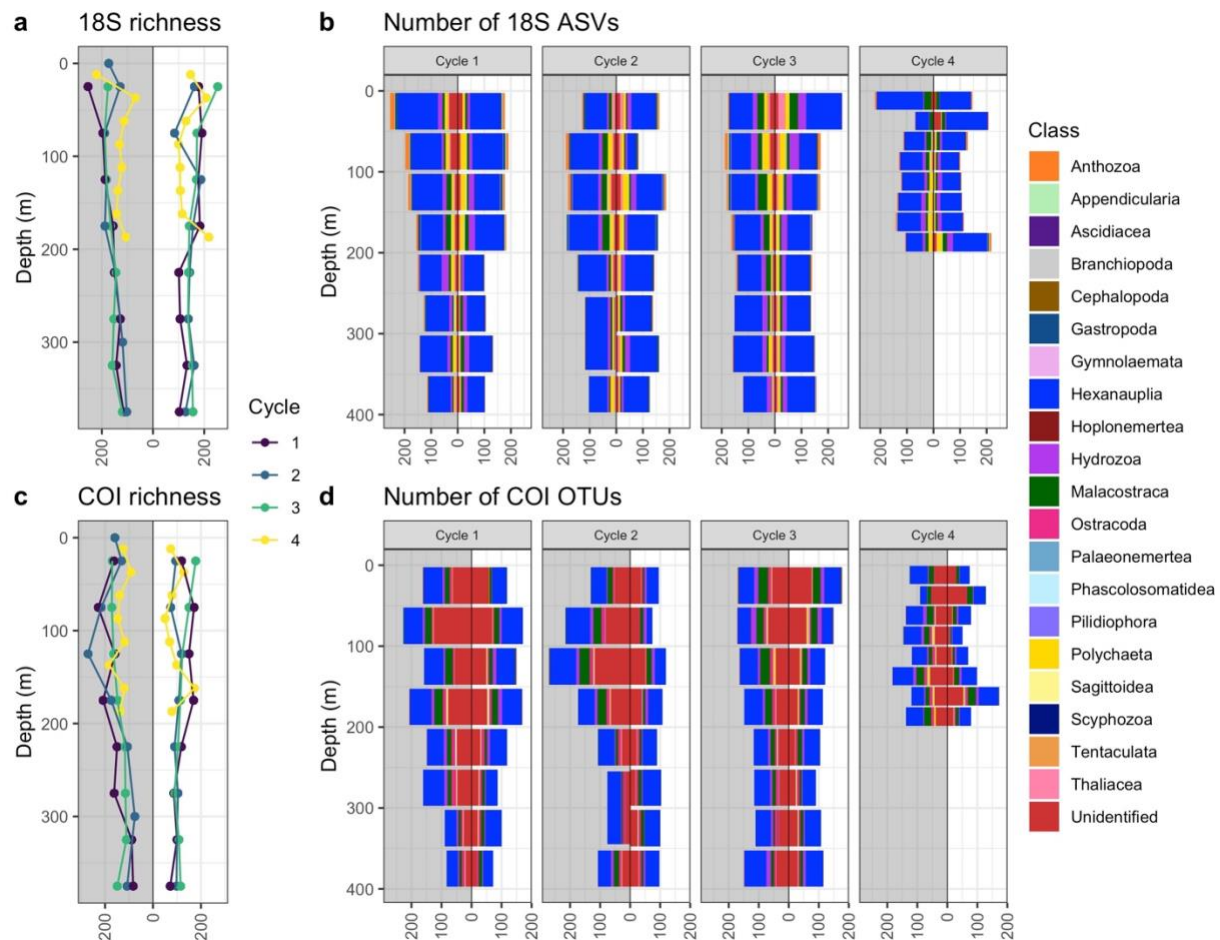


Figure 2.2. Total richness (number of ASVs or OTUs observed within a sample) with depth at (a) 18S and (c) COI, and number of OTUs assigned to each taxonomic Class at (b) 18S and (d) COI. Colors in (a) and (c) indicate different sampling Cycles. Colors in (b) and (d) indicate taxon. Within Cycles, nighttime samples are shown on the shaded left panel, and daytime samples on the right.

We observed cross-shore changes in community composition within the epipelagic zone, with distinct zooplankton communities at each Cycle, both day and night (Fig. 2.3). In contrast, in the upper mesopelagic (200-400m), cross-shore community composition was more uniform. The night-time community exhibited somewhat deeper cross-shore vertical extent (compression of navy blue), while mesopelagic day-time vertical distributions showed less change along the environmental gradient. Vertical displacement at the community level was stronger within the epipelagic zone, but the lower limit of the epipelagic community influenced the upper mesopelagic community, particularly at night.

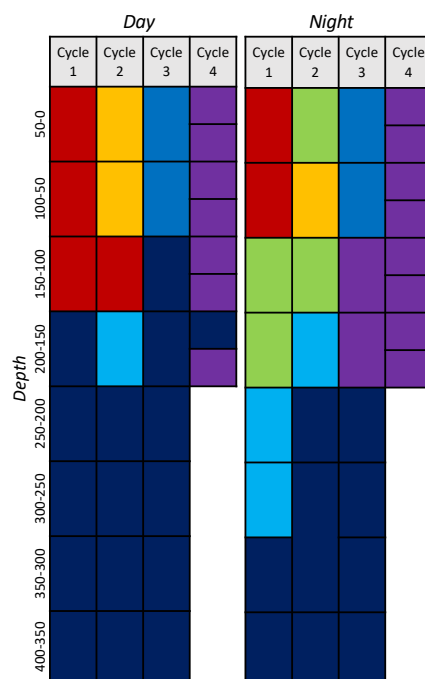


Figure 2.3. Day (left) and night (right) samples colored by Chao similarity clusters (Fig. S2). Samples were clustered based on Jaccard similarity after accounting for unseen species pairs, then divided into 7 distinct clusters (function cutree, R stats). Similar colors across the two panels represent samples that clustered together.

Turning to taxon-specific responses, copepods comprised a large proportion of the zooplankton community assessed via read abundance (Fig. 2.4), in addition to being the most diverse taxon and dominating carbon biomass. Within the copepod community, there were clear

cross-shore changes in composition at the family level. Nearshore, the family Calanidae dominated the community, and while they remained present in the epipelagic zone, they became less abundant in the offshore; in the offshore the Clausocalanidae were relatively more important in the surface waters. There were some differences between copepod communities at the two metabarcoding markers, with generally higher richness at 18S. The Eucalanidae were dominant at both markers but were a larger proportion at COI than 18S, while 18S marker higher abundances of Calanidae and Metridinidae. Euchaetidae were only detected at COI, and Oithonidae only at 18S. Despite these differences in taxonomic resolution, overall community-level copepod richness and community composition showed similar patterns between markers.

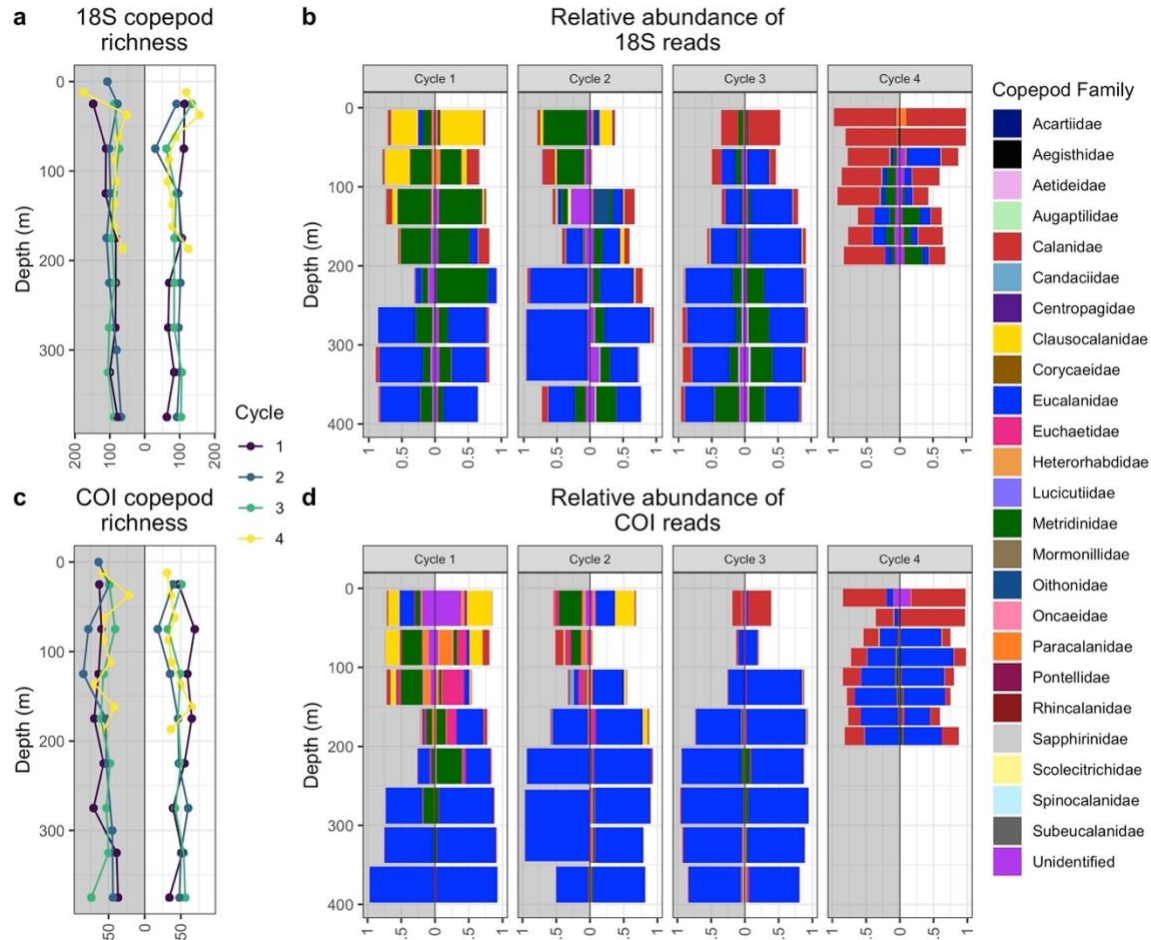


Figure 2.4. Total copepod richness detected (a) by 18S, and (c) by COI, and the relative abundance of each copepod family as a proportion of the total zooplankton community (b) for the 18S community and (d) for the COI community. Colors in (a) and (c) indicate different sampling cycles. Colors in (b) and (d) indicate taxon. Within Cycles, nighttime samples are shown on the shaded left panel, and daytime samples on the right.

The observed cross-shore changes in copepod community composition were the result of individual copepod family distributions, which in some cases had distinct cross-shore shifts in vertical distributions. Eucalanid copepod reads were broadly distributed across the region, with the vertical mode shifting to deeper waters offshore. Carbon biomass of eucalanid copepods analyzed by ZooScan showed a similar pattern, confirming that relative read abundance for this

dominant group reflected shifts in the biomass distribution of these animals determined by an independent method (Fig. 2.5).

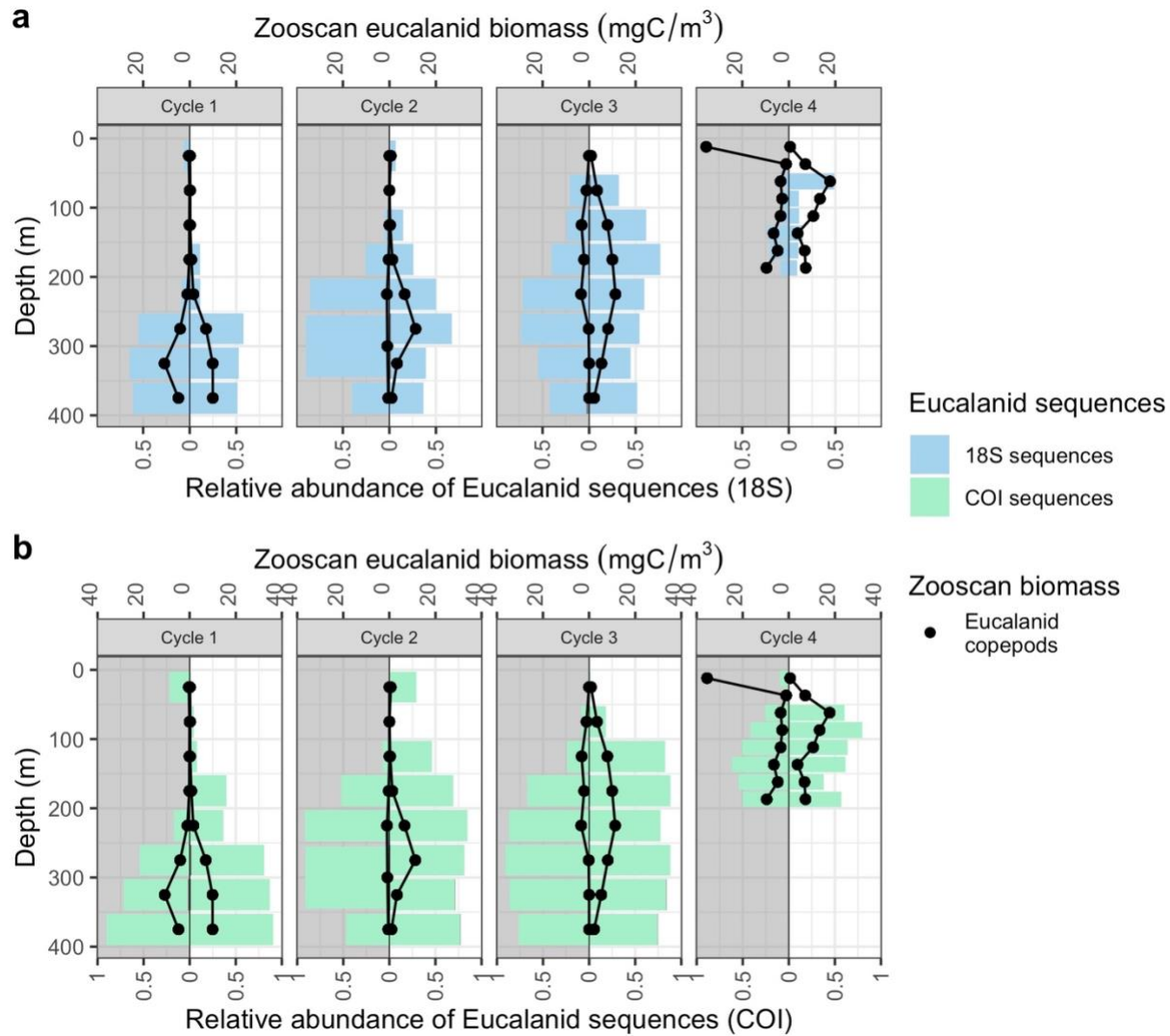


Figure 2.5. The relative abundance and distribution of Eucalanid copepod reads at (a) 18S and (b) COI, shown together with eucalanid copepod carbon biomass calculated via Zooscan imaging (black lines, a and b). Within Cycles, nighttime samples are shown on the shaded left panel, and daytime samples on the right.

Siphonophores exhibited suborder-specific patterns in habitat partitioning among species. Siphonophore species in the suborder Calycophorae tended to have restricted depth distributions, with vertical partitioning of the water column. Calycophorans exhibited a diverse epipelagic assemblage (*Amphicaryon acaule*, *Lensia campanella*, *L. conoidea*, *Muggiאה atlantica*) (Fig.

2.6a), transitioning with depth to a mesopelagic community dominated by *Lensia achilles*, which was broadly distributed across all four sampling locations. The calycophoran assemblage also exhibited a cross-shore shift in vertical habitat, with most species moving deeper offshore, and with the maximum richness in the upper mesopelagic zone offshore. In comparison, physonect siphonophores exhibited lower overall richness, and had restricted or patchy distributions with no clear vertical habitat partitioning among species, and no cross-shore shifts in vertical distributions (Fig. 2.6b).

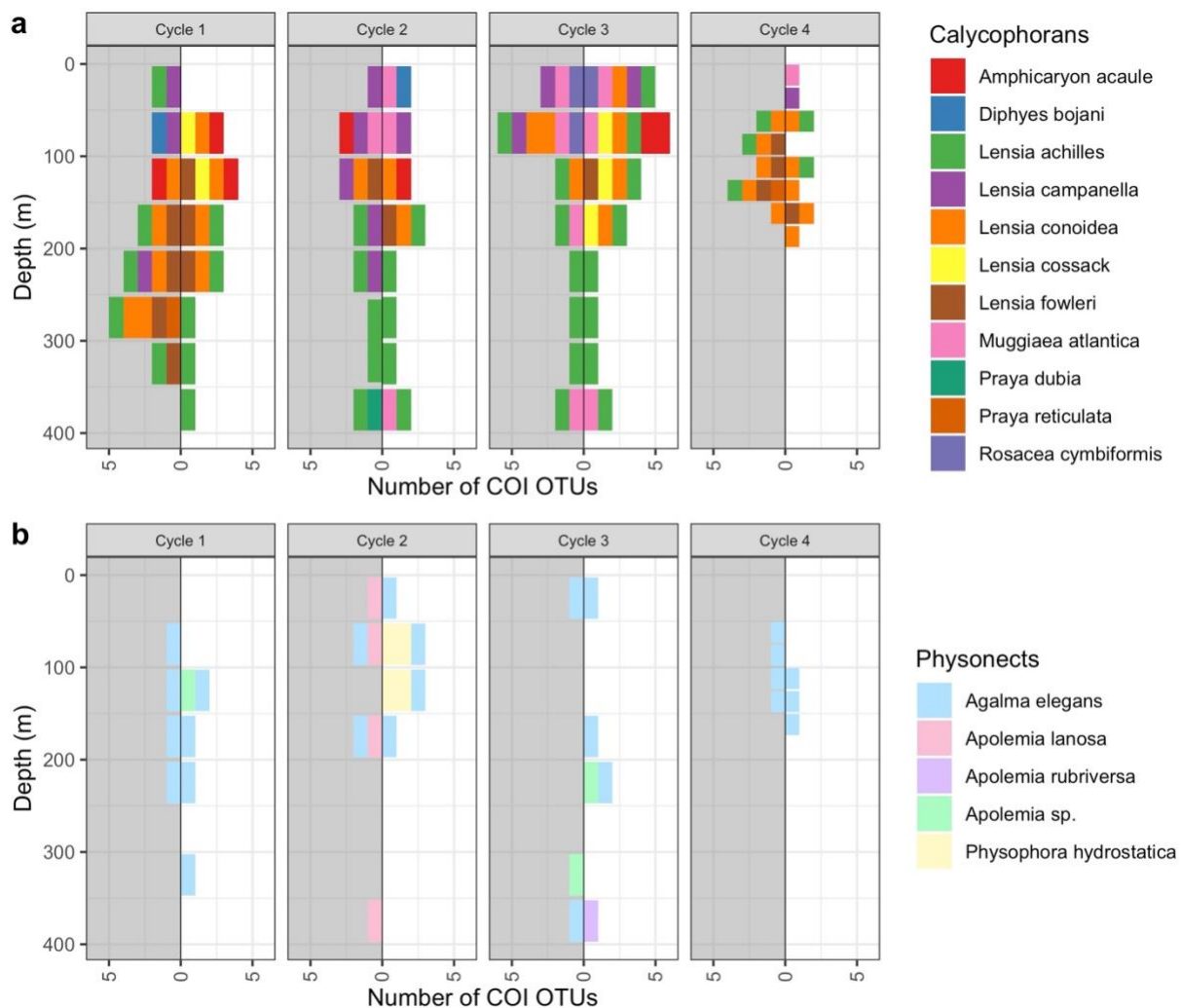


Figure 2.6. Observed richness of COI siphonophore OTUs (a) within the calycophoran group, and (b) within the physonect group. Colors indicate taxon. Within each Cycle, nighttime samples are shown on the grey-shaded left panel, and daytime samples on the right panel.

Euphausiid biomass was highest nearshore, and euphausiid distributions shifted deeper in the water column with distance offshore, as detected by both ZooScan biomass and metabarcoding reads (Figs. 2.7, S2.3). In addition to shifting vertical distributions of individual species (primarily *Euphausia pacifica* and *Nematoscelis difficilis*), there was cross-shore and vertical turnover in euphausiid species composition. The euphausiid community in the upwelling region (Cycles 3-4) was dominated by *Euphausia pacifica*, which was also observed offshore in lower relative abundances and deeper waters. *Nematoscelis difficilis* was broadly distributed across the region, but deeper in the water column than *E. pacifica*. *Nyctiphanes simplex* was restricted to the nearshore, while *Thysanoessa gregaria* was observed primarily in Cycle 2 and the deeper-dwelling *Thysanopoda acutifrons* was almost entirely restricted to Cycle 1. In total, the euphausiid community composition showed species turnover, in addition to the family-level shift toward deeper waters in the offshore region (Fig. 2.7).

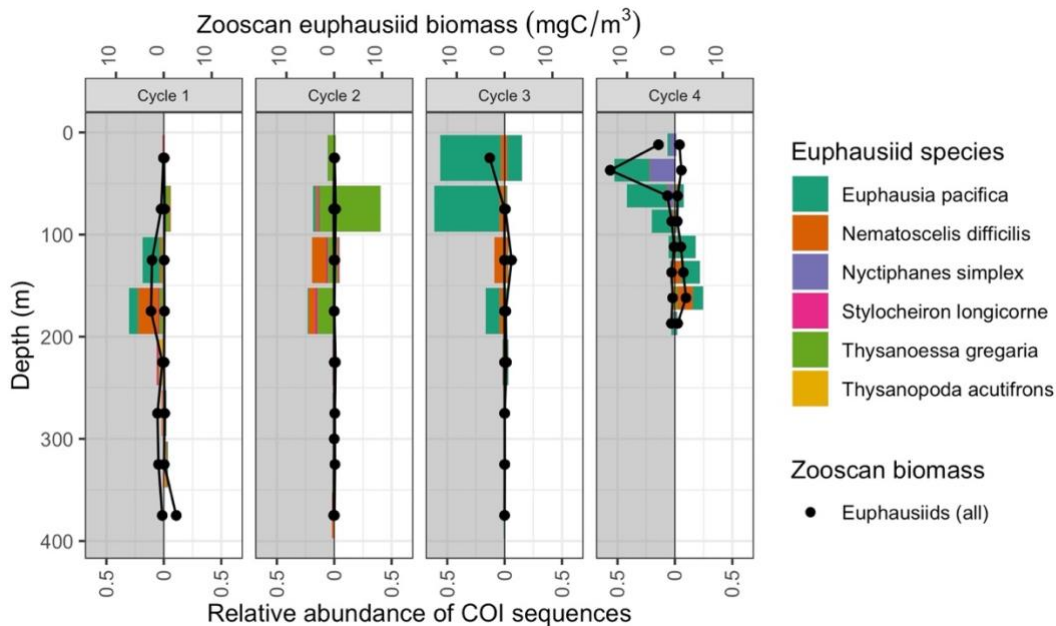


Figure 2.7. Relative read abundance of all euphausiid species detected at COI (colored bars), as well as total euphausiid biomass from digital ZooScan imaging (black lines). Colors represent taxa. Within Cycles, nighttime samples are shown on the shaded left panel, and daytime samples on the right.

In total, cross-shore shifts in vertical distributions across Cycles 1-3 were detectable in 161 ASVs at 18S (10%), and 165 OTUs at COI (13%) (Table 2.2, S2.2). Within these vertically shifting taxa, 9 and 11 (5.6% and 6.6%) respectively exhibited vertical shifts both day and night. Of taxa observed in all three Cycles, 65.4% and 73.3% of ASVs and OTUs exhibited vertical shifts in MDO, while only 30% and 34% of taxa observed in two Cycles exhibited vertical shifts (Table 2.2). Vertical shifts were overrepresented ($>1.5\times$ the percentage observed across all taxa) within Calanoida, Euphausiacea, Trachymedusae, Ctenophora and Mollusca (Table S2.2).

Table 2.2. The number of 18S ASVs and COI OTUs that exhibited shifts in mean depth of occurrence (MDO), and the number of Cycles in which they were observed. Some amplicons at each marker were not observed at all, as this analysis includes only Cycles 1-3, all of which were sampled to 400m.

	18S			COI		
# of Cycles						
Observed	Shifting	Fixed	Total	Shifting	Fixed	Total
0	0	370	370	0	166	166
1	0	912	912	0	757	757
2	76	177	253	88	166	254
3	85	45	130	77	28	105

To identify potential causes of the observed vertical habitat changes, we examined environmental variables that vary in the cross-shore direction. A one-way ANOVA showed a significant difference in mean MDO among all taxa cycles (18S: $F_{2, 1071} = 11.609$, $p < 0.001$; COI: $F_{2, 1912} = 10.602$, $p < 0.001$). We used AIC model selection to rank linear models of the relationship between MDO and depth of the chlorophyll maximum, depth of the 1% light level, depth of particle maximum, depth of the 100 $\mu\text{M kg}^{-1}$ oxygen threshold, depth of the thermocline, and depth of the pycnocline (measured by the maximum in buoyancy frequency,

N²), treating each OTU or ASV independently within day and night tows. AIC model selection revealed that shifts in MDO in the 18S-resolved community were best correlated with the depth of the pycnocline across the upper 400m (32% of model weight, adjusted $R^2 = 0.659$, $F_{1296, 807} = 4.133$, $p < 0.001$, Fig. 2.8a), and the depth of the chlorophyll maximum within the epipelagic (99% of model weight: $R^2_{adj} = 0.580$, $F_{1396, 750} = 3.3.122$, $p < 0.001$). Linear models for the COI-resolved community found the strongest effect of the depth of the 1% light level, both across the upper 400m (86% of model weight, $R^2_{adj} = 0.644$, $F_{1117, 797} = 4.102$, $p < 0.001$, Fig. 2.8b) and within the epipelagic (100% of model weight, $R^2_{adj} = 0.404$, $F_{1099, 741} = 2.131$, $p < 0.001$). The sum of model weights across the four model comparisons indicated that depth of the 1% light level was the strongest explanatory variable (53% of total model weights), followed by depth of the chlorophyll maximum (27%) and depth of the pycnocline (11%) (Table S2.3).

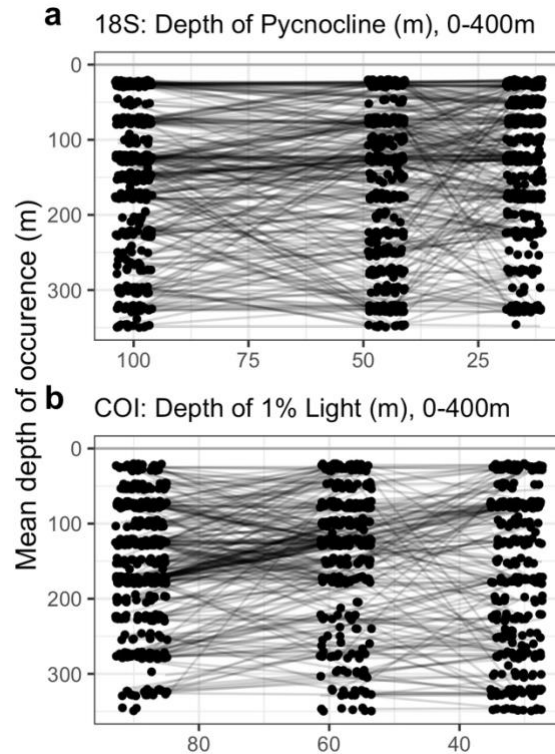


Figure 2.8. Mean depth of occurrence for each ASV or OTU is shown against the best explanatory variable. MDO is best correlated with (a) the depth of the pycnocline for 18S, and (b) the depth of the 1% light level for COI. Points from the same ASV/OTU are connected. Day and night MDOs are plotted independently for each ASV/OTU.

In addition to environmental variables, we tested whether species traits affect changes in zooplankton vertical habitat use. For this analysis, total number of reads assigned to each trait state was summed, and total relative abundance of reads belonging to each trait state was treated as a single group. We tested whether trait groups changed vertically in the cross-shore direction and found strong differences among diets (Fig. 2.9). Carnivorous zooplankton exhibited strong cross-shore deepening in vertical distribution, especially at night, while the peak in herbivore read abundance remained in the epipelagic zone both day and night across all four Cycles (Fig. 2.9). Carnivores (296 18S ASVs, 207 COI OTUs) were dominated by copepods, hydrozoans, polychaetes, and eucaraid and peracarid malacostracans. Herbivorous taxa (387 18S OTUs, 62 COI OTUs) were primarily calanoid copepods (Tables S2.4, S2.5). Omnivores were broadly

distributed throughout the water column and across Cycles, but thaliacean omnivores exhibited herbivore-like distributions primarily restricted to the epipelagic zone (Fig. S2.4).

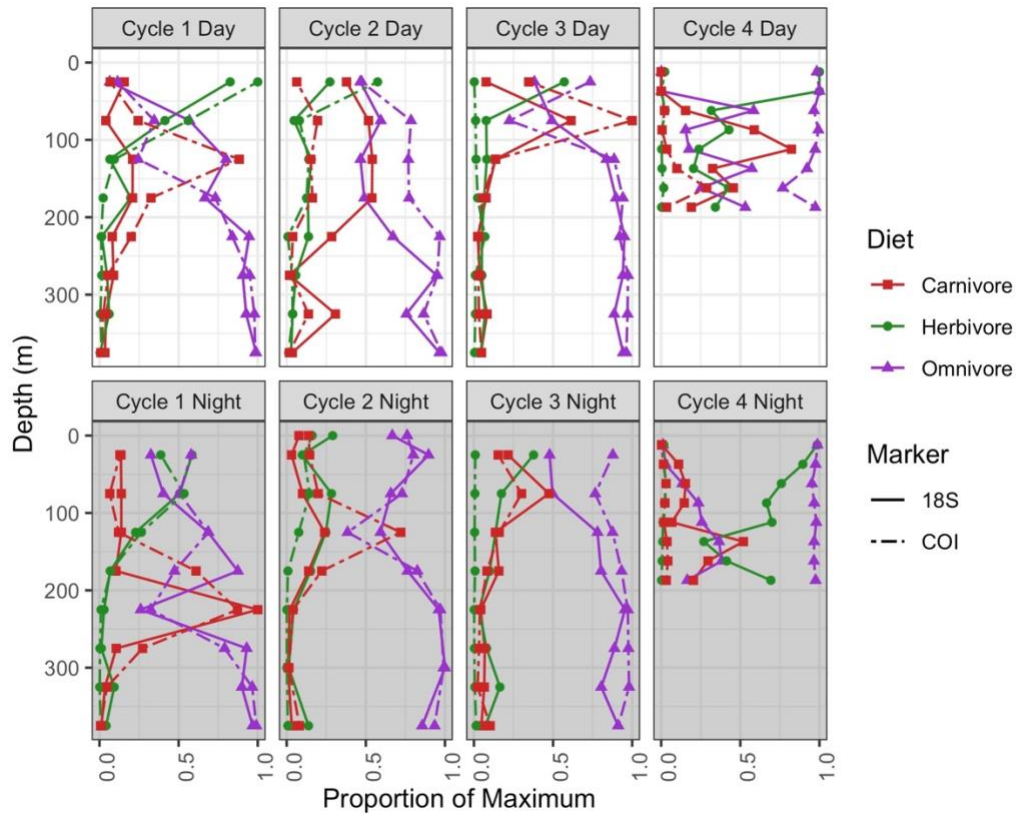


Figure 2.9. Vertical distribution of dietary traits at each Cycle. Upper row: daytime (white background), lower row: nighttime (grey background) Relative abundance of COI and 18S reads assigned to each dietary characteristic is scaled relative to the maximum abundance for the characteristic (carnivores in red; herbivores in green, and omnivores in purple).

We also observed cross-shore changes in trait composition across the full zooplankton community sampled (Fig. 2.10). Asexual reproduction and brooding behavior were lowest nearshore, with the proportion of asexual reproduction peaking in Cycles 3 and 1 at 18S and COI respectively (Figs. 2.10a, S2.5), and a monotonic increase in brooding behavior with distance offshore (Fig. 2.10b). The proportion of reads belonging to taxa exhibiting DVM diverged between the COI and 18S marker, with the nearshore community dominated by DVM in the 18S-resolved community and showing the lowest occurrence of DVM in the COI-resolved

community (Figs. 2.10c, S2.6). Across all four Cycles, the most common feeding mode was current feeding, but offshore there was an increase in cruise feeding and ambush feeding (Fig. 2.10d). Offshore dietary modes also became more heterogeneous, with increased contributions from carnivores and herbivores and decreasing proportions of omnivores (Fig. 2.10f). Mean maximum size and mean transparency were lowest in the nearshore upwelling region, increased in the intermediate Cycles 2-3, and decreased again offshore (Fig. 2.10e, 2.10g). The zooplankton community was primarily non-gelatinous across all locations, with a slight increase in of gelatinous taxa beyond the continental shelf (Fig. 2.10h).

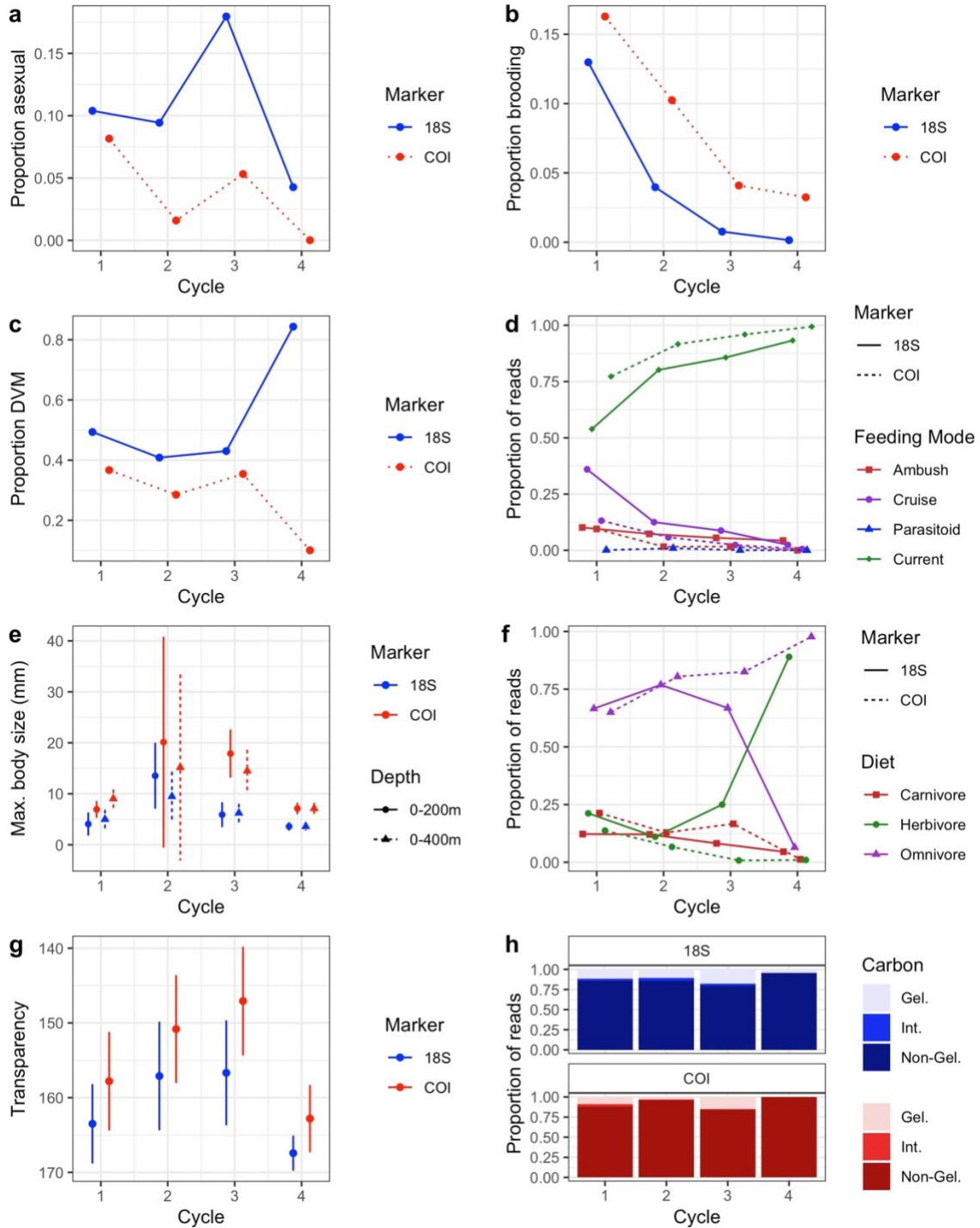


Figure 2.10. Proportions of zooplankton reads that were identified as (a) exhibiting asexual reproduction; (b) brooding gametes or juveniles; (c) exhibiting DVM behavior; (d) exhibiting each feeding strategy; (e) the mean maximum body size, weighted by proportion of reads for each species; (f) proportion of reads with each dietary group; (g) the mean transparency, measured by greyscale; and (h) read proportions by carbon composition. Error bars for (e) and (g) are standard error. Transparency was assessed within the upper 200m at each Cycle, while all other traits included the full extent of the sample (0-200m or 0-400m).

2.4. Discussion

A trait-based analysis of metabarcoding sequences from two marker regions complemented by digital imaging with a ZooScan revealed clear cross-shore trends in community composition and zooplankton vertical habitat use. Our study area extended from the nearshore active upwelling ecosystem, across the California Current, to offshore stratified mesotrophic waters. The 18S and COI markers resolved comparable total richness and similar spatial trends in richness, taxonomic composition, and trait composition. Cross-shore shifts in vertical habitat use were exhibited by individual ASVs and OTUs and across the full zooplankton community, with deeper distributions in the offshore region. Vertical shifts varied between diet modes, and there were cross-shore changes in reproductive strategies, DVM behavior, feeding behaviors, body size, and transparency. As discussed below, species traits are key to understanding the ecological relationship between zooplankton and their environment and to predicting potential effects of ocean changes on zooplankton communities.

2.4.1 *Community composition and species richness*

We observed comparable levels of species richness at 18S and COI, with both communities dominated by calanoid copepods. Previous metabarcoding analyses of metazoans in the California Current Ecosystem (CCE) have reported OTU or ASV counts ranging from 21% to 2700% of the counts we observed (342 to 44,837 at 18S and 1,596 to 21,402 at COI) (Pitz et al. 2020), likely due to bioinformatic differences. The dominance of copepods, malacostracans, cnidarians, and thaliaceans in our study, both in richness and abundance, is a slight departure from morphologically-determined community composition which is dominated by copepods, malacostracans, chaetognaths and tunicates, but not cnidarians (Lavaniegos and Ohman 2007).

The richness of cnidarians in our study reflects the ability to easily detect small, hard to identify specimens through genetic sequencing, as well as the finer mesh size we used (202mm rather than 505mm). We were not able to identify most cnidarian sequences to species, but our results indicate a need for additional reference specimen sequencing, as well as morphological and ecological studies on this group.

Vertical zooplankton species richness patterns appear not to have been previously documented within the CCE. We observed the highest richness within the epipelagic zone, with the depth of the maximum varying between the surface and 150 m, depending on the marker and sampling location. Within copepods and siphonophores, we also observed higher richness in the epipelagic than in the mesopelagic zone. Previous work in other systems has found the maximum in total zooplankton species diversity in the epipelagic (tropical Eastern Pacific, ~50 m), upper mesopelagic (North Pacific Subtropical Gyre, ~400-600 m), and lower mesopelagic (Arctic Basin, 500-1000 m) (Longhurst 1985; Kosobokova and Hopcroft 2010; Sommer et al. 2017). This variability in the depth of the richness maximum reflects the importance of physical environment in structuring zooplankton communities, as the richness maximums across these diverse ecosystems tend to be generally associated with thermoclines.

2.4.2 Vertical habitat use along an environmental gradient

The vertical distribution of many individual OTUs, some complete groups, and the overall zooplankton community shifted to deeper depths in offshore, more transparent, and more stratified waters. Within copepods, similar vertical changes have previously been observed in adult females of *Pleuromamma* species, with vertical distributions shifting deeper in the water column offshore (Haury 1988). Euphausiid species are closely associated with water masses, and

the shifting community composition we observed in the hydrographically complex southern California Current likely reflects the vertical and horizontal water mass structure of the region (Lilly and Ohman 2018). While cross-shore shifts in community composition are well documented within CCE, vertical distributions of eucalanid copepods and siphonophores do not appear to be previously reported from the study region, and the observed cross-shelf species turnover together with species-specific changes in vertical habitat have not previously been reported.

Vertical shifts in mean depth of occurrence were best correlated with optical environment, defined by depth of the 1% light level, highlighting the importance of visual predation risk in shaping species distributions. Optical environment has previously been identified as a predictor of copepod vertical distributions, based on ZooScan imaging and assessment of all copepod species together (Ohman and Romagnan 2016; Robison et al. 2020; Benoit-Bird and Moline 2021; Hobbs et al. 2021). Our metabarcoding analysis finds that vertical habitat use changes within individual species, which were previously unresolved by digital imaging (Ohman and Romagnan 2016). Rather than species turnover along an environmental gradient, we found that individual species alter their vertical habitat. We also observed larger horizontal distributions among taxa with vertically shifting distributions, indicating that active vertical habitat selection can increase the biogeographic range of a species. The strongest cross-shore vertical changes were in the nighttime epipelagic community, the lower boundary of which encroaches on the habitat of the more stable upper mesopelagic community, indicating that changes in epipelagic optical environment can influence vertical distributions deeper in the water column.

2.4.3 *Species traits and vertical habitat use*

In addition to vertical shifts associated with light availability, chlorophyll maximum depth, and hydrographic structure, we also observed taxon-specific variability in vertical shifts, implying that physiological or behavioral differences among species can influence responses to environmental gradients. Cross-shore vertical changes varied among dietary strategies, with stronger vertical descents for carnivores than for herbivores, as well as offshore increases in the relative importance of carnivory and herbivory. While some zooplankton taxa exhibit visual acuity (e.g., heteropods, some pontellid copepods), zooplankton vision is typically restricted to detection of light and movement (Kiørboe 2011) and prey detection by zooplankton largely depends on mechanosensory or chemosensory reception rather than vision (Martens et al. 2015). We did not observe differences in cross-shore vertical habitat among feeding modes, indicating that changes in prey detection potential is unlikely to drive the response of carnivorous taxa to the optical environment. Our analysis focused on zooplankton taxa, but the distributions of many larval fishes closely track the optical environment (Job and Bellwood 2000). It is possible that prey items targeted both by fish and zooplankton are driven deeper in clearer waters due to predation risk from visual predators, and deeper distributions of carnivorous zooplankton are due to non-visual carnivores following potential prey into deeper waters.

Herbivores also appear to be associated with their food source, with vertical distributions associated with the chlorophyll maximum. Additionally, the stronger relationship between MDO and the depth of the chlorophyll maximum at 18S reflects greater proportions of herbivorous taxa at this marker (23% of 18S ASVs, 5% of COI OTUs). Examination of thaliaceans, detected only by 18S, indicate that the three feeding modes we assigned may insufficiently describe the ecology of some taxa. We classified thaliaceans as omnivores, due to their inability to

discriminate among prey caught in feeding webs or mucus nets (Bone 1998). However, vertical distributions of thaliceans were primarily restricted to the upper epipelagic zone, most similar to herbivores.

2.4.4 Trait shifts along an environmental gradient

Offshore, feeding behavior became more heterogeneous, with cruise and ambush comprising 24-48% of the community offshore of the California Current. Increased diversity of feeding modes reflects increasing complexity of zooplankton community structure in the offshore waters, as primary productivity decreases in nutrient-depleted stratified waters and trophic complexity increases (Takimoto and Post 2013). Carnivory increased in the offshore as well, with marker-specific differences in herbivory and omnivory due to muted amplification of herbivorous calanoids at the COI marker. Zooplankton feeding modes and diet have previously been identified as key trait axes that may influence the effects of community change on ecosystem function (Saint-Béat et al. 2022). Our analysis indicates that current feeders are sensitive to ocean stratification, but that carnivores actively shift their vertical distributions and become relatively more abundant in stratified ecosystems.

Brooding behavior increased with distance offshore, detected by both marker regions. Taxa with the potential for asexual reproduction were most abundant in Cycles 1 and 3, and least abundant in nearshore Cycle 4. Together, this suggests that reproductive strategies in productive upwelling waters tend toward production of large numbers energetically inexpensive larvae. In these opaque coastal waters, food availability for juveniles is high, and the relative risk of predation is lower. Offshore, brooding behavior becomes more common, reflecting increased parental investment likely due to greater predation risk to vulnerable larvae and juveniles, as well

as lower probability of mate encounter. Asexual reproduction peaks overall at Cycle 3, which may reflect a community adapted to respond rapidly to transient pulses in food availability due to intermittent upwelling and horizontal transport of chlorophyll-rich coastal waters (Chabert et al. 2021).

Cross-shore trends in DVM behavior differed between the 18S and COI resolved communities and calculation methods. Nearshore, differences between COI and 18S are due to low relative abundances of calanoid copepods in the COI-resolved community. Relative read abundance, a measure of the proportion of migratory biomass, was highly variable between markers, while the number of taxa at each marker exhibiting migratory behavior exhibited similar cross-shore trends. We expected increased DVM behavior in transparent stratified waters where predation risk and prey availability co-occur in surface waters, and we did observe more taxa exhibiting DVM in the offshore stratified waters.

Body size and transparency exhibited complementary cross-shore shifts, with the smallest and darkest animals in productive nearshore waters, larger and more transparent taxa in Cycles 2 and 3, and the offshore community decreasing in size and transparency. In addition to mean maximum size, we examined relative read abundances within 5 size ranges (0-1 mm, 1-2 mm, 2-5 mm, 5-10 mm, >10 mm), and found no significant changes (X^2 (495, N = 208) = 534.35, $p > 0.05$).

Overall, comparisons among the eight traits assessed across two metabarcoding datasets showed differential vertical habitat use among zooplankton with different diets, and revealed cross-shore shifts toward an offshore community with more heterogeneous feeding strategies and increased parental investment. Feeding (or growth), survival, and reproduction are the three fundamental activities each individual must accomplish, and our results highlight the impact of

changing ocean environments on all three mandates (Litchman et al. 2013). We found vertical habitat use is primarily influenced by optical environment, a proxy for predation risk, reflecting pressure to avoid predation. However, differential effects of light between trophic groups reflects differences in prey availability, emphasizing the need to balance predator avoidance with prey availability. In more nutrient limited transition and offshore waters, reproductive strategies shift, with increased brooding and greater potential for asexual reproduction reflecting greater parental investment and decreased rates of mate encounter.

2.4.5 Future priorities

Our results suggest that future oceans will have increased rates of carnivory, cruise feeding, egg brooding, and asexual reproduction, but additional work is needed to identify the relative importance of these traits and to identify potential tradeoffs between them. Continued development of both sequencing databases and trait databases are vital for future analyses of molecular data through a trait-based lens. Reference databases for assigning taxonomy to metabarcoding sequences are vital to linking molecular data with the wealth of ecological observations that exist for many species. Our results also highlight the importance of a comprehensive zooplankton traits database to facilitate analyses of trait interactions and ecological tradeoffs among zooplankton functional groups.

2.4.6 Implications for changing oceans

The observed diet-specific responses to changing water column structure along a gradient of upwelling, stratification, and prey availability indicate that zooplankton responses to ocean warming and stratification will vary among species with different dietary strategies. Cross-shore

shifts in vertical habitat use, DVM behavior, feeding strategy, and reproductive strategy indicate that increased ocean stratification may lead to a zooplankton community that dwells deeper in the water column, with increased carnivory, more heterogeneous feeding strategies, and greater parental investment. The observed changes are concentrated in the epipelagic ocean across the range of environmental conditions we examined, but can extend into the upper mesopelagic. In a space-for-time framework, the environmental gradient across the CCE, ranging from active upwelling to more stratified mesotrophic habitats, indicates that more stratified future oceans will restructure zooplankton community composition and habitat use, and that the effects will vary among functional groups.

Data availability

ZooScan data are available through CCE-LTER DataZoo. Raw 18S and COI sequences are under NCBI SRA PR-JNA679794. Code, ZooScan data, CTD data, and sample metadata is available at <https://github.com/samathews/VerticalHabitatShiftsCCE>.

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Chapter 2, in full, is currently being revised for resubmission for publication of the material in Limnology & Oceanography. Matthews, Stephanie A.; Ohman, Mark D. The dissertation author was the primary researcher and author of this paper.

Author contributions

SAM and MDO designed the research. MDO collected samples. SAM conducted laboratory and statistical analyses. SAM wrote the manuscript. SAM and MDO edited the final version of the manuscript.

Supplemental figures

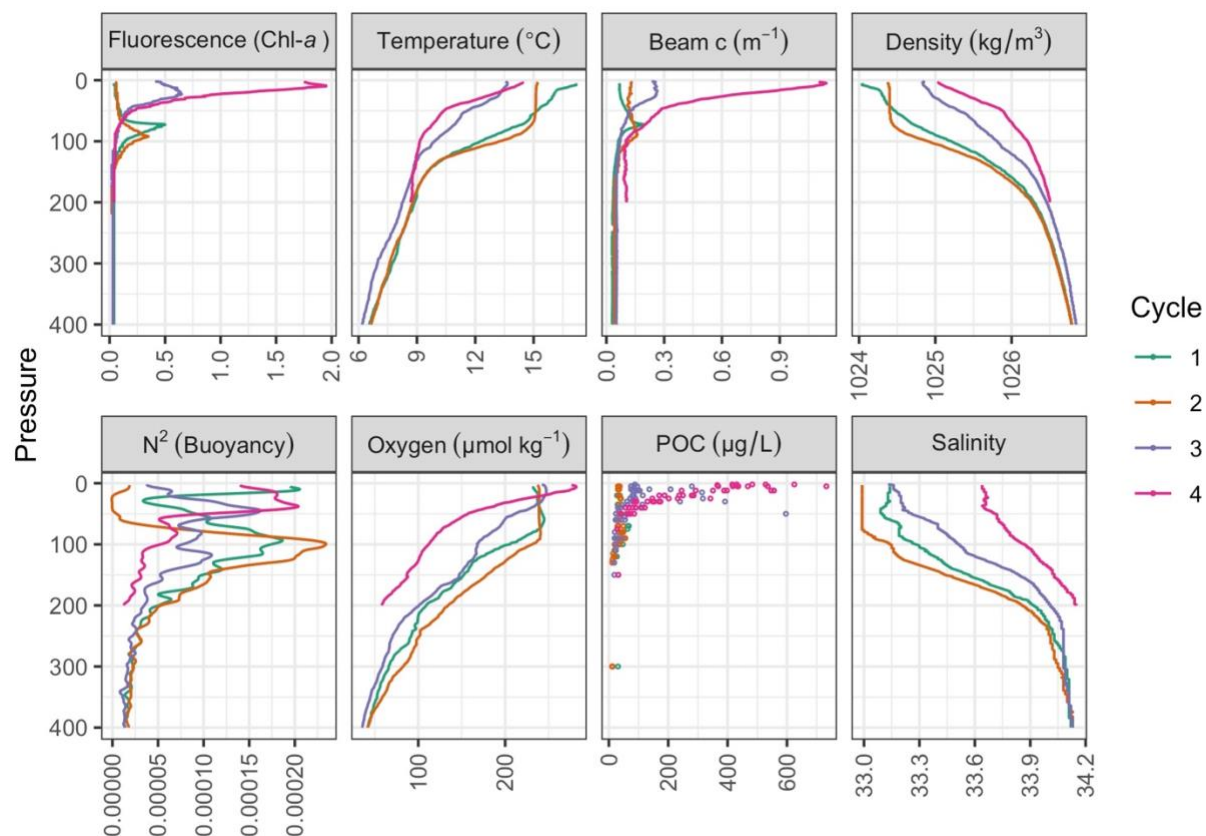


Fig S2.1. Vertical environmental profiles for each of the four Cycles sampled. Profiles for fluorescence, temperature, beam c , density, oxygen, and salinity reflect the average of all unique CTD casts within the Cycle. N^2 profiles were calculated from density and pressure. POC values are all measurements from bottle-collected samples for each Cycle. Beam c is the beam attenuation coefficient, a measure of light extinction.

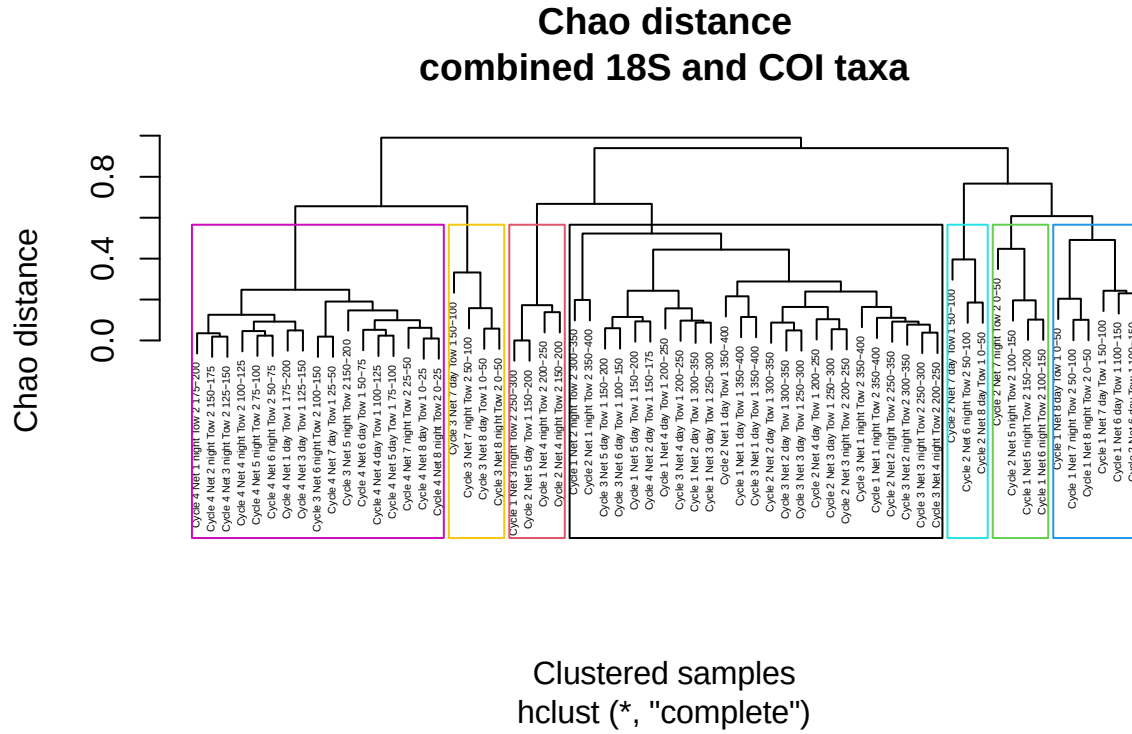


Fig S2.2. All samples clustered based on Chao distance calculated from combined 18S ASVs and COI OTUs. Seven distinct clusters are visible, outlined in colored boxes which correspond to the clusters illustrated in Fig. 3. Samples were clustered using complete linkage.

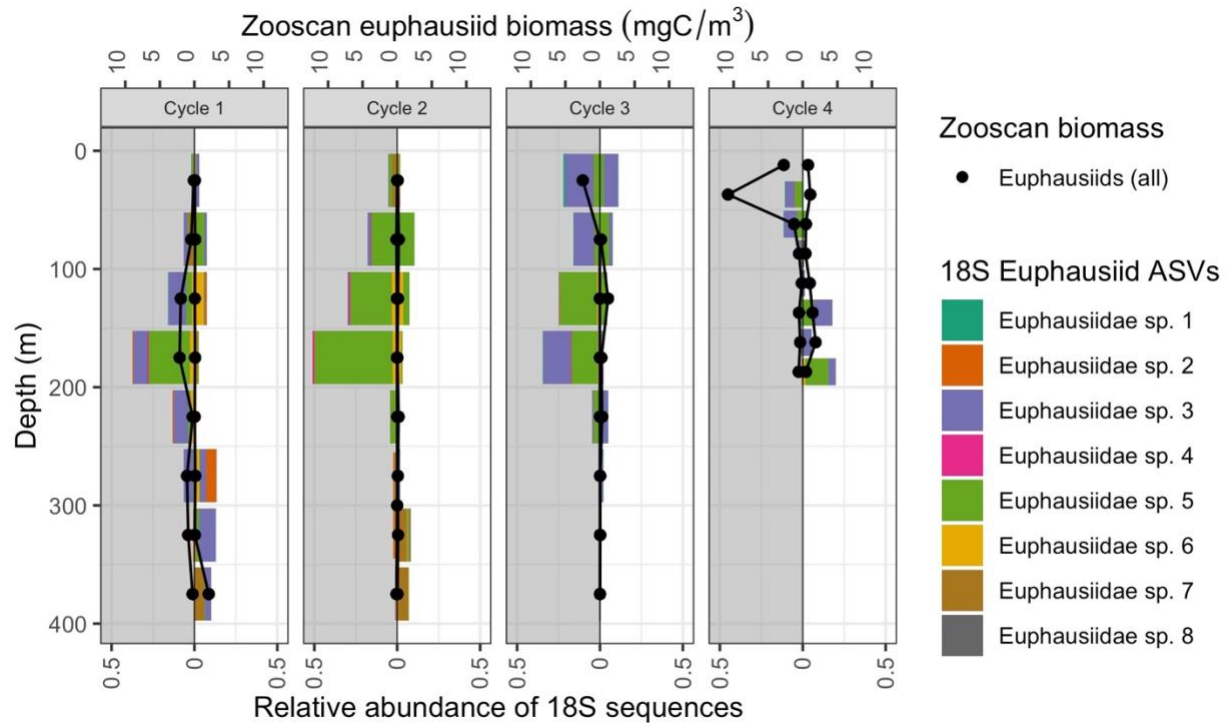


Fig S2.3. Relative abundance of ASVs assigned to the order Euphausiacea (colored bars), none of which were classified to species at 18S, and total euphausiid biomass calculated from digital Zooscan imaging (dots and lines). The cross-shore shift to deeper waters and shifting species composition mirror the patterns observed at COI.

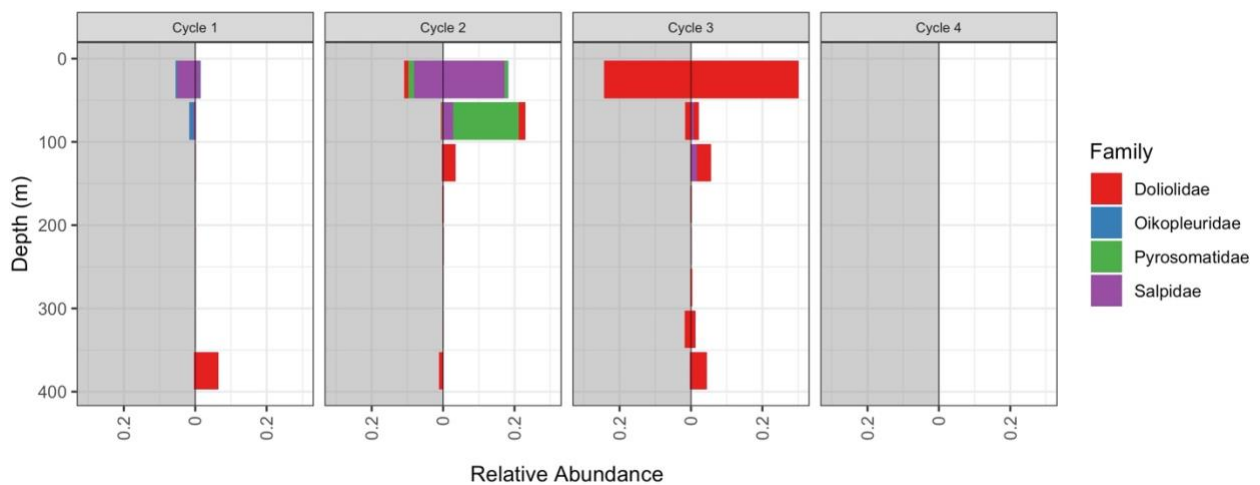


Figure S2.4. The vertical distributions of pelagic tunicates as detected by 18S reads. Tunicates were largely restricted to the surface waters, exhibiting herbivore-like distributions. There is a notable cross-shore difference in the taxonomic composition of the tunicate community, with doliolids dominating nearshore and salps offshore.

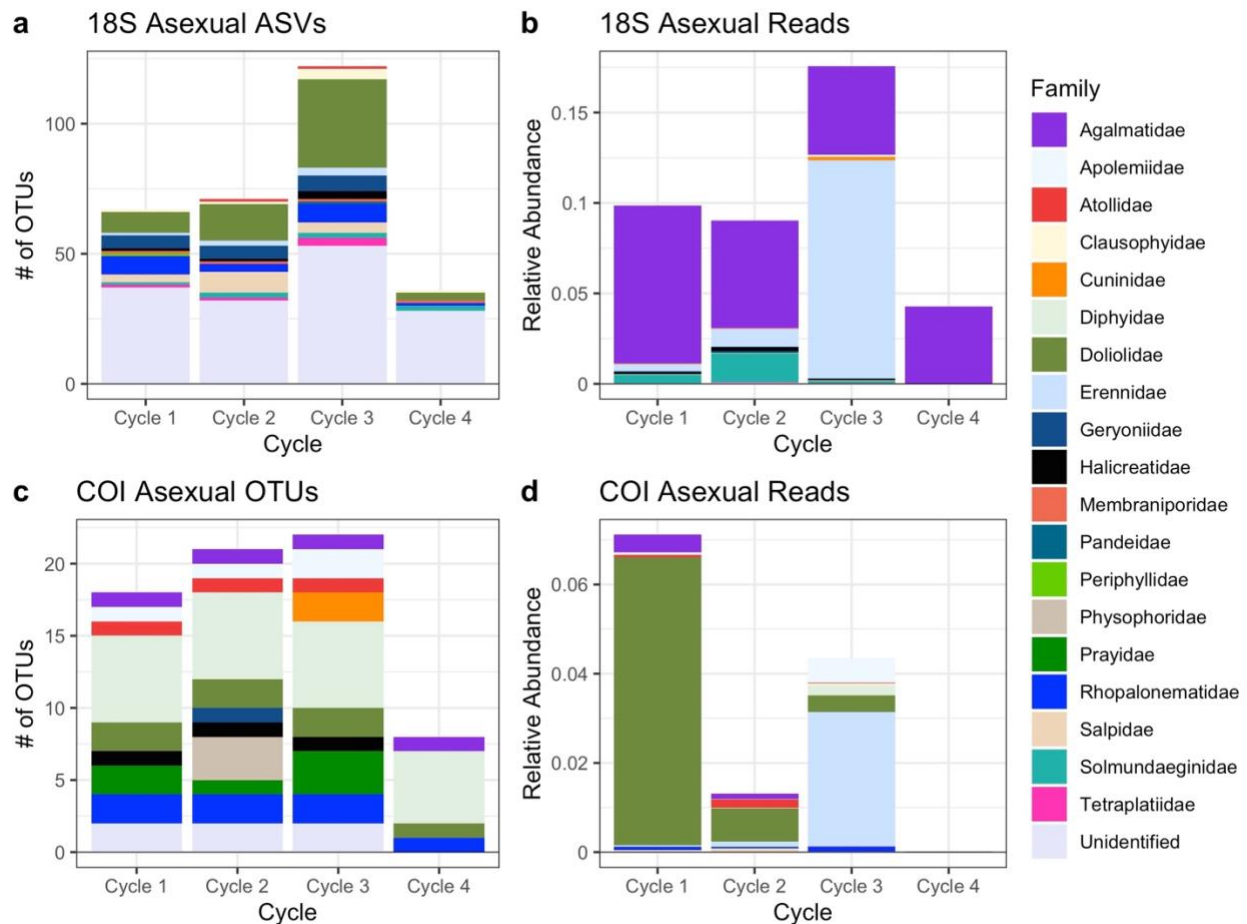


Figure S2.5. Taxa with evidence for asexual reproduction at 18S and COI. ASVs observed within each Cycle, by (a) presence and by (b) relative abundance; and OTUs observed within each Cycle by (c) presence and (d) relative abundance. OTUs are colored based on taxonomic assignment at family level. Presence and relative read abundance were calculated from the total sum of reads across all depths and day and night samples.

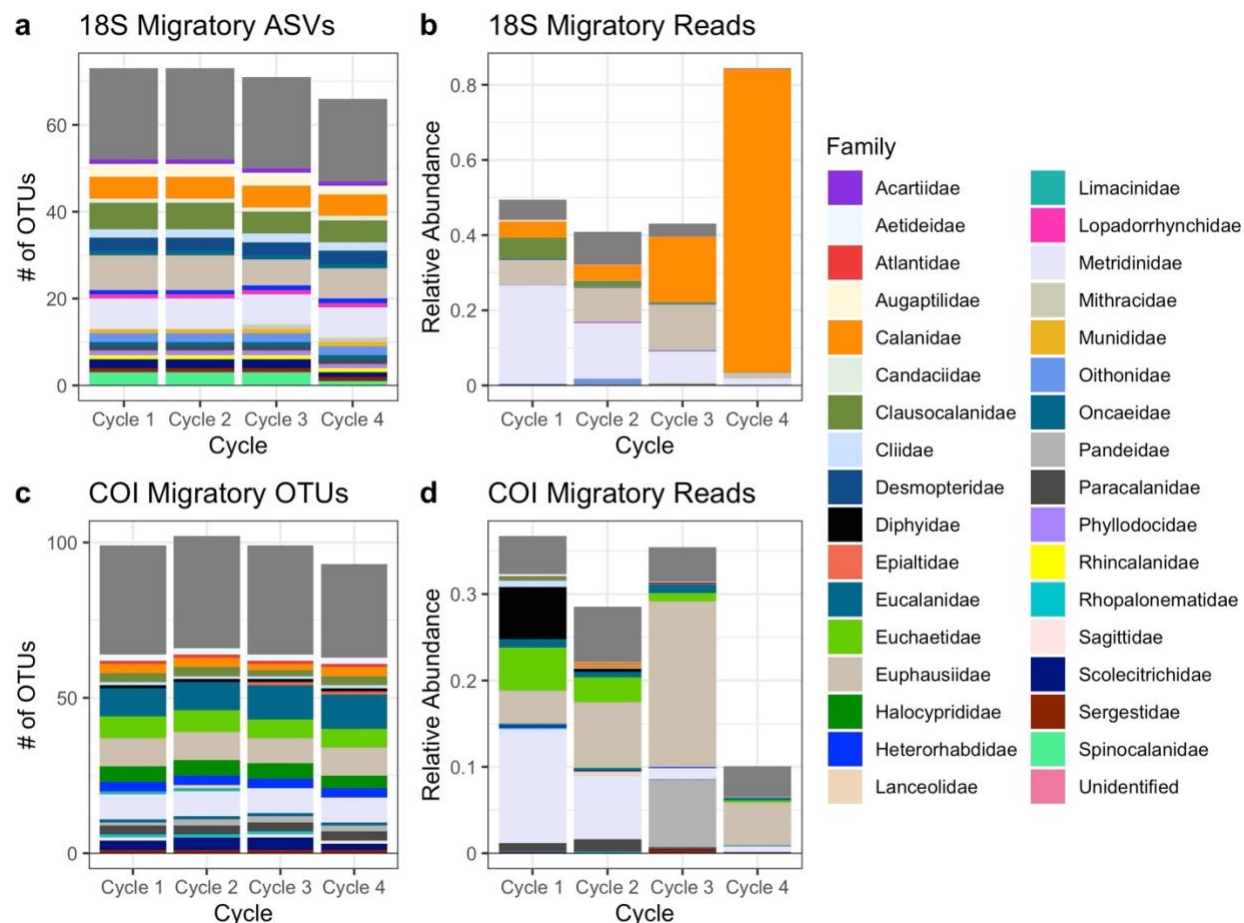


Figure S2.6. Diel vertical migratory ASVs observed within each Cycle, by (a) presence and (b) relative abundance; and diel vertical migratory OTUs observed within each Cycle by (c) presence and (d) relative abundance. OTUs are colored based on taxonomic assignment at family level. Presence and relative read abundance were calculated from the total sum of reads across all depths and day and night samples.

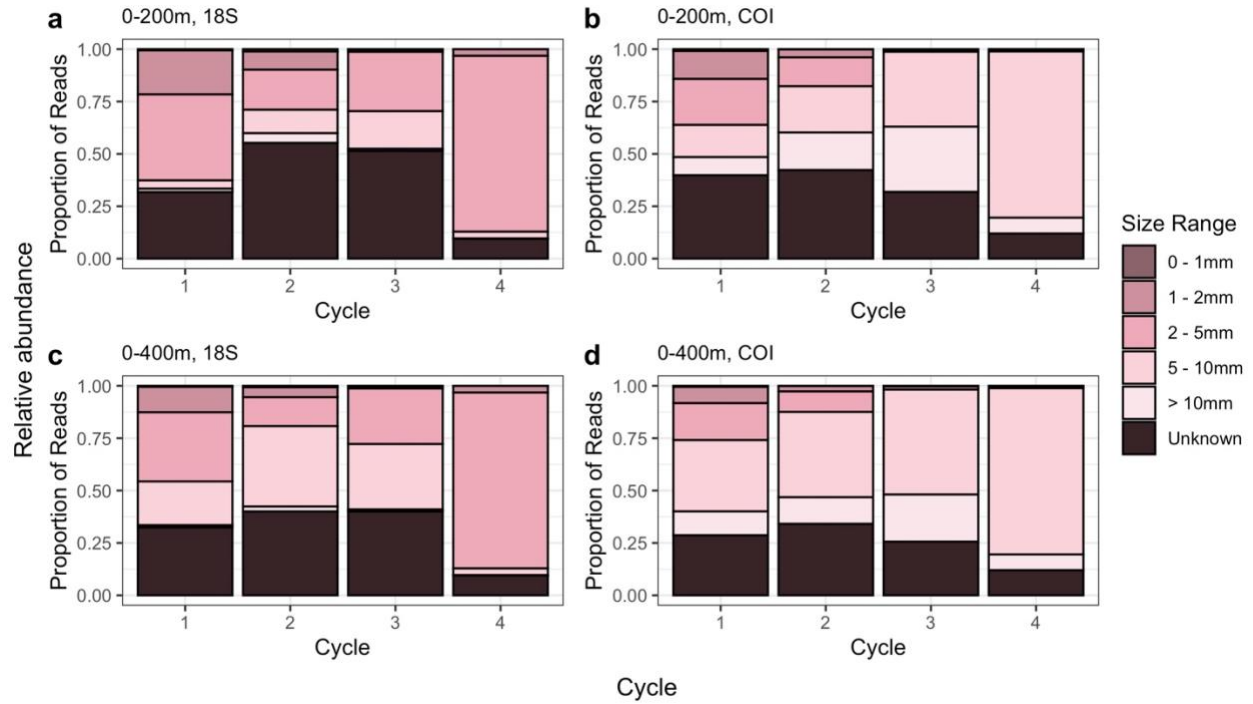


Figure S2.7. The relative abundance of reads within each of 5 bins for maximum size (0-1 mm, 1-2 mm, 2-5 mm, 5-10 mm, >10 mm) and reads with unknown size (unknown). Reads are summed (a, b) within the epipelagic for each Cycle, and (c, d) across the full sampling depth for each Cycle.

Supplemental tables

Table S2.1. The morphologically identifiable taxonomic groups from digital Zooscan images, average carbon biomass of each group across the study samples, and the proportion of the total zooplankton biomass for each group.

Class	Mean Carbon Biomass (mg C m⁻³)	Proportion of Total Biomass
Calanoid Copepods	21.5	0.694
Eucalanid Copepods	4.32	0.139
Euphausiids	1.1	0.0354
Doliolids	0.828	0.0267
Multiples	0.628	0.0202
Chaetognaths	0.589	0.019
Others	0.544	0.0175
Cnidarians	0.286	0.00922
Eggs	0.252	0.0081
Appendicularians	0.243	0.00784
Poecilostomatoid Copepods	0.159	0.00511
Oithona-like Copepods	0.131	0.0042
Nauplii	0.087	0.0028
Pteropods	0.0767	0.00247
Ostracods	0.0745	0.0024
Salps	0.0516	0.00166
Other Copepods	0.0447	0.00144
Polychaetes	0.0411	0.00132
Other Crustaceans	0.0322	0.00104
Pyrosomes	0.0194	0.000623
Harpacticoid Copepods	0.00652	0.00021
Bryozoan Larvae	0.00305	0.0000981
Radiolarians	21.5	0

Table S2.2. The number of Amplicon Sequence Variants (ASVs, 18S) and Operational Taxonomic Units (OTUs, COI) exhibiting cross-shore vertical shifts in mean depth of occurrence, as well as the total number of amplicons within each phylum and within orders for arthropods and cnidarians. Vertical shifts were defined as a change of at least 50 meters deeper in offshore between consecutive Cycles, or at least 100m deeper from Cycle 3 to Cycle 1.

Phylum (Order)	18S ASVs			COI OTUs		
	Shifting (No., %)		Total	Shifting (No., %)		Total
Unknown Animalia	11	8.3%	133	33	6.1%	541
Annelida	14	9.9%	141	0	0.0%	12
Arthropoda	92	9.5%	970	112	18.5%	607
Calanoida	67	8.9%	750	60	21.2%	283
Cyclopoida	7	12.3%	57	1	33.3%	3
Decapoda	2	7.1%	28	4	6.7%	60
Euphausiacea	13	13.5%	96	11	26.2%	42
Harpacticoida	1	25.0%	4	-	-	-
Lepadiformes	1	20.0%	5	-	-	-
Lophogastrida	-	-	1	-	-	1
Mormonilloida	-	-	1	-	-	-
Mysida	-	-	4	-	-	-
Onychopoda	-	-	1	-	-	-
Sessilia	-	-	3	-	-	2
Chordata	4	5.3%	75	0	0.0%	3
Cnidaria	28	10.0%	280	15	18.3%	82
Actiniaria	1	2.8%	36	-	-	1
Coronatae	1	50.0%	2	1	100.0%	1
Narcomedusae	2	28.6%	7	1	33.3%	3
Penicillaria	1	100.0%	1	1	100.0%	1
Scleractinia	1	11.1%	9	-	-	-
Siphonophorae	6	8.1%	74	2	8.7%	23
Trachymedusae	7	33.3%	21	1	25.0%	4
Alcyonacea	-	-	1	-	-	-
Anthoathecata	-	-	22	-	-	8
Corallimorpharia	-	-	2	-	-	-
Leptothecata	-	-	2	1	50.0%	2
Spirularia	-	-	1	-	-	-
Zoantharia	-	-	1	-	-	-
Ctenophora	2	18.2%	11	0	0.0%	1
Mollusca	9	21.4%	42	3	16.7%	18
Sipuncula	1	16.7%	6	0	0.0%	1
Bryozoa	0	0.0%	1	0	-	0
Nemertea	0	0.0%	4	1	16.7%	6
Platyhelminthes	0	0.0%	2	0	0.0%	5
Chaetognatha	0	-	0	1	16.7%	6
Total	161	9.7%	1667	165	12.9%	1282

Table S2.3. Model weights for comparison of linear models for six environmental variables. Italicized values denote the best model. Models were ranked within each marker and depth range (rows) based on Akaike Information Criteria. Vertical profiles for each environmental variable are shown in Fig S1. Depth of the 1% light level was calculated from light extinction in the upper 50m.

Marker	Depth	Depth of 1% Light Level	Depth of Chlorophyll Maximum	Depth of Pycnocline (Maximum N ²)	Depth of Particle Maximum	Depth of 100um/kg Oxygen	Depth of Thermocline
18S	0-200m	0	<i>0.991</i>	0	0.009	0	0
	0-400m	0.255	0.104	<i>0.317</i>	0.106	0.113	0.105
COI	0-200m	<i>1</i>	0	0	0	0	0
	0-400m	<i>0.863</i>	0	0.136	0	0	0
Sum of weights		2.118	1.095	0.453	0.115	0.113	0.105
Percent of total		53%	27%	11%	3%	3%	3%

Table S2.4. The number of ASVs (18S) in each taxonomic class that exhibit each trait mode for all categorical traits. Traits were assigned based on the best taxonomic identification available (Table S1) according to published databases, published data for individual species and taxonomic groups, and expert judgement. See Methods section for full explanations of each trait mode. *(Table continued on next page)*

		Diet			Feeding Mode				Spawning Strategy			Asexual Reproduction	
	Total # ASVs (18S)	Carni-vore	Herbi-vore	Omni-vore	Ambush Feeder	Cruise Feeder	Parasitoid	Current Feeder	Broadcast	Brooding	Absent	Present	
Anthozoa	182	-	-	-	-	-	-	-	42	-	-	-	
Appendicularia	84	-	-	-	-	-	-	-	84	-	-	-	
Branchiopoda	14	-	-	14	-	-	-	14	14	-	14	-	
Cephalopoda	1	-	-	-	-	-	-	-	-	-	-	-	
Gastropoda	5	5	-	-	-	-	-	-	-	-	5	-	
Gastropoda	37	6	-	7	-	6	-	7	13	-	35	-	
Gymnolaemata	1	-	-	-	-	-	-	-	1	-	-	1	
Hexanauplia	828	61	384	184	21	111	-	410	515	135	824	-	
Hoploneurtemtea	1	1	-	-	-	-	-	-	1	-	1	-	
Hydrozoa	152	152	-	-	73	49	-	-	152	-	6	116	
Malacostraca	134	1	3	16	-	1	-	100	15	5	134	-	
Multicrustacea	2	-	-	-	-	-	-	-	-	-	2	-	
Palaeonemertea	1	1	-	-	-	-	-	-	1	-	1	-	
Phascolosomatidea	6	-	-	-	-	-	-	-	-	-	-	-	
Pilidiophora	2	2	-	-	-	-	-	-	2	-	2	-	
Polychaeta	141	54	-	-	-	39	-	-	36	-	40	-	
Scyphozoa	2	2	-	-	2	-	-	-	2	-	-	2	
Tentaculata	11	11	-	-	-	-	-	5	11	-	11	-	
Thaliacea	61	-	-	61	-	-	-	61	61	-	9	52	
Total	1665	296	387	282	96	206	0	597	950	140	1084	171	

Table S2.4. (continued) The number of ASVs (18S) in each taxonomic class that exhibit each trait mode for all categorical traits. Traits were assigned based on the best taxonomic identification available (Table S1) according to published trait databases, published data for individual species and taxonomic groups, and expert judgement. See Methods section for full explanations of each trait mode.

Class	Total # ASVs (18S)	DVM Behavior		Carbon			Transparency				
		Migratory	NonMigratory	Gelatinous	Intermediate	Nongelatinous	1	2	3	4	5
Anthozoa	182	3	179	3	179	7	-	-	-	-	-
Appendicularia	84	1	83	1	83	-	-	-	-	-	-
Branchiopoda	14	-	14	-	14	-	-	-	-	14	-
Cephalopoda	1	-	1	-	1	1	-	-	1	-	-
Gastropoda	5	-	5	-	5	-	-	-	-	-	-
Gymnolaemata	37	7	30	7	30	-	-	35	-	-	-
Hexanauplia	1	-	1	-	1	-	-	-	-	-	-
Hoploneurtea	828	46	782	46	782	824	-	-	-	816	-
Hydrozoa	1	-	1	-	1	-	1	-	-	-	-
Malacostraca	152	4	148	4	148	-	-	-	-	131	21
Multicrustacea	134	11	123	11	123	134	-	134	-	-	-
Palaeonemertea	2	-	2	-	2	2	-	2	-	-	-
Phascolosomatidea	1	-	1	-	1	-	1	-	-	-	-
Pilidiophora	6	-	6	-	6	-	6	-	-	-	-
Polychaeta	2	-	2	-	2	-	2	-	-	-	-
Scyphozoa	141	5	136	5	136	-	141	-	-	-	-
Tentaculata	2	-	2	-	2	-	-	-	2	-	-
Thaliacea	11	-	11	-	11	-	-	-	-	11	-
	61	-	61	-	61	-	-	-	-	61	-
Total	1665	77	1588	77	1588	968	151	171	3	1033	21

Table S2.5. The number of OTUs (COI) in each taxonomic class that exhibit each trait mode for all categorical traits. Traits were assigned based on the best taxonomic identification available (Table S1) according to published databases, published data for individual species and taxonomic groups, and expert judgement. See Methods section for full explanations of each trait mode. (*Table continued on next page*)

Class	Total # OTUs (COI)	Diet			Feeding Mode				Spawning Strategy		Asexual Repro.	
		Carni-vore	Herbi-vore	Omni-vore	Ambush Feeder	Cruise Feeder	Parasitoid	Current Feeder	Broadcast	Brooding	Absent	Present
Anthozoa	686	1	-	-	-	-	-	-	19	-	1	-
Asciacea	2	-	-	-	-	-	-	1	2	-	-	2
Cephalopoda	1	-	-	1	-	-	-	1	1	-	1	-
Gastropoda	17	1	-	-	-	-	-	-	-	-	1	-
Hexanauplia	299	4	-	3	-	3	-	3	6	-	15	-
Hoploneurtea	1	50	56	114	11	32	-	136	178	43	296	-
Hydrozoa	61	1	-	-	-	-	-	-	1	-	1	-
Malacostraca	159	61	-	-	20	10	-	-	61	-	2	28
Ostracoda	26	38	6	25	-	-	22	43	40	33	159	-
Palaeonemertea	3	-	-	25	-	-	-	25	25	-	25	-
Phascolosomatidea	1	3	-	-	-	-	-	-	3	-	3	-
Pilidiophora	1	-	-	-	-	-	-	-	-	-	-	-
Polychaeta	12	1	-	-	-	-	-	-	1	-	1	-
Sagittoida	6	7	-	-	-	-	-	-	-	-	-	-
Scyphozoa	1	6	-	-	6	-	-	-	6	-	6	-
Tentaculata	1	1	-	-	1	-	-	-	1	-	-	1
Thaliacea	2	1	-	-	-	-	-	-	1	-	1	-
		-	-	2	-	-	-	2	2	-	-	2
Total	1280	175	62	170	38	45	22	211	347	76	512	33

Table S2.5. (continued) The number of OTUs (COI) in each taxonomic class that exhibit each trait mode for all categorical traits. Traits were assigned based on the best taxonomic identification available (Table S1) according to published trait databases, published data for individual species and taxonomic groups, and expert judgement. See Methods section for full explanations of each trait mode.

Class	Total # OTUs (COI)	DVM Behavior		Carbon			Transparency				
		Migratory	NonMigratory	Gelatinous	Intermediate	Nongelatinous	1	2	3	4	5
Anthozoa	686	26	660	19	-	123	4	-	-	-	-
Asciacea	2	-	2	2	-	-	-	-	-	-	-
Cephalopoda	1	-	1	1	-	-	-	-	-	1	-
Gastropoda	1	-	1	-	1	-	-	-	-	-	-
Hexanauplia	17	3	14	-	15	-	-	14	-	-	-
Hoploneurtea	299	53	246	-	-	299	-	-	-	296	-
Hydrozoa	1	-	1	1	-	-	1	-	-	-	-
Malacostraca	61	4	57	61	-	-	-	-	-	57	4
Ostracoda	159	15	144	-	-	159	20	139	-	-	-
Palaeonemertea	26	7	19	-	-	25	25	-	-	-	-
Phascolosomatidea	3	-	3	3	-	-	3	-	-	-	-
Pilidiophora	1	-	1	-	-	-	1	-	-	-	-
Polychaeta	12	-	12	-	-	-	12	-	-	-	-
Sagittroidea	6	1	5	-	6	-	-	-	6	-	-
Scyphozoa	1	-	1	1	-	-	-	-	-	1	-
Tentaculata	1	-	1	1	-	-	-	-	-	-	-
Thaliacea	2	-	2	2	-	-	-	-	-	1	-
Total	1280	109	1171	92	34	606	67	153	7	357	4

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CHAPTER 3

Divergent patterns of zooplankton connectivity in the epipelagic and mesopelagic zones of the eastern North Pacific

Abstract

Due to historical under-sampling of the deep ocean the distributional ranges of mesopelagic zooplankton are not well documented, leading to uncertainty about the mechanisms that shape midwater zooplankton community composition. We characterized zooplankton diversity and community composition in the upper 1000m of the northeast Pacific Ocean using DNA metabarcoding of two markers (18S-V4 and mtCOI). We tested whether the North Pacific Transition Zone is a biogeographic boundary region for mesopelagic zooplankton. We also tested whether zooplankton taxa occupying different vertical habitats and exhibiting different ecological traits differed in the ranges of temperature, Chl-*a*, and dissolved oxygen conditions inhabited. The depth of the maximum taxonomic richness deepened with increasing latitude in the North Pacific. Community similarity in the mesopelagic zone also increased in comparison with the epipelagic zone, and no evidence was found for a biogeographic boundary between previously delineated mesopelagic biogeochemical provinces. Epipelagic zooplankton exhibited broader temperature and Chl-*a* ranges than mesopelagic taxa. Within the epipelagic, taxa with broader temperature and Chl-*a* ranges also had broader distributional ranges. However, mesopelagic taxa were distributed across wider dissolved oxygen ranges, and within the mesopelagic only oxygen ranges covaried with distributional ranges. Environmental and distributional ranges also varied among traits both for epipelagic taxa and mesopelagic taxa. The

strongest differences in both environmental and distributional ranges were observed for taxa with or without diel vertical migration behavior. Our results suggest that species traits can influence the differential effects of physical dispersal and environmental selection in shaping biogeographic distributions.

3.1. Introduction

The mesopelagic zone of the ocean is understudied compared to the epipelagic zone. Despite growing interest in the deep ocean, biodiversity and ecosystem structure in the mesopelagic zone are not well documented. Analyses of environmental climatology have defined thirteen mesopelagic biogeochemical provinces with variable vertical extents, most of which occur in multiple ocean basins (Reygondeau et al., 2018). An alternative classification of mesopelagic biogeography used a combination of water masses, environmental climatology, and biotic partitioning to define thirty-three mesopelagic ecoregions (Sutton et al., 2017). While the biogeographic distributions of pelagic species do not always map cleanly onto biogeochemical provinces, there tend to be distinct pelagic communities in different provinces even when individual species are distributed across multiple provinces (Reygondeau & Dunn, 2018). Despite the disparity in total number of provinces, there is good agreement in the location of mesopelagic biogeographic and biogeochemical boundaries in the North Pacific (Reygondeau et al., 2018; Sutton et al., 2017). The basin-scale structure of mesopelagic biogeochemical provinces is broadly similar to that of overlying epipelagic biogeochemical provinces, but the biogeochemical boundaries in the mesopelagic zone may be more permeable to species distributions than observed in the epipelagic zone (Longhurst, 2007; Sutton et al., 2017).

In the North Pacific, differences in epipelagic zooplankton community composition are well documented among biogeochemical provinces, with many zooplankton species restricted to the North Pacific Transition Zone (NPTZ), the Subpolar gyre, or the Subarctic gyre (Costello et al., 2017; McGowan & Williams, 1973). Within the epipelagic zone, the NPTZ, which coincides with the eastward flowing West Wind Drift, is a distinct biogeochemical province and is home to a characteristic zooplankton community with moderate levels of endemism (Pearcy, 1991). This community is supported by enhanced primary production due to mesoscale mixing within this hydrodynamically complex region (Miyamoto et al., 2022). Within the mesopelagic zone there are also distinct Subarctic and Subtropical Provinces, but no intervening Transition Province has been recognized (Reygondeau et al., 2018; Sutton et al., 2017). The degree to which the border between Subarctic and Subtropical biogeochemical provinces at midwater depths acts as a biogeographic boundary for mesopelagic zooplankton is unknown, as are the mechanisms by which species distributions might be limited in this region.

Among global ocean biogeochemical provinces, the extent of differentiation between zooplankton communities can vary. Physical dispersal by ocean circulation can increase connectivity among planktonic communities, and genetic breaks in population connectivity often co-occur with hydrographic barriers to dispersal (Cornils et al., 2017; González et al., 2020). Physical circulation and mixing are slower in the mesopelagic zone than in the epipelagic zone (Kawabe & Fujio, 2010; Reid et al., 1978), which could decrease connectivity and promote geographic divergence among zooplankton communities. However, biogeographic distributions are also shaped by habitat suitability (Goetze et al., 2017). While physical transport can introduce expatriates into new locations, in suboptimal habitats these individuals may not establish autochthonous populations. Compared to the epipelagic zone, the mesopelagic zone has

significantly lower variability in temperature and food availability, particularly at basin scales and after averaging short-term mesoscale variability (Robinson et al., 2010). Dissolved oxygen and pH may be the exception to this rule, with significant oxyclines occurring within the mesopelagic zone in many pelagic environments. However, the overall greater environmental homogeneity in the mesopelagic zone leads to a contrasting prediction: that there could be broader species distributions and greater community similarity at mesopelagic than epipelagic depths.

The biogeographic distributions of mesopelagic zooplankton will thus reflect the combined influences of greater environmental homogeneity and decreased physical mixing relative to the surface ocean. The relative importance of each can be modified by species-specific physiological tolerances and behavior. Differences among taxa in ecological strategies and environmental ranges tolerated may lead to variability in the effects of dispersal and environmental heterogeneity. In the trait-based approach, species-specific traits that influence a species' ability to feed, survive predation, or reproduce can be used to identify potential trade-offs in ecological strategies among taxa (Litchman et al., 2013). Individual characteristics such as body size can be compared across taxa to identify emergent general principles (Ohman & Romagnan, 2016), or combinations of traits can be used to identify functional groups that share ecological strategies and have similar ecosystem function (Venello et al., 2021). Within zooplankton, trait-based analyses have primarily been applied to copepods, leading to characterization of functional groups that have similar ecological responses to environmental changes, and have identified latitudinal gradients in feeding behavior, diet, and body size, among other traits (Benedetti et al., 2021; Brun et al., 2016). Trait-based analyses can be strengthened

by comparing traits across multiple major taxonomic groups, expanding the range of trait states considered.

DNA metabarcoding uses targeted amplicon sequencing of bulk zooplankton samples to characterize zooplankton community composition (Bucklin et al., 2016). Primers are designed to amplify sequences from multiple species at specific marker regions, and so-called ‘universal primers’ can resolve a broad array of taxonomic groups. The inclusion of multiple marker regions can further increase taxonomic breadth and resolution (Questel et al., 2021). While primer biases can lead to skewed representations of total community composition, comparison of read proportions across samples can reasonably approximate changes in species proportions (Matthews et al., 2021). Detection sensitivity can be quite high, making metabarcoding particularly useful for inclusion of rarer species. When used in combination with reference sequence databases and trait databases, metabarcoding sequences can be identified taxonomically and then linked to species traits.

Here, we analyze zooplankton community composition between the surface and 1000m in the Subarctic Gyre, the NPTZ, and the Subtropical Gyre, in order to compare epipelagic and mesopelagic biogeographic distributions across this region. We test 1) whether the North Pacific Transition Zone is a biogeographic boundary region for mesopelagic zooplankton communities, 2) whether epipelagic and mesopelagic taxa have different biogeographic and environmental ranges in the Northeastern Pacific Ocean, and 3) whether biogeographic and environmental ranges vary among zooplankton with different traits.

3.2. Materials and methods

3.2.1. Sampling

Samples were collected in the Northeast Pacific Ocean between 8 August – 18 September, 2012 onboard the R/V *New Horizon* (Fig. 3.1). At each sampling location, paired day and net MOCNESS tows (Wiebe et al., 1985) were conducted targeting the following 8 depth strata: 1000-800m, 800-600m, 600-400m, 400-200m, 200-100m, 100-50m, 50-25m, and 25-0m. Net mesh size was 150 μm and net area 1m². Upon recovery, samples were held on ice, and large fish were fixed and stored separately. On some samples, a few (less than 10 per sample; in general one per sample) pteropods and euphausiids were removed before splitting and preservation (see <https://www.bco-dmo.org/dataset/3546>). The sample was then quantitatively split with a Motoda box-splitter. One half of the sample was preserved in 95% ethanol, one quarter in 5% buffered formalin, and one quarter in 70% non-denatured ethanol.

At each sampling location, hydrographic profiles were collected as a part of standard casts with a CTD rosette. The rosette included sensors for temperature, conductivity, and depth (DigiQuartz), as well as for dissolved oxygen (SBE43, SeaBird Electronics), a transmissometer (Wet Labs C*, 660nm wavelength), and a chlorophyll-*a* fluorometer (Wet Labs ECO-AFL). Vertical profiles were collected on continuous downcasts (to 1000m), and all data averaged into 1-meter vertical bins for subsequent calculations of environmental ranges and environmental means in discrete MOCNESS sampling depths.

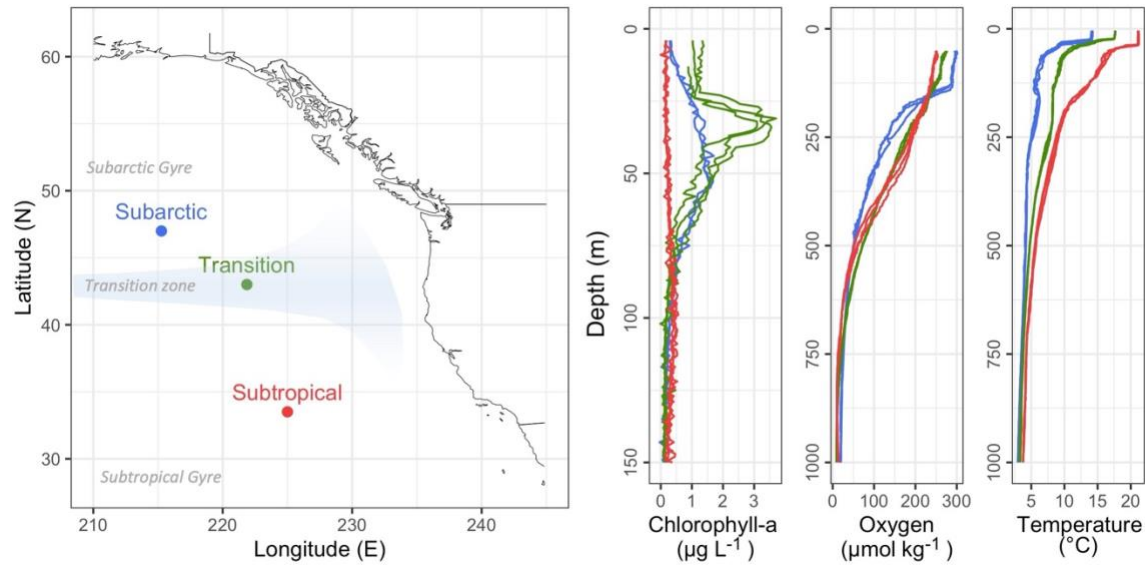


Figure 3.1. Locations of MOCNESS tows in the Northeast Pacific, and CTD profiles of chlorophyll-a fluorescence, temperature, and dissolved oxygen at each of the sampling locations (red: Subarctic Province; blue: Transition Province; green: Subtropical Province). Major oceanographic regions are labelled in grey. Chl-a fluorescence profiles are shown from 0-150m, and oxygen and temperature profiles from 0-1000m. Ocean circulation features from Reid et al. 1978.

3.2.2. Extraction, amplification, and sequencing

DNA extraction followed previously published protocols for ethanol-preserved bulk zooplankton tissue (Blanco-Bercial, 2020). In short, after removal of ethanol and milliQ washing, the zooplankton pellet was weighted and transferred to 15 - 25 mL of SDS buffer (Tris-HCl 10 mM, EDTA 100 mM pH 8.0, NaCl 200 mM, SDS 1 %). Samples were vortexed to break up the pellet and then ground using a Fisher Scientific Homogenizer FSH125. After the grinding protocol, proteinase K (Sigma-Aldrich; St. Louis, MO) was added to the buffer (0.2 mg mL⁻¹ final concentration), as well as 5 mL of sterilized stainless-steel Shot, 3/32" Ellipse (RioGrande, CA). The tubes were incubated at 60° C for four hours, with vortexing every 30 min, then centrifuged to pellet the tissue. Three (pseudo)replicates of 400 µL of the supernatant were transferred to 1.5 mL Eppendorf tubes. After this step, DNA was extracted following the

E.Z.N.A.® Mollusc DNA Kit (Omega Bio-tek, Norcross, GA), from the addition of chloroform:Isoamyl alcohol 24:1 step onward.

Concentrations of extracted DNA were normalized in deionized water to concentrations of 4 ng/μl. Each sample was amplified in triplicate using the 18S-V4 (primers Uni18S/Uni18SR) (Zhan et al., 2013) and mtCOI (primers mlCOIintF/jgHCO2198) (Leray et al., 2013) marker regions, with two-step PCR and library preparation as described previously (Matthews et al., 2021). Duplicate dual indexes were used for each sample, with independent indexes for PCR replicates. All PCR reactions were cleaned with AMPure XP magnetic beads (Beckman Coulter), then DNA concentrations were quantified with Picogreen assays. Samples were pooled in equimolar concentrations within each marker region, with 20% higher concentrations for the 18S marker to compensate for preferential sequencing of the shorter COI sequences. The pooled library was sequenced on an Illumina MiSeq with 300bp paired end V3 chemistry.

3.2.3. Bioinformatic processing

Bioinformatic processing followed previously described pipelines (Matthews et al., 2021). In brief, samples were demultiplexed based on unique combinations of forward and reverse indexes, with only sequences with perfect matches to all four indexes retained. Demultiplexed sequences were visually assessed for quality, then trimmed and denoised into ASVs using *dada2* in QIIME2 (Bolyen et al., 2019). COI data were subsequently clustered into OTUs at 97% similarity using *vsearch* in QIIME2. Sequences recovered from negative controls were used to removal potential contaminants through the R package *decontam* (Davis et al., 2018). Technical replicates were visually assessed based on total richness, taxonomic composition, and nMDS clustering. As all replicates with successful PCRs were similar,

replicates for each unique biological sample were merged by summing the total reads from each replicate. Taxonomic assignments for 18S ASVs and COI OTUs were made with the *sklearn* classifier implemented in QIIME2. The classifier was trained on a custom database created from the global MetaZooGene COI and 18S databases (Bucklin et al., 2021), supplemented with 242 COI sequences and 283 18S sequences collected from 53 zooplankton species identified from the southern California Current System (Appendix B). Prior to classifier training, all sequences were trimmed with appropriate primers using virtual PCR implemented with the R package *insect*, with primer regions retained. Taxonomic assignments were retained if they had at least 80% confidence levels. All species-level classifications were manually compared against previously described biogeographic distributions for the species (Horton et al., 2022; Razouls et al., 2005; *The Deep-Sea Guide (DSG)*, 2023). If sequences were identified as a species that does not occur in the North Pacific, the taxonomic assignment was only retained at the genus level. Sequences unidentified to phylum were removed from the analysis, as were all protistan, mammal, and fish sequences.

3.2.4. Trait assignment

Traits were mapped to taxonomic assignments as previously described (Appendix A) with traits defined both by taxon-specific trait databases (Benedetti et al., 2016; Brun et al., 2017) and from the primary literature. The following traits were assigned where possible: body size (maximum reported for the species), diet (carnivore, omnivore, or herbivore; detritivores were included as omnivores), feeding behavior (ambush, cruise, parasitoid, or suspension), spawning strategy (broadcast spawning or brooding), presence of asexual reproduction (yes, no), DVM behavior (yes, no), and body composition (gelatinous, intermediate, or nongelatinous).

For species with spatial or temporal plasticity (e.g. species with a preference for herbivory but the capacity for omnivory), we used the most inclusive trait. For each unique taxon that we observed, we assigned all traits that were possible for the specificity of the taxonomic assignment. For genera and families with consistent traits among all species, traits were assigned even if the amplicon was unidentified at species level; for taxonomic groups with variable traits, that trait was not assigned, and the amplicon was not included in trait-based comparisons.

3.2.5. Statistical analyses

All statistical analyses were conducted in R 4.2.1 (2022-06-23), primarily using base R stats as well as the packages *vegan*, *phyloseq*, *ggpubr*, and *tidyverse*. To facilitate comparisons between samples with variable sequencing depths, ASV and OTU tables were converted to relative abundance but were not rarefied (McMurdie & Holmes, 2014, but see also Schloss, 2020). Taxonomic richness for each region and taxon biogeographic ranges were calculated from presence, with parallel analyses for rarefied and non-rarefied data. Mean depth of occurrence (MDO) was calculated for each taxon by averaging net midpoints for all nets in which the taxon was observed, weighted by relative read abundance within the net. MDO was calculated independently for each MOCNESS tow, as well as overall across all sampling locations. Taxon body size and MDO were tested for normality using Shapiro-Wilk tests and found to be non-normally distributed even after logarithmic or square-root transformations, so correlations of untransformed data were tested using ties-corrected non-parametric Spearman rank correlation. To visualize community similarity among samples, non-metric multidimensional scaling was used as amplicon data are non-normally distributed and relative abundances are not independent of each other (Ramette, 2007). Beta-dispersion was calculated from Bray-Curtis distance and

Jaccard distance, and within-group dispersions for epipelagic and mesopelagic samples were compared with a one-way analysis of variance (Anderson et al., 2006).

All CTD values from each net depth range within a province were averaged to describe the mean conditions. The environmental range inhabited by each taxon was calculated from the difference between the maximum and minimum of the mean environmental property for the nets in which the taxon occurred. To allow for uneven sample sizes among groups and non-normal distributions of environmental ranges, non-parametric Kruskal-Wallis rank sum tests were used to test for differences in environmental and biogeographic ranges. For comparisons with more than two groups, significant differences between groups were identified with two-sided post-hoc Dunn's test of multiple comparisons with Bonferroni adjustment for family-wise error rate. For ease of interpreting visualizations, ranges were plotted with 20% scatter around categorical values and 0% scatter around numerical values. All data analysis scripts, including bioinformatic processing, statistical analyses, and plotting are available at <https://github.com/samatthews/NorthPacificMidwater/>.

3.3. Results

In total, we recovered 4.05 million COI sequences and 2.44 million 18S sequences, 2.56 million (COI) and 2.34 million (18S) of which passed quality control and were identified as zooplankton, (Table S3.1, S3.2). After bioinformatic processing and removal of contaminants and non-zooplankton sequences, 718 OTUs were detected at the COI marker (1.8×10^6 reads, 97% similarity clustering) and 491 ASVs at the 18S marker (2.3×10^6 reads). Average sequencing depth per biological sample after all quality control and filtering was 5.1×10^4 reads ($7.4 \times 10^3 - 1.3 \times 10^5$) at the COI marker and 4.6×10^4 reads ($9.5 \times 10^3 - 8.9 \times 10^4$) at the 18S

marker. We found no relationship between sequencing depth and richness at the COI marker, and a negative relationship at the 18S marker (Fig. S3.1).

3.3.1 Zooplankton community composition

Despite higher overall richness in the COI-resolved community, vertical and horizontal patterns in richness were similar at the two markers (Fig 3.2a, b). In the Subtropical Province, richness peaked in the 50-100m net and decreased with depth, apart from a secondary peak in the 600-800m net in the daytime COI samples (Fig. 3.2a). In contrast, vertical richness in the Transition and Subarctic Provinces was lowest between the surface and 50-100m, then increased with depth, both day and night. The richness maxima in both the Transition and Subarctic Provinces were observed in the 600-800m and 800-1000m nets. In total, zooplankton richness within the upper 1000m was highest in the Subtropical Province, moderate within the Transition Province, and lowest in the Subarctic Province (Fig. 3.2c). Richness patterns were similar for rarefied data, confirming that these patterns are not artefacts of sequencing depth (Fig. S3.2).

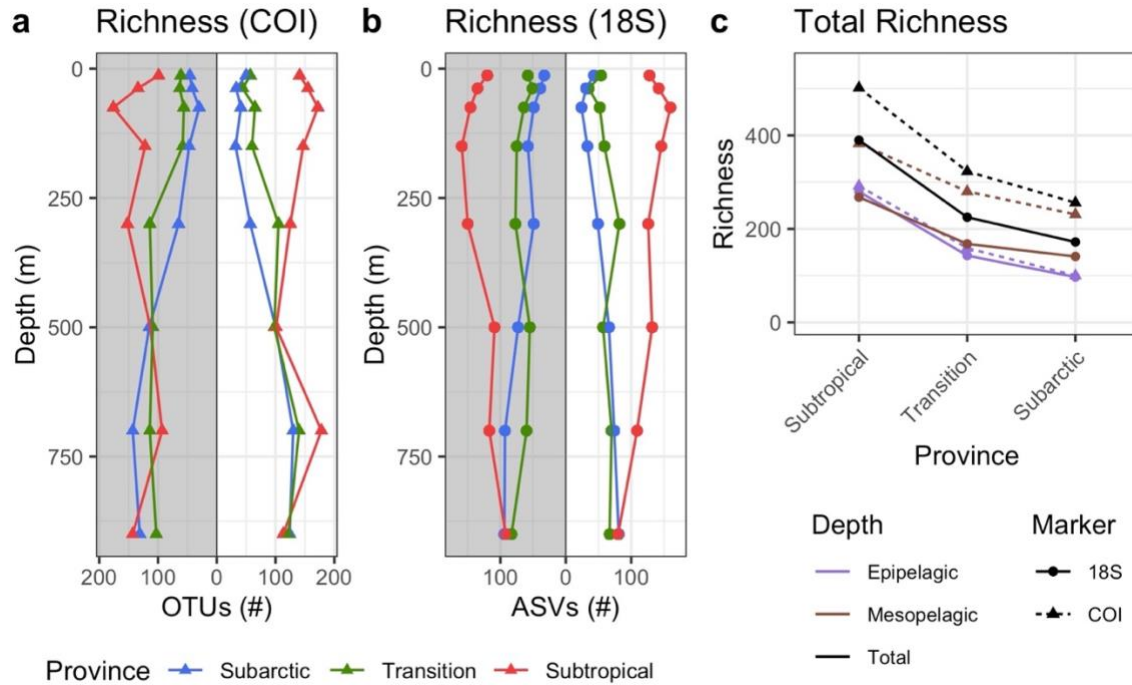


Figure 3.2. Taxonomic richness across the three biogeographic provinces for (a) COI OTUs across depth (b) 18S ASVs across depth, and (c) total richness within the epipelagic zone, mesopelagic zone, and across the full 0-1000m sampled. For panels (a) and (b), points are plotted at the midpoint of each discrete zooplankton net range, with nighttime samples on the left (gray background) and daytime samples on the right (white background). For panel (c), colors indicate depth zones and line types denote marker. Richness is the total number of OTUs/ASVs for COI or 18S, respectively.

Across all depths and all provinces, the zooplankton community was dominated by the class Copepoda (Fig. 3.3a). The lowest relative abundance of copepods was observed in the epipelagic zone of the Transition Province, which was characterized by relatively higher proportions of hydrozoans, thaliaceans, and malacostracans. Within the class Copepoda, there were notable shifts among provinces in the relative abundance of taxonomic families within the epipelagic community (Fig. 3.3b, upper panel). The highest diversity of copepod families was observed within the Subtropical epipelagic zone, which was comprised primarily of Calanidae and Paracalanidae, followed by Euchaetidae and Clausocalanidae. Clausocalanidae were also relatively abundant in the epipelagic Transition Province, although in lower proportions than Metridinidae. In the Subarctic province, Metridinidae were the largest proportion of epipelagic

copepod reads, followed by Eucalanidae and Calanidae. In the mesopelagic zone, copepod community composition was less variable among provinces (Fig. 3.3b, lower panel). Eucalanidae were the most abundant group in all three provinces, followed by Metridinidae and Calanidae. The relative contributions of Metridinidae and Calanidae reads varied across provinces, with more Metridinidae in the Subtropical Province mesopelagic zone, a mixture of Metridinidae and Calanidae in the Transition Province mesopelagic zone, and greater proportions of Calanidae in the mesopelagic zone of the Subarctic Province.

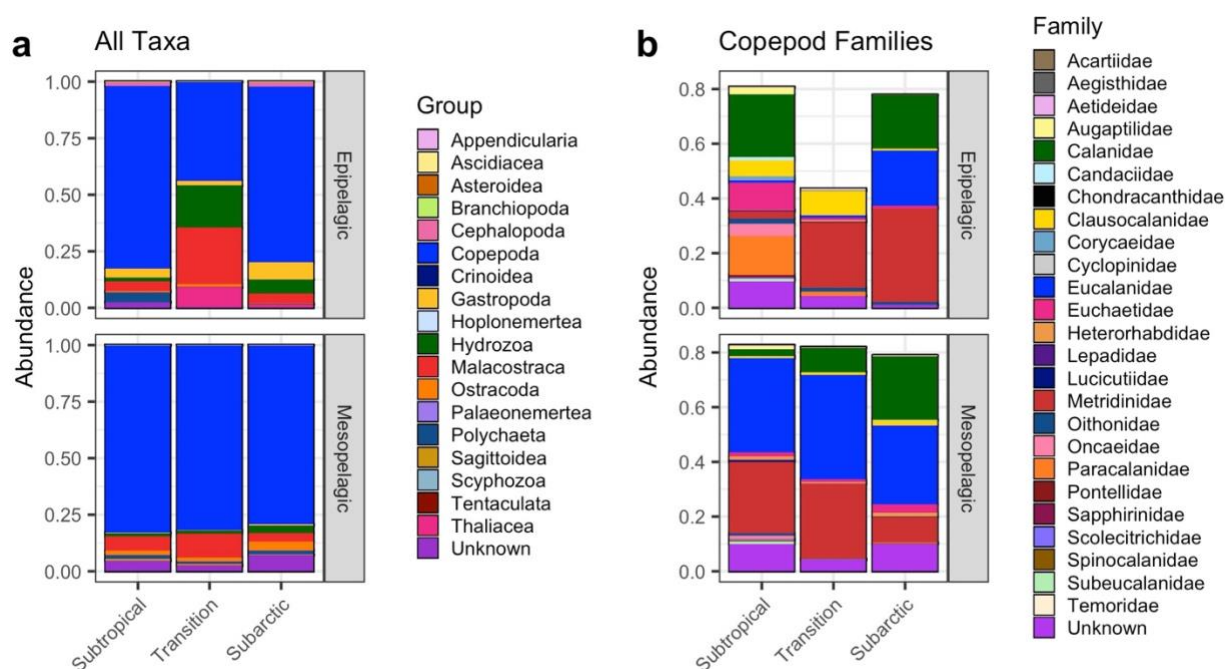


Figure 3.3. Relative read abundance of (a) taxonomic classes; and (b) families within the class Copepoda. Relative read abundance reflects the sum of COI OTUs and 18S ASVs across day and night samples. Colors represent taxonomic groups. Epipelagic samples (0-200m) are plotted in the upper sub-panel of each set, and mesopelagic samples (200-1000m) in the lower sub-panels.

For the traits of diet, feeding behavior, spawning strategy, presence of asexual reproduction, and body carbon composition, relative read abundance of trait states among biogeographic provinces was more variable within the epipelagic zone than within the mesopelagic zone (Fig. 3.4). Within the epipelagic zone, diets shifted from a carnivore- and

herbivore-dominated community in the Subtropical Province to an almost entirely omnivorous community in the Subarctic Province, while the mesopelagic community consisted primarily of omnivores in all three provinces (Fig. 3.4a). The most common feeding behavior across all three provinces and both depth zones was suspension feeding, followed by moderate levels of cruise feeding, but ambush feeding was relatively more common in the epipelagic than in the mesopelagic and peaked in relative abundance in the Transition Province (Fig. 3.4b). Brooding behavior and asexual reproduction were both more common in the epipelagic zone than in the mesopelagic zone. Brooding was primarily observed in the epipelagic zone of the Subtropical Province and to a lesser extent in the epipelagic zone of the Transition Province (Fig. 3.4c). Asexual reproductive strategies were primarily observed in the epipelagic zone of the Transition Province and to a lesser extent in the epipelagic zone of the Subarctic Province (Fig. 3.4d). Most reads were classified as belonging to non-gelatinous taxa, but gelatinous reads comprised approximately $\frac{1}{4}$ of all reads in the epipelagic zone of the Transition Province, and a small number of reads in the Subarctic Province epipelagic zone (Fig. 3.4e). Intermediate body compositions were primarily observed in the Subtropical Province within the epipelagic zone. Approximately 50% of all reads belonged to taxa with detectable DVM behavior, and in the Subtropical Province there was a slight decrease in the relative abundance of reads to corresponded to taxa with DVM behavior (Fig. 3.4f).

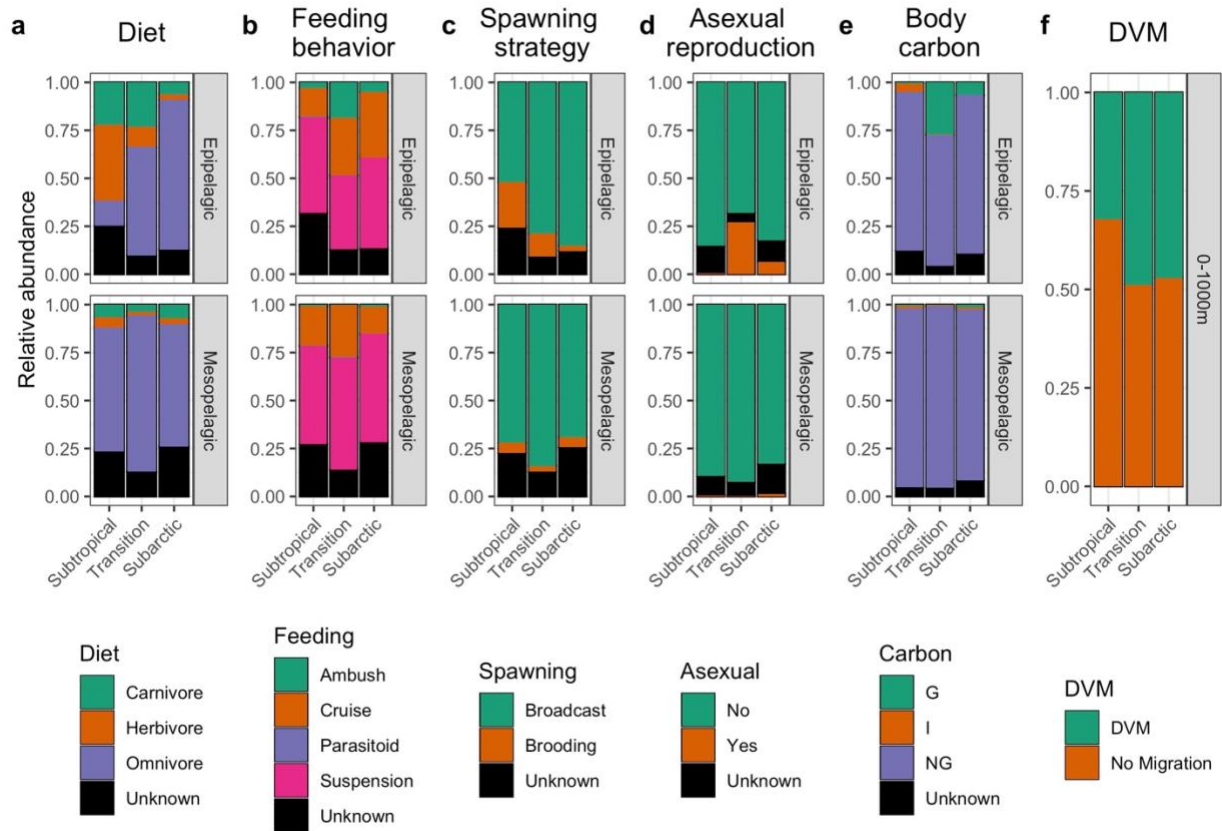


Figure 3.4. Relative read abundance assigned to each trait state within the epipelagic zone (upper subpanels) and mesopelagic zone (lower subpanels) for (a) dietary preference; (b) feeding behavior; (c) spawning strategy; (d) presence or absence of asexual reproduction; (e) body carbon composition; and (f) presence or absence of detectable DVM behavior. DVM behavior is not divided into vertical strata, as animals migrate between strata.

Taxon body size was significantly correlated with mean depth of occurrence for both daytime and nighttime vertical distributions in the Subtropical and Transition Provinces (ties-corrected Spearman rank correlation; Subtropical Province day: $\rho = 0.38$, $p < 0.0001$; Subtropical Province night: $\rho = 0.41$, $p < 0.0001$; Transition Province day: $\rho = 0.22$, $p < 0.0001$; Transition Province night: $\rho = 0.32$, $p < 0.0001$; Fig 3.5). A *loess* non-parametric locally fitted regression showed that the positive correlations between size and depth were restricted to taxa smaller than 10mm. In the Subarctic Province, we found no significant relationship between maximum reported taxon size and observed mean depth of occurrence. Among provinces, we found no

significant differences in epipelagic or mesopelagic taxon body sizes (Kruskal-Wallis rank sum test $p > 0.05$, both depth zones). We also tested whether size was correlated with mean depth of occurrence within major zooplankton groups and found larger taxa at deeper depths for copepods (Class Copepoda) and euphausiids (Class Malacostraca) (Fig. S3.3).

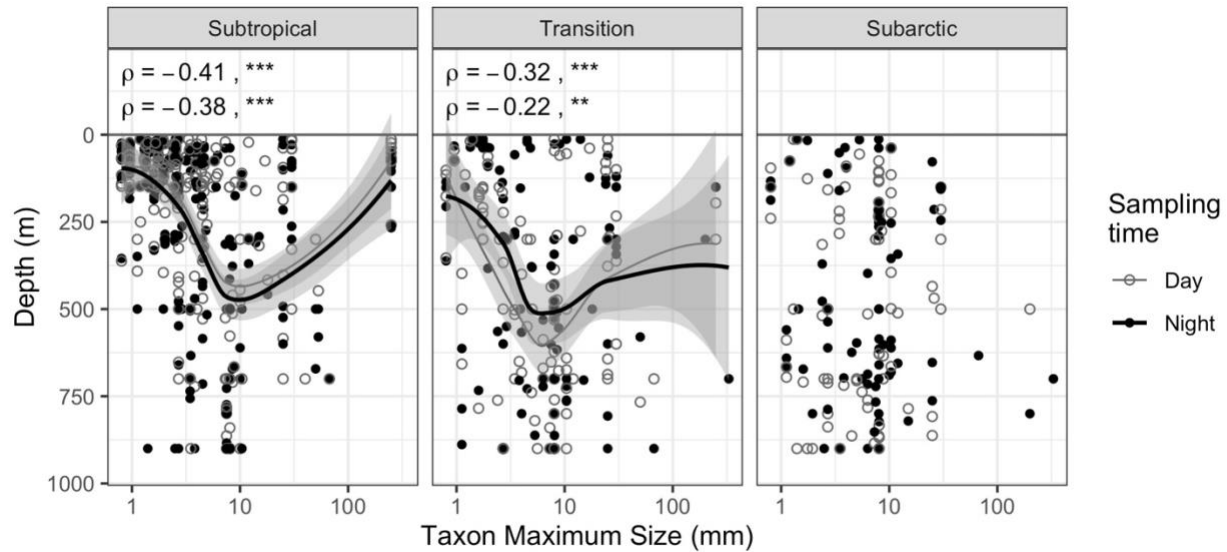


Figure 3.5. Relationship between the maximum reported body size and observed mean depth of occurrence, in each of the three provinces. Loess best-fit lines are shown for the significant relationships observed for day (open) and night (closed) samples in the Subtropical and Transition provinces. Shading surrounding lines represents the 95% CI. Daytime mean depth of occurrence is plotted in grey, and nighttime mean depth of occurrence in black. (Spearman's rank: ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3.3.2. Taxa distributions across provinces

We examined whether taxa were observed in a single province, distributed across two provinces, or extended across all three biogeographic provinces (Fig. 3.6). Across the full 0-1000m sampled, the largest group of taxa in our study was observed only in the Subtropical Province (529), followed by taxa observed across all three provinces (201) (Fig. 3.6a). More taxa were shared between the Subtropical and Transition Provinces (131) than between the Transition and Subarctic Provinces (107), and the smallest number of taxa was those observed in the

Subtropical Province and Subarctic Province but not the Transition Province (31). When we divided the water column into vertical strata, we found increased rates of endemism in the epipelagic zone (35%, 33%, and 76% of taxa in the Subarctic, Transition, and Subtropical Provinces respectively), compared with the mesopelagic zone (19%, 17%, and 51%). Across the full water column, 17% of all taxa were cosmopolitan, appearing in all three stations. The percentage of cosmopolitan taxa decreased to 5% within the epipelagic zone and increased to 21% within the mesopelagic zone.

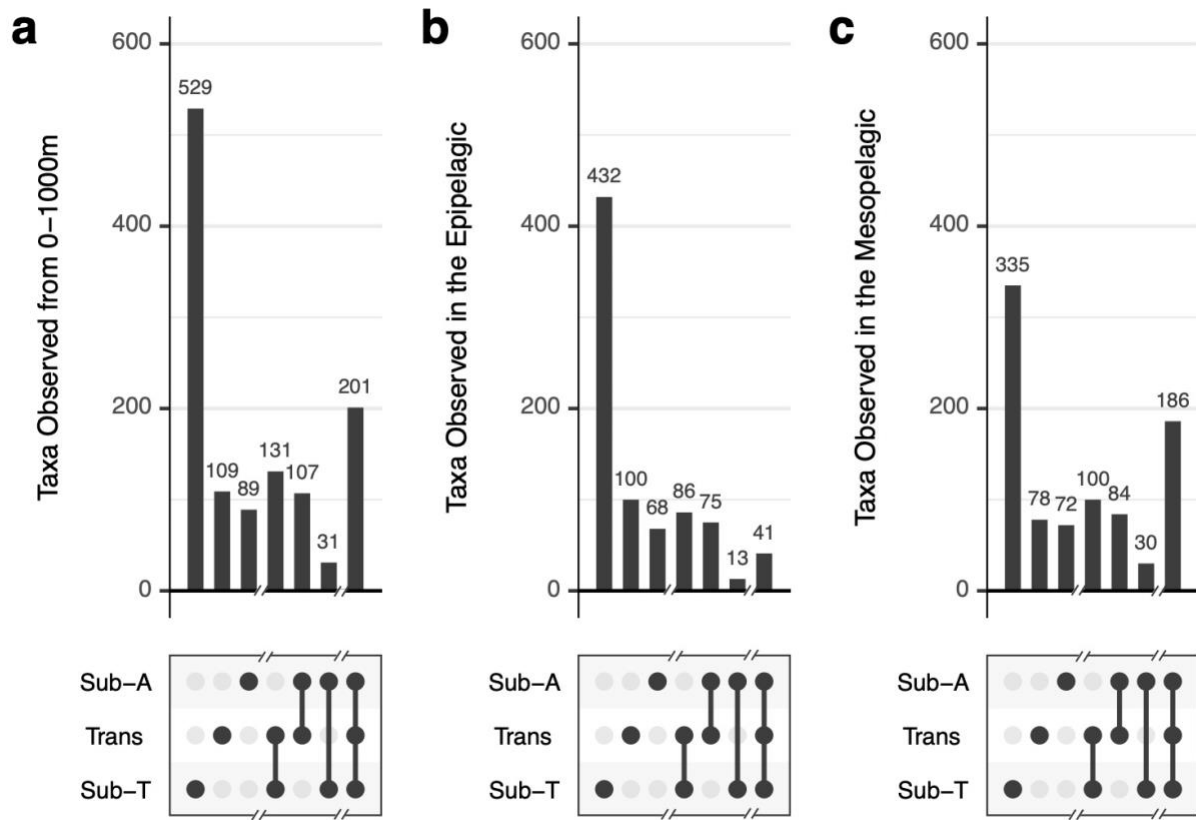


Figure 3.6. Number of taxa (OTUs and ASVs) observed within only one province or shared between multiple provinces for (a) the full water column (0-1000m), (b) the epipelagic zone (0-200m) and (c) the mesopelagic zone (200-1000m). Bar heights in the upper panel represent the number of taxa observed within each province or combination of provinces. Filled circles and connecting lines in the lower panel represent the province or provinces in which the taxa were observed. Sub-a: Subarctic Province; Trans: Transition Province; Sub-T: Subtropical Province.

When samples were clustered using non-metric multidimensional scaling of Bray-Curtis distance between samples, mesopelagic samples clustered more closely than epipelagic samples (Fig. 3.7). To test whether community similarity across the three provinces was higher in the mesopelagic than in the epipelagic, we calculated the beta-dispersion within samples from each depth zone. Mesopelagic samples had lower within-group beta-dispersion, confirming that mesopelagic communities exhibit fewer differences across provinces than epipelagic communities ($F(1,46) = [9.3719]$, $p < 0.01$, Fig. 3.7). Parallel analyses using Jaccard distance calculated from presence/absence similarly found higher similarity among mesopelagic samples than among epipelagic samples ($F(1,46) = [8.2681]$, $p < 0.01$, not shown).

In addition to dividing the zooplankton community into discrete epipelagic and mesopelagic depth zones, we classified each taxon as primarily epipelagic or primarily mesopelagic, based on mean read depth across all provinces. Taxa classified as mesopelagic (MDO > 200m) were significantly more likely to be observed in multiple provinces than taxa classified as epipelagic (MDO < 200m) ($\chi^2 = 152.01$, $p < 0.001$) (Fig. 3.8).

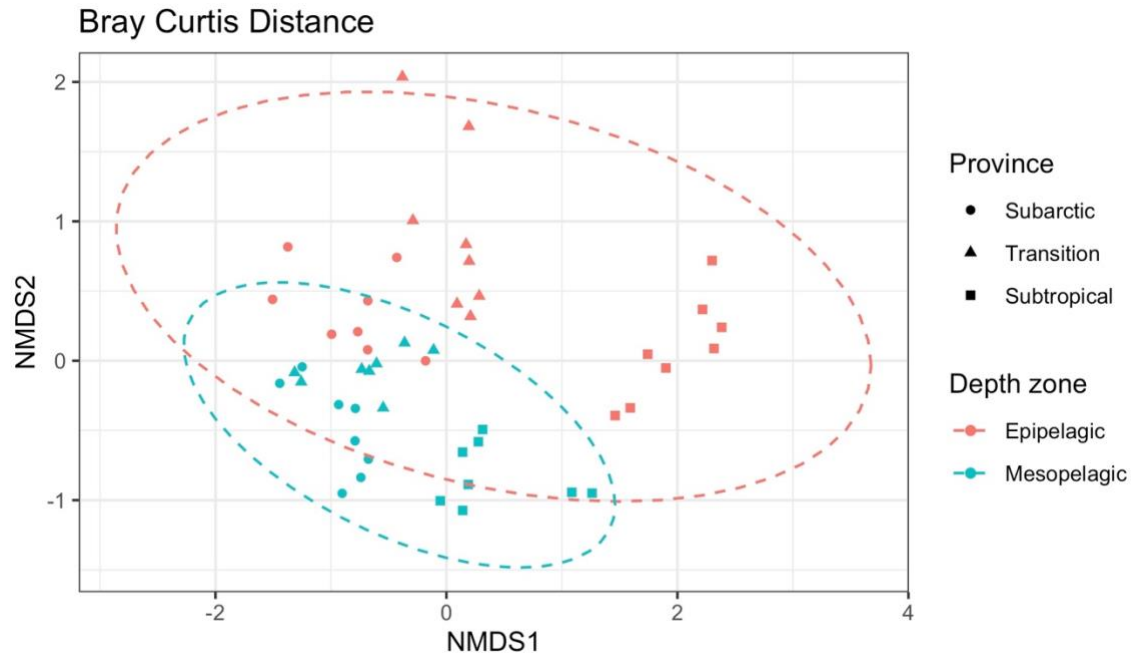


Figure 3.7. Non-metric multidimensional scaling of the Bray-Curtis distance between samples, calculated from combined 18S and COI amplicons. Symbol shapes indicate different biogeographic provinces and colors denote vertical depth zones.

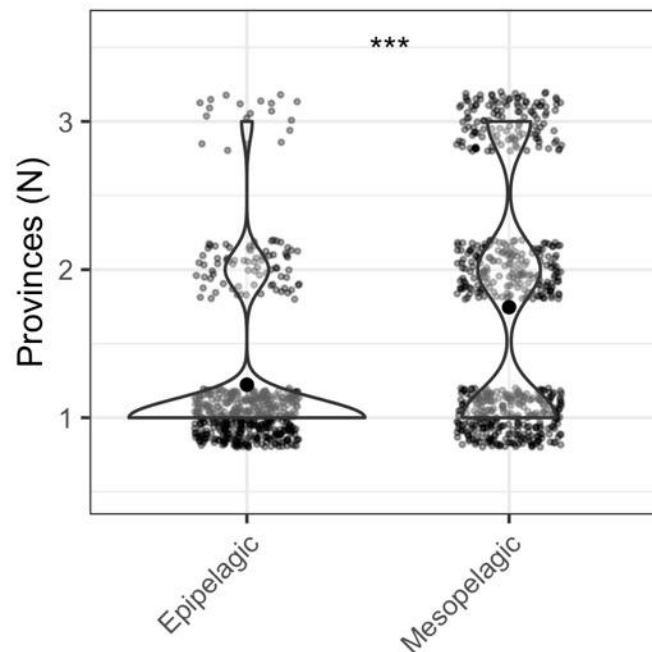


Figure 3.8. Epipelagic and mesopelagic OTUs and ASVs and the number of provinces in which each taxon was observed. Violin plots show density distribution, data points jittered within each categorical value. Data points are semi-opaque, variability in color intensity reflects overlapping points. (Kruskal-Wallis: ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

3.3.3. Environmental range and ecological strategies

We used differences in the mean environment observed within each sample to test whether there were differences between epipelagic and mesopelagic taxa in the ranges of environmental properties inhabited by each taxon. Kruskal-Wallis tests revealed that the ranges of epipelagic and mesopelagic taxa were significantly different, with larger temperature ranges for epipelagic taxa ($\chi^2 = 21.992$, 1 d.f, $p < 0.001$), larger Chl-*a* ranges for epipelagic taxa ($\chi^2 = 39.292$, 1 d.f, $p < 0.001$), and larger oxygen ranges for mesopelagic taxa ($\chi^2 = 43.982$, 1 d.f, $p < 0.001$) (Fig. S3.4). To investigate whether these wider environmental ranges are associated with greater biogeographic ranges, we tested whether taxa observed in one, two, or three provinces were distributed across wider ranges of temperature, Chl-*a*, or dissolved oxygen (Fig. 3.9). We found significant differences in temperature ranges of epipelagic taxa observed in one, two, or three provinces ($\chi^2 = 37.715$, 2 d.f, $p < 0.001$) but no differences among mesopelagic taxa (Fig 3.9a). The Chl-*a* ranges of epipelagic taxa observed in only one province were significantly narrower than the Chl-*a* ranges of taxa observed in two or three provinces ($\chi^2 = 174.23$, 2 d.f, $p < 0.001$), and mesopelagic taxa observed only in a single province had narrower Chl-*a* ranges than taxa observed in two provinces, but not significantly different than taxa observed in three provinces ($\chi^2 = 7.2583$, 2 d.f, $p < 0.05$) (Fig 3.9b). Both epipelagic and mesopelagic taxa found in two or three provinces were found across significantly wider oxygen ranges than taxa found only in a single province (epipelagic: $\chi^2 = 67.868$, 2 d.f, $p < 0.001$; mesopelagic: $\chi^2 = 17.978$, 2 d.f, $p < 0.001$) (Fig 3.9c).

To determine whether epipelagic and mesopelagic zooplankton with different ecological traits have different environmental and biogeographic ranges, we examined the environmental

ranges across which taxa with different trait states for diet, feeding behavior, spawning strategy, presence of asexual reproduction, or DVM behavior were observed. Among epipelagic taxa, we found significant differences in the Chl-*a* ranges and distributional ranges of taxa with and without the capacity for asexual reproduction, with asexually reproducing taxa found across wider Chl-*a* ranges and more provinces (Chl-*a*: $\chi^2 = 13.804$, 1 d.f, $p < 0.001$; number of provinces: $\chi^2 = 31.195$, 1 d.f, $p < 0.001$) (Fig. 3.10a, b). We also found significant differences in the number of provinces in which taxa with different body carbon compositions were observed, with gelatinous taxa more likely to be distributed across multiple provinces than either taxa with non-gelatinous or intermediate compositions ($\chi^2 = 14.418$, 2 d.f, $p < 0.001$) (Fig. 3.10c).

Among mesopelagic taxa, we found significant differences in the breadth of Chl-*a* and dissolved oxygen ranges between taxa with different diets, with omnivores inhabiting wider ranges than herbivores (Chl-*a*: $\chi^2 = 7.6532$, 2 d.f, $p < 0.05$; oxygen: $\chi^2 = 6.8479$, 2 d.f, $p < 0.05$) (Fig. 3.11a, b). We also observed differences in the oxygen ranges and distributional extent of taxa with different feeding strategies, with suspension feeding taxa occurring across a wider range of oxygen concentrations than parasitoid feeding taxa ($\chi^2 = 8.2206$, 3 d.f, $p < 0.05$) and cruise-feeding taxa distributed across more provinces than either parasitoid taxa or suspension feeders ($\chi^2 = 13.613$, 3 d.f, $p < 0.01$) (Fig. 3.11c, d).

For taxa with and without DVM behavior, we tested for differences in environmental and biogeographic ranges of taxa across the full water column, as these taxa utilize both epipelagic and mesopelagic habitats. We found that taxa exhibiting DVM behavior were distributed across wider ranges of temperature, Chl-*a*, and oxygen, and were more likely to occur in multiple biogeographic provinces than taxa without DVM behavior (temperature: $\chi^2 = 111.23$, 1 d.f, $p <$

0.001; Chl-*a*: $\chi^2 = 105.11$, 1 d.f, $p < 0.001$; oxygen: $\chi^2 = 91.611$, 1 d.f, $p < 0.001$; number of provinces: $\chi^2 = 118.02$, 1 d.f, $p < 0.001$) (Fig. 3.12).

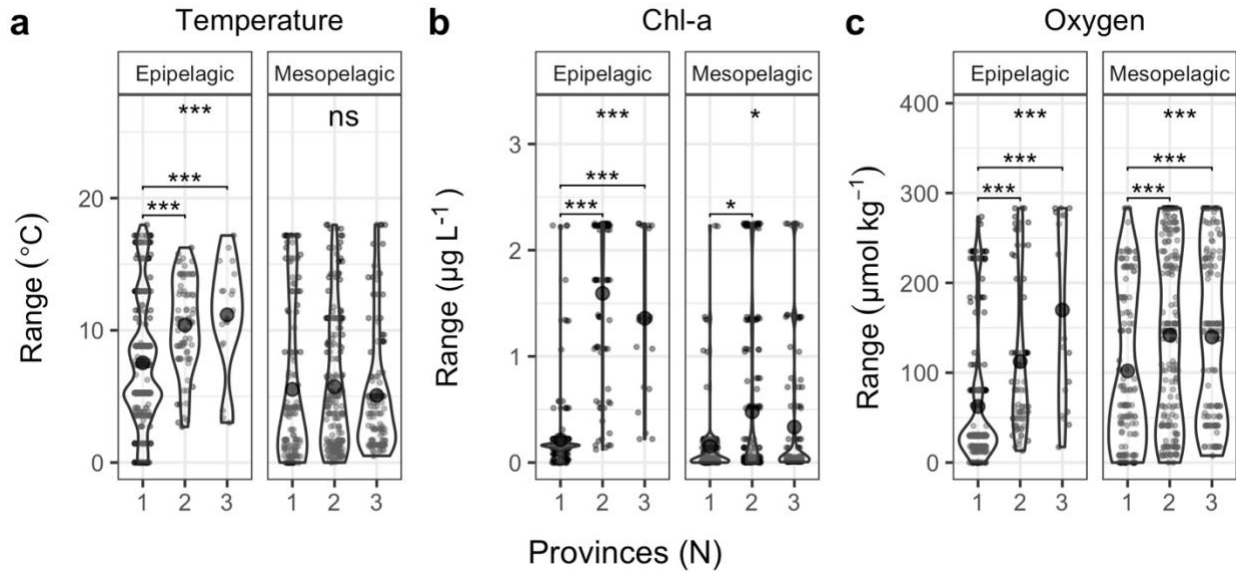


Figure 3.9. Environmental ranges across which epipelagic and mesopelagic taxa were distributed, for taxa observed in one, two, or three biogeographic provinces. (a) temperature ranges; (b) Chl-*a* ranges; (c) oxygen ranges. Violin plots and data points as in Figure 3.8. Global significance of Kruskal-Wallis tests; pairwise comparisons from Bonferroni-corrected post-hoc Dunn's test results. (ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

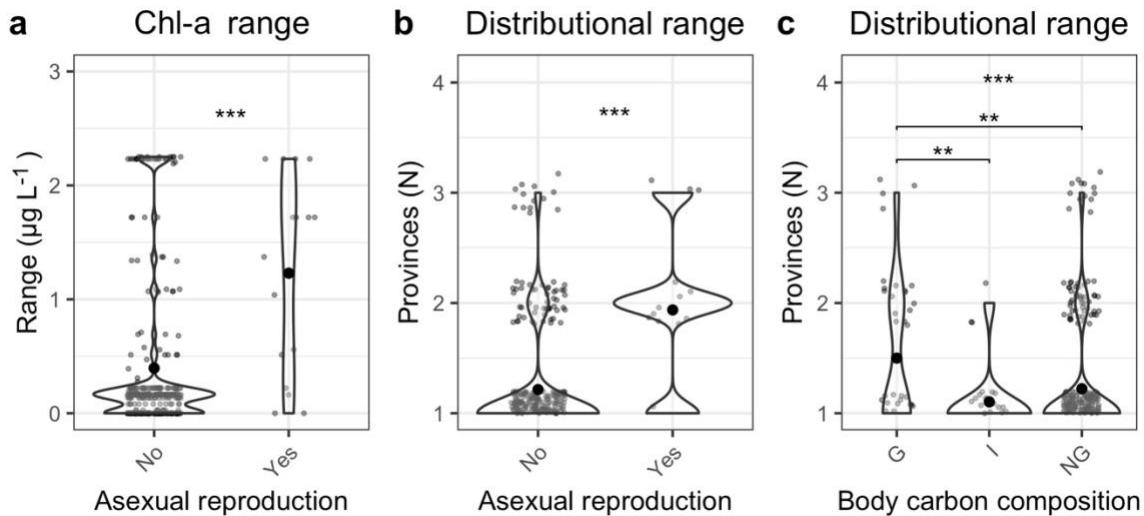


Figure 3.10. For epipelagic taxa, environmental and biogeographic ranges that differed among traits. (a) Chl-*a* ranges for epipelagic taxa with and without asexual reproduction. (b) Number of biogeographic provinces across which taxa with and without asexual reproduction were distributed; (c) number of biogeographic provinces across which taxa with different body carbon compositions were distributed. Plots and tests as in Figure 3.9. (ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

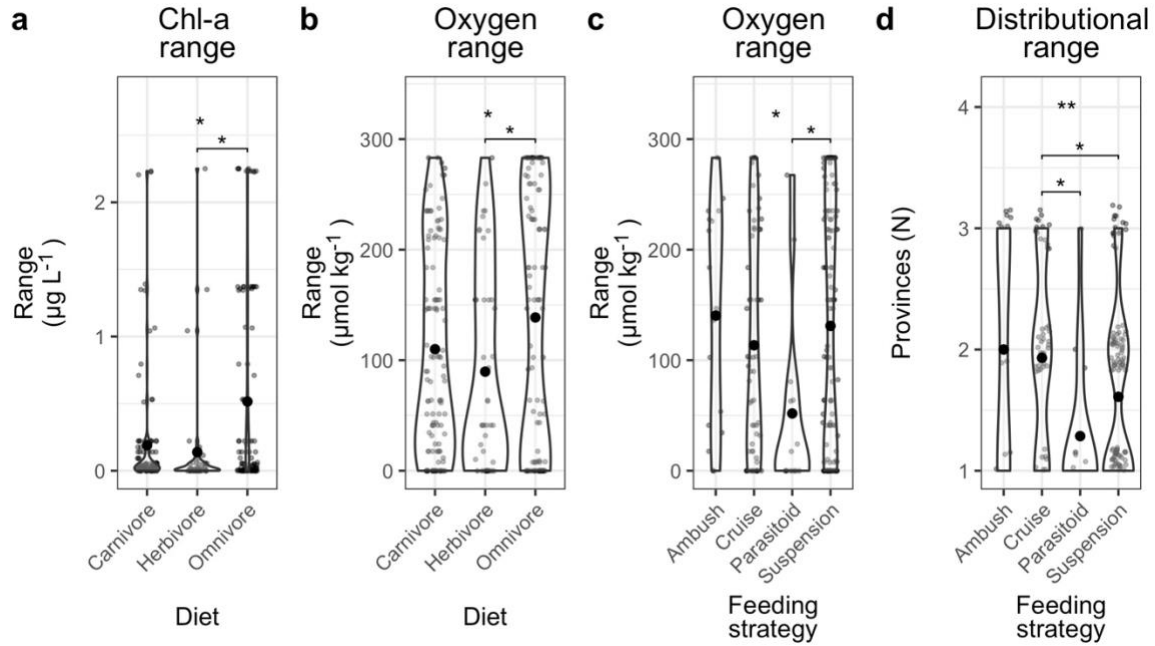


Figure 3.11. For mesopelagic taxa, the environmental and biogeographic ranges that differed among traits (a) Chl-a ranges for mesopelagic taxa with different diets; (b) Oxygen ranges for mesopelagic taxa with different diets; (c) Oxygen ranges for mesopelagic taxa with different feeding strategies; and (d) Number of biogeographic provinces across which taxa with different feeding strategies were distributed. Plots and tests as in Figure 3.9. (ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

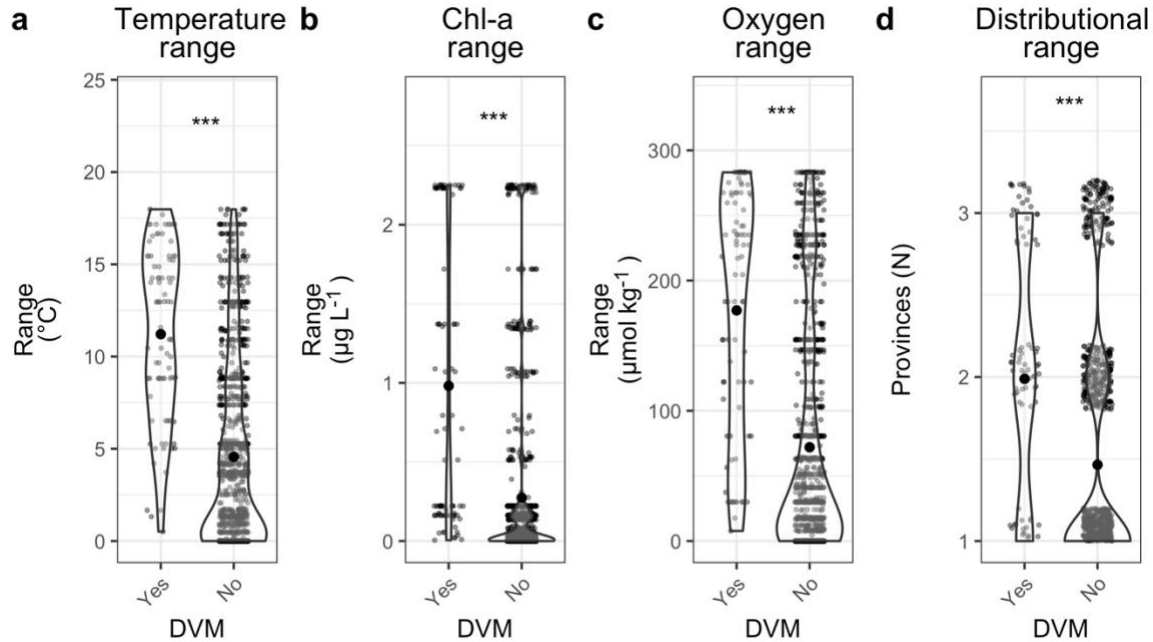


Figure 3.12. For taxa with and without Diel Vertical Migration, (a) temperature ranges; (b) Chl-a ranges; (c) oxygen ranges; and (d) number of provinces each taxon was observed within. Violin plots show density distribution, data points jittered within each categorical value. Data points are semi-opaque, variability in color intensity reflects overlapping points. Global significance reflects results of Kruskal-Wallis test. (ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

3.4. Discussion

To explore potential drivers of biogeographic distributions, we examined epipelagic and mesopelagic environmental ranges, species traits, and distributions across the Subtropical, Transition, and Subarctic biogeochemical provinces in the North Pacific Ocean. Our results suggest that differences in traits among zooplankton species can lead to differential effects of physical dispersal and environmental selection on biogeographic distributions. We found that the temperature, dissolved oxygen, and Chl-a ranges of zooplankton in the epipelagic and mesopelagic North Pacific reflected the environmental variability observed within each taxon's primary vertical habitat, with oxygen levels appearing as the main environmental driver in the mesopelagic zone. Within each vertical depth zone larger environmental ranges were associated

with broader biogeographic distributions, but overall we observed larger distributional ranges of mesopelagic taxa compared to the ranges of epipelagic taxa. We discuss potential implications of each of these results below.

3.4.1 Community composition

Within both the epipelagic and mesopelagic zones, we observed highest species richness in the Subtropical Province and decreasing richness at higher latitudes, with comparable decreases for the 18S and COI resolved zooplankton communities. Subtropical richness maxima and decreases in richness with increasing latitude are expected in pelagic ecosystems, although most previous analyses have focused on specific zooplankton groups and have often been restricted to the epipelagic (Angel, 1993; Brinton et al., 1999; Rombouts et al., 2009; van der Spoel et al., 1997). Our results reveal that latitudinal species richness in the mesopelagic parallels these previously described epipelagic trends. Additionally, vertical patterns of species richness varied among provinces, with mesopelagic richness maxima in the Transition and Subarctic Provinces, but epipelagic richness maxima in the subtropics. Previous analyses of vertical gradients in zooplankton richness have found richness peaks at approximately 50m in the Tropical Pacific (Longhurst, 1985), between 150-200m in the Subtropical North Pacific (Sommer et al., 2017), and 500-1000m in the Arctic basin (Kosobokova et al., 2011). Our data combined with these previously published values, reveal a latitudinal trend of increasing depth of richness maxima with increasing latitude (Fig. 3.13). While the magnitude of richness cannot be compared among studies with different methodologies, the depths of the richness maxima can. We caution against over interpretation of the slightly deeper richness maximum at lower

subtropical latitudes due to variability in the longitudinal locations of these studies. Future studies should test the strength of this pattern.

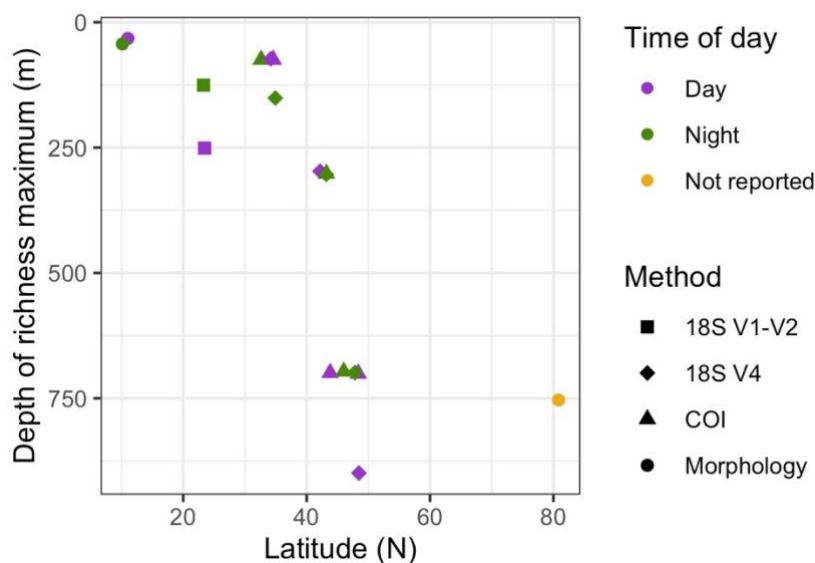


Figure 3.13. The depth of maximum zooplankton richness for sampling locations in the Pacific Basin, extending from the tropics to the Arctic. 18S V4 and COI data are from this study. 18S V1-V2 data are from Sommer et al. 2017. Morphological data are from Longhurst 1985 (tropics) and Kosobokova et al. 2011 (Arctic). Points are plotted at the midpoint for reported depth bins from each study.

Taxonomically, the zooplankton were dominated by copepod reads, reflecting the importance of this major taxonomic group throughout the pelagic zooplankton community. The high proportions of Eucalanidae reads likely reflect preferential amplification of this family at the metabarcoding markers we used, rather than true dominance of this group (Matthews et al., 2021). Previous analyses of zooplankton community composition have reported that copepods comprise 60 – 90 % of the zooplankton community by both abundance and biomass in the Subtropical and Subarctic Pacific (Mackas & Tsuda, 1999; Steinberg et al., 2008). In the epipelagic Transition Province, the abundant malacostracan, hydrozoan, and thaliacean reads were primarily identified as euphausiids, siphonophores, and salps, respectively. This aligns with previous reports of increased euphausiid abundance in this region (Brodeur et al., 1999), and association of thaliaceans with productive regions of ocean mixing (Ishak et al., 2022). Within

the mesopelagic zone, the change from a community dominated by Metridinidae in the Subtropical Province to one dominated by Calanidae in the Subarctic Province agrees with previously described distributions. The genera *Gaussia*, *Metridia* and *Pleuromamma* (all within the family Metridinidae) are associated with warmer subtropical regions (Soh et al., 1998), and mesopelagic Calanidae reads in the Subarctic Province reflect the tendency of high-latitude herbivores to use the deep ocean as a refuge during diapause (Miller et al., 1984).

The trait composition of the full zooplankton community has not been previously well-characterized for the depth ranges we examined. However, copepod species distribution models based on records from the upper 500m have predicted that with increasing latitudes, copepods will shift toward larger body sizes, away from sac spawning, from carnivory to omnivory, and from ambush feeding to current and cruise feeding (Benedetti et al., 2022). Our results do not cleanly align with these predicted latitudinal gradients. However, our analysis included the full zooplankton community rather than targeting copepod species, and it is possible that different taxonomic groups exhibit different spatial trends. The positive relationship between body size and depth was restricted to taxa smaller than 10mm and was stronger within specific zooplankton groups than across all zooplankton taxa combined. There can be significant variability in size within a species (Kobari et al., 2003; Sasaki & Dam, 2019), but we used maximum reported size for each species rather than measurements of individual specimens, another factor that could obscure latitudinal gradients. As expected, we observed decreasing rates of brooding (sac-spawning) with increasing latitude as well as latitudinal increases in omnivory. In our data, ambush feeding peaked in the Transition Province rather than in the Subtropical Province as predicted (Benedetti et al., 2022). Additionally, ambush feeding was more prevalent in the epipelagic than in the mesopelagic. This pattern may reflect decreased physical mixing in the

deep ocean, requiring animals to encounter prey items through motile cruise and suspension feeding.

There are few previously published observational reports of epipelagic zooplankton trait composition in the North Pacific. In the Subtropical Province, a previous analysis of morphologically identified communities focused on the upper 200m (Ge et al., 2022). We calculated relative abundances of traits from metabarcoding reads rather than morphological identifications. Despite different methods, we found similar proportions of feeding behaviors and dietary strategies. In comparison, we identified nearly 85% of reads as broadcast spawners in our study, while sac spawning was previously reported for about 50% of the zooplankton community. Within copepods, broadcast spawning species often have larger body sizes than brooding species, and the increased relative abundance of reads associated with broadcast spawning in our study may represent increased representation of higher biomass from these species.

3.4.2 Biogeographic boundaries

We found no evidence for a biogeographic boundary between the mesopelagic Subarctic and Subtropical biogeochemical provinces. Across the three locations sampled, we observed greater community similarity in the mesopelagic zone than in the epipelagic zone and we found broader distributional ranges for mesopelagic zooplankton. Decreased rates of endemism and greater community similarity could be created by increased connectivity, decreased environmental variability, or wider environmental tolerance windows for mesopelagic. However, connectivity through large-scale ocean currents and physical mixing is muted in the mesopelagic, compared to surface waters (Kawabe & Fujio, 2010; Reid et al., 1978; Scott et al., 2010). Greater

similarity between mesopelagic communities than between epipelagic communities is paralleled by greater environmental similarity at mesopelagic depths than at epipelagic depths (Figs. S3.5, S3.6). Together with taxon-specific environmental ranges that mirror the primary environmental variability found within each depth zone, greater community similarity and decreased rates of endemism suggest that midwater zooplankton community composition is more strongly influenced by environmental selection than by physical dispersal due to ocean mixing. Our results contrast with recent observations of decreased community similarity at depth and stronger effects of physical distance than of environmental distance on community similarity (Canals et al., 2023). However, our sampling locations comprise a strong latitudinal gradient in productivity, while the Pacific Ocean locations sampled by the Malaspina expedition were restricted to primarily equatorial and subtropical environments. The apparent conflicts between these two studies may indicate region-specific patterns of similarity, reflecting varying strengths of physical connectivity and environmental dissimilarity.

To assess whether the North Pacific Transition Province is more strongly influenced by the northern Subarctic Province or southern Subtropical Province, we examined Bray-Curtis community similarity and the absolute number of taxa shared among provinces. Within both the epipelagic and mesopelagic zones, we found higher proportions of shared taxa between samples from the Subtropical and Transition provinces, but greater Bray-Curtis similarities (incorporating relative abundance) between the Subarctic and Transition provinces. The proportion of species shared between provinces and the overall community similarity between provinces will have different responses to physical dispersal, environmental similarity, and environmental tolerances of species. The number of species shared between provinces is a better measure of dispersal and physical connectivity, but can reflect expatriated individuals that are reproductively inactive.

Similarity in community composition, incorporating relative abundance of different species, is a better measure of the sum effects of dispersal, environment, and environmental tolerances. Previous latitudinal analyses of copepod community composition have found that the northern boundary of the Transition Province is a region of decreased physical mixing and muted community connectivity (Miyamoto et al., 2022), aligning with our finding that species within the Transition Province were more likely to be shared with the southern Subtropical Province than with the northern Subarctic Province. Conversely, the increased community-level similarity we observed between the Transition and Subarctic Provinces mirrored the increased environmental similarity between these two provinces in temperature, salinity, Chl-*a*, and dissolved oxygen, particularly within the epipelagic zone (Figs. S3.5, S3.6). The increased community-level similarity between the Transition and Subarctic provinces reflects higher abundances of taxa from the north, which may be better adapted to the cold, productive Transition Province. We discuss the potential role of environmental tolerance below.

3.4.3 Traits and habitat ranges

To investigate potential effects of environmental tolerance on biogeographic ranges, we examined the temperature, Chl-*a*, and oxygen ranges of epipelagic and mesopelagic taxa with varying biogeographic extent. The strongest environmental gradients in the pelagic ocean occur vertically, and gradients in temperature and Chl-*a* are concentrated in the epipelagic zone (Miller, 2004), while strong oxygen gradients are more commonly found within the mesopelagic zone (Robinson et al. 2010). Epipelagic taxa were observed across broader temperature and Chl-*a* ranges than mesopelagic taxa, and broader temperature and Chl-*a* ranges were associated with larger biogeographic distributions. Similarly, mesopelagic taxa were observed across broader

ranges of dissolved oxygen than epipelagic taxa, and mesopelagic taxa with broader dissolved oxygen ranges also exhibited larger biogeographic distributions.

Temperature is well established as a structuring variable for epipelagic zooplankton distributions (Beaugrand et al., 2014; Mackas et al., 2007) and community composition (Costello et al., 2017; Hirai et al., 2020). The importance of oxygen to mesopelagic community composition and species distributions is also well-documented (Bertrand et al., 2011; Ekau et al., 2010; Wishner et al., 2013, 2020). The ranges of Chl-*a* values observed for primarily mesopelagic taxa and the ranges of dissolved oxygen concentrations observed for epipelagic taxa indicate that these taxa are not restricted to their primary depth habitat. For both epipelagic and mesopelagic zooplankton, survival in and utilization of multiple vertical habitats appear to be associated with larger biogeographic ranges.

Among epipelagic taxa, we found significantly wider Chl-*a* ranges and broader distributional ranges for zooplankton with the capacity for asexual reproduction. The mechanistic link between asexual reproduction and distributional range is unclear. Asexual reproduction may allow for reproduction even by expatriated individuals that are unlikely to encounter a mate, which could increase the effectiveness of physical dispersal processes. However, many sexually reproducing zooplankton taxa have strategies for sperm storage and reproduction by previously mated females in the absence of available mates (Hirche, 1997; Miller & Clemons, 1988; Næss & Nilssen, 1991). The larger biogeographic distributions of asexual taxa may reflect mechanistic effects of other traits that covary with this trait. Future analyses should prioritize traits related to feeding and energy requirements, as Chl-*a* ranges were also larger for asexually reproducing zooplankton.

Among mesopelagic taxa, we found the strongest differences in environmental and distributional ranges among taxa with different dietary strategies and feeding behaviors. Chl-*a* and dissolved oxygen ranges were larger for omnivores than for herbivores. Dissolved oxygen ranges were also larger for suspension feeding taxa than for parasitoid taxa, but the widest biogeographic ranges were found in cruise feeding taxa. As far as we are aware, there are no previous observational studies of the environmental ranges associated with zooplankton feeding behaviors. Among studies of dietary strategies, there are conflicting results. Analyses of copepod taxa in the North Atlantic and Southern Oceans found no difference in the environmental niche breadth of omnivores and herbivores, but found narrower environmental niches for carnivores (McGinty et al., 2018). The environmental ranges of herbivore and omnivore dominated cool-water copepod assemblages in the western North Pacific are narrower than the ranges of more carnivorous warm-water assemblages in the Subtropical North Pacific (Tang et al., 2022). While there are incongruent outcomes between previous studies and our results, there are also notable differences among studies. Differences in dietary definitions reflect differences in the taxonomic specificity and breadth of zooplankton taxa analyzed. We also note that both of these analyses focused on biogeographic distributions within the epipelagic zone, while our observations extend through the mesopelagic zone. The environmental ranges of zooplankton with different dietary strategies and feeding behaviors appear to vary among ecosystems and taxonomic groups.

Across all taxa, we found differences in the temperature, dissolved oxygen, Chl-*a*, and distributional ranges of taxa with or without Diel Vertical Migration behavior (DVM), with larger ranges for taxa with DVM. Due to the strong vertical gradients found in the pelagic ocean, DVM exposes taxa to multiple habitats and to a wide range of environmental conditions. It is unclear whether the wider environmental ranges associated with DVM reflect transit through

low-oxygen layers or residence within hypoxic habitats. Similarly, DVM behavior can allow primarily herbivorous taxa to reside in habitats far below the euphotic zone for a portion of each day (Cohen & Forward, 2016). However, the larger distributional ranges we observed for taxa with DVM behavior indicate that this vertical habitat flexibility is associated with increased species ranges.

In addition to the traits that we explicitly tested, there are other characteristics that may be associated with specific depth zones or may influence the environmental ranges of zooplankton species. The variability we observed in the environmental and distributional ranges of taxonomic classes and copepod families may be used to identify additional traits that should be considered (Figs. S3.7, S3.8). Notably, generation time is highly variable among zooplankton: the generation time of crustaceans is typically on the order of 30-180 days, while generation times for thaliaceans in particular, can be much shorter (Bone, 1998). Duration of developmental stages, total generation time, and flexibility in development can vary even within a species depending on temperature and food availability. Species inhabiting deeper depth zones may have longer lifespans, as animals typically exhibit lower metabolic rates at colder mesopelagic depths (Ikeda et al., 2007). Longer lifespans could result in larger distributional ranges due to increased dispersal potential over the lifetime of individual animals. As our metabarcoding methods do not resolve the life history stage of the animals collected or the reproductive potential of the individuals detected, we did not include generation time, development time prior to reproductive maturity or capacity for indeterminate growth in our analysis. Future work could consider differences in dispersal potential and environmental tolerance due to variability in generation time and growth strategies.

Conclusion

We found that the mesopelagic zooplankton community in the eastern North Pacific Ocean has greater similarity across biogeographic provinces than the epipelagic community. Mesopelagic taxa were observed across more biogeographic provinces than epipelagic taxa, and there was less differentiation among mesopelagic than among epipelagic zooplankton communities across the NPTZ. The temperature, dissolved oxygen, and Chl-*a* ranges of epipelagic and mesopelagic taxa reflected the environmental variability observed within each taxon's primary vertical habitat. Larger environmental ranges were associated with broader biogeographic distributions, but the extent of environmental and distributional ranges varied among traits. These findings confirm the influence of physical dispersal regimes, environmental variability, and species traits in shaping zooplankton distributions throughout the upper 1000m of the pelagic ocean. Physical dispersal can introduce zooplankton into new environments, but our results suggest that tolerance to those new environments may play a larger role in shaping overall zooplankton community composition.

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Chapter 3, in full, has been submitted for publication of the material in Ecology and Evolution. Matthews, Stephanie A.; Blanco-Bercial, Leocadio. The dissertation author was the primary researcher and author of this paper.

Data accessibility

Raw sequences are deposited to the NCBI nucleotide database under BioProject PRJNA954464. All code, CTD data, and sample metadata can be found at <https://github.com/samatthews/NorthPacificMidwater>.

Author contributions

Stephanie A. Matthews: Conceptualization, Methodology, Data curation, Investigation, Formal analysis, Visualization, Writing. *Leocadio Blanco-Bercial*: Sample collection, Investigation, Resources.

Supplemental figures

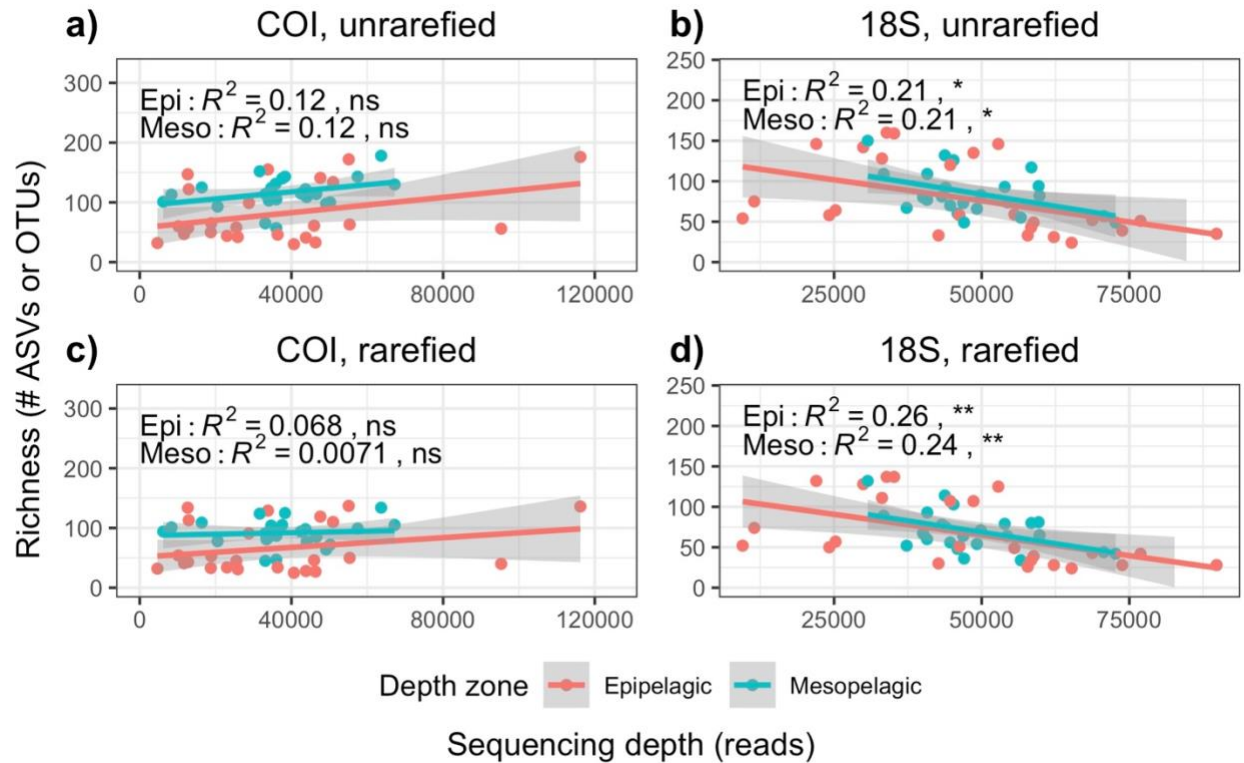


Figure S3.1. The Pearson correlation between sequencing depth prior to rarefaction and observed richness of a) and c): COI OTUs pre- and post- rarefaction; and b) and d): 18S ASVs pre- and post-rarefaction. Coral points and lines represent epipelagic samples (0-200m) and teal points and lines represent mesopelagic samples (200-1000m). Rarefied richness in (c) and (d) are plotted against the original sequencing depth, as samples have identical numbers of reads post-rarefaction. Shading surrounding lines represents the 95% CI. (ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

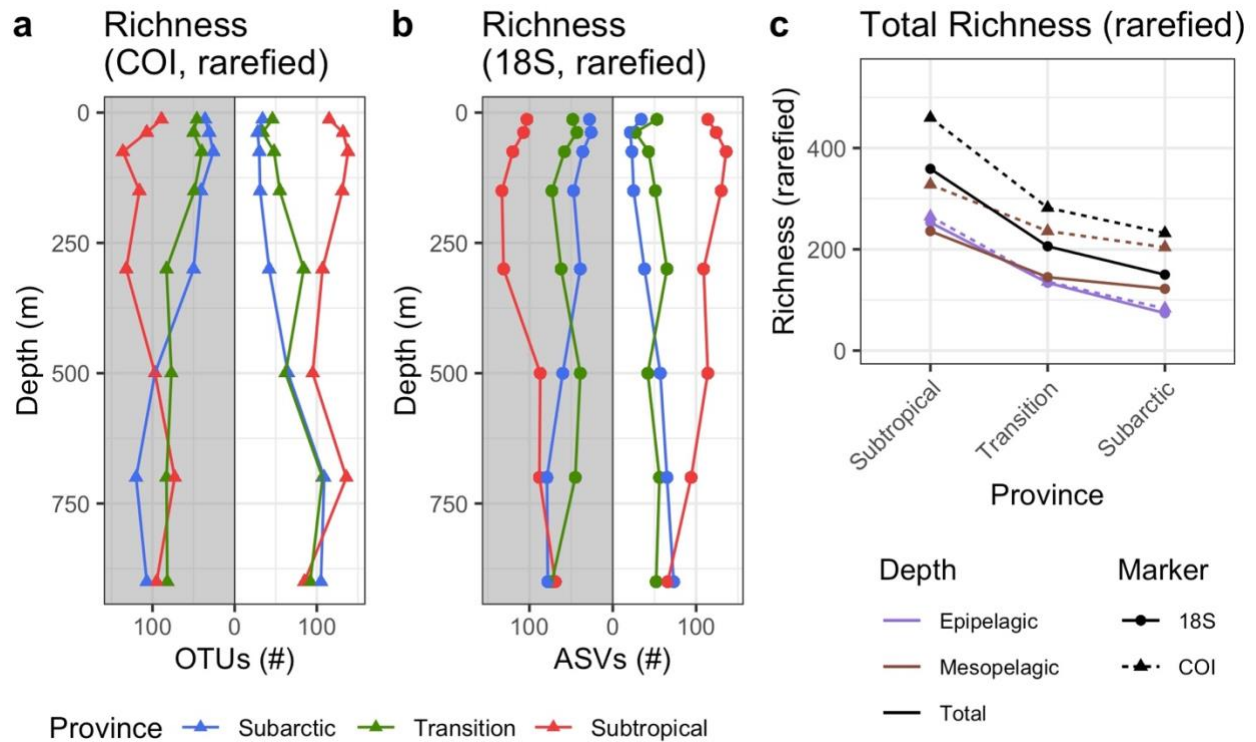


Figure S3.2. Taxonomic richness, calculated from rarefied datasets, across the three biogeographic provinces for (a) COI OTUs across depth (b) 18S ASVs across depth, and (c) total richness within the epipelagic zone, mesopelagic zone, and across the full 0-1000m sampled. For panels (a) and (b), points are plotted at the midpoint of each discrete zooplankton net range, with nighttime samples on the left (gray background) and daytime samples on the right (white background). For panel (c), colors indicate depth zones and line types denote marker. Richness is the total number of OTUs/ASVs for COI or 18S, respectively.

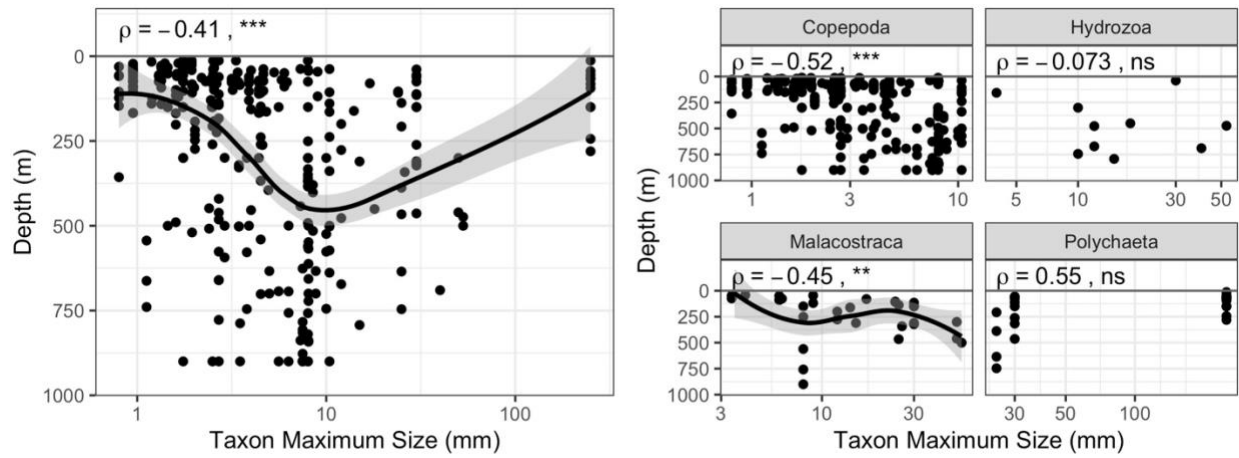


Figure S3.3. The Spearman rank correlation between the maximum reported size and the observed mean depth of occurrence for (a) all ASVs and OTUs together; and (b) ASVs and OTUs within taxonomic classes with at least 10 unique taxa. Loess best fit lines are shown for significant relationships. Shading surrounding lines represents the 95% CI. (ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

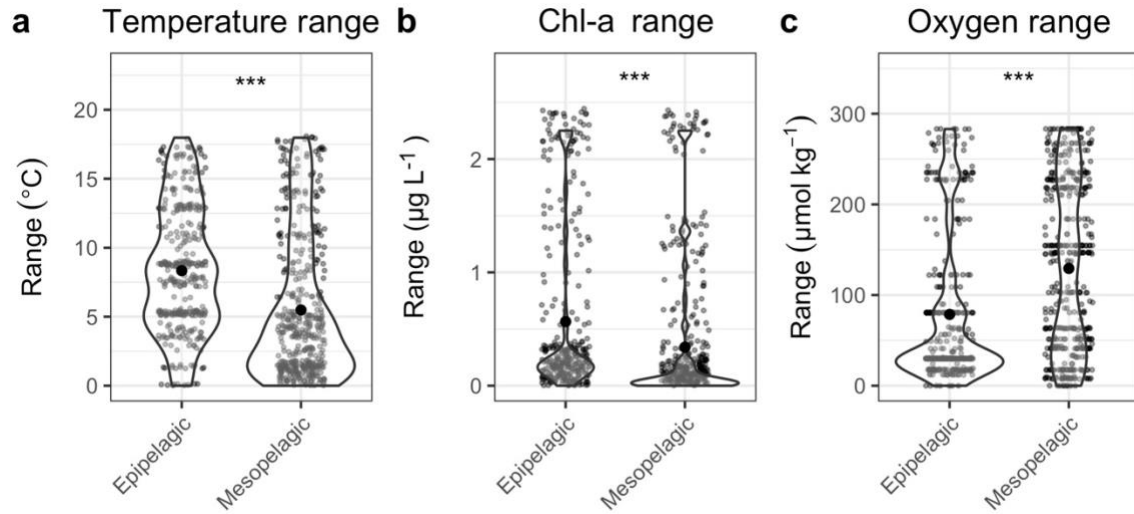


Figure S3.4. Epipelagic and mesopelagic OTUs and ASVs and the environmental ranges across which they were observed, for (a) temperature; (b) Chl-a fluorescence; and (c) dissolved oxygen. Violin plots showing the density distribution of points are overlaid on jittered data. Points are semi-opaque, variability in the intensity of color is due to overlapping data points. (Kruskal-Wallis: ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

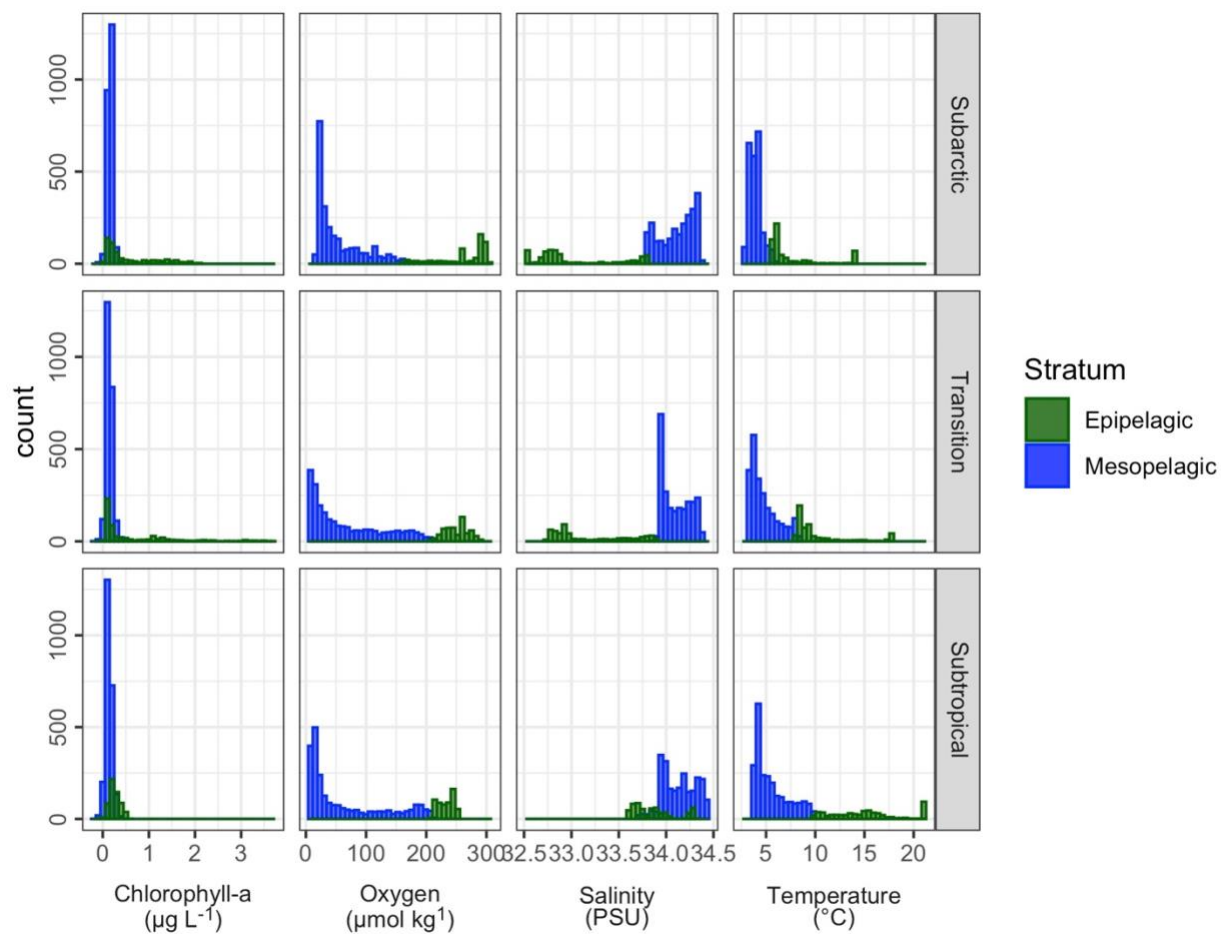


Figure S3.5. Histograms of the observed physical environment within the epipelagic zone (0-200m, green) and mesopelagic zone (200-1000m, blue) for temperature, salinity, Chl-a fluorescence, and dissolved oxygen (columns), within the Subarctic, Transition, and Subtropical biogeographic provinces (rows). Data are 1-meter averaged values from 0-1000m CTD casts.

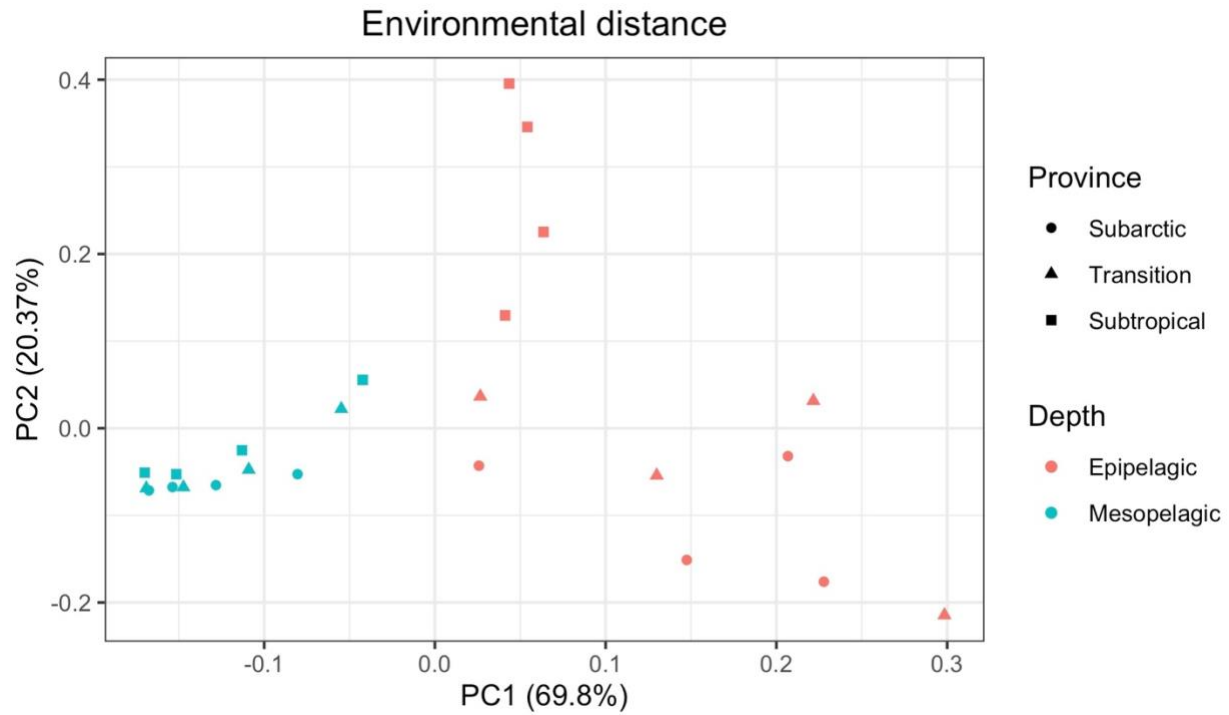


Figure S3.6. Principal component analysis of the environment observed within each net, including mean temperature, chl-a fluorescence, salinity, and dissolved oxygen. All environmental variables were scaled and centered prior to calculating distance. Shapes indicate different biogeographic provinces, and colors denote vertical depth zones.

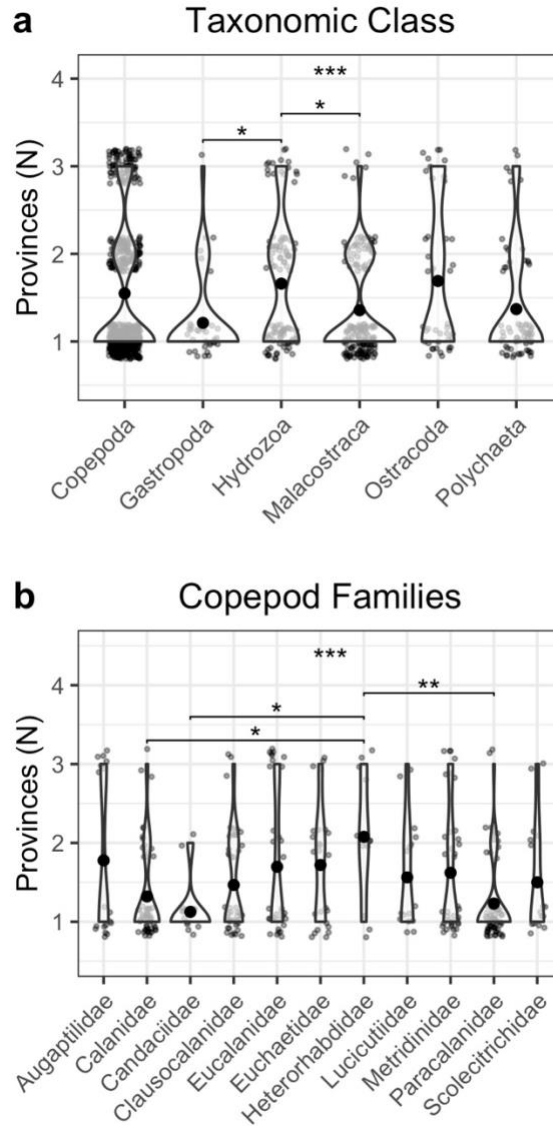


Figure S3.7. Number of provinces in which OTUs and ASVs were observed, divided by (a) taxonomic class; and (b) taxonomic family within the class Copepoda. Only taxonomic groups with at least 10 unique taxa are shown. Violin plots showing the density distribution of points are overlayed on data points jittered within each categorical value. Data points are semi-opaque, variability in color is due to overlapping data points. (ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

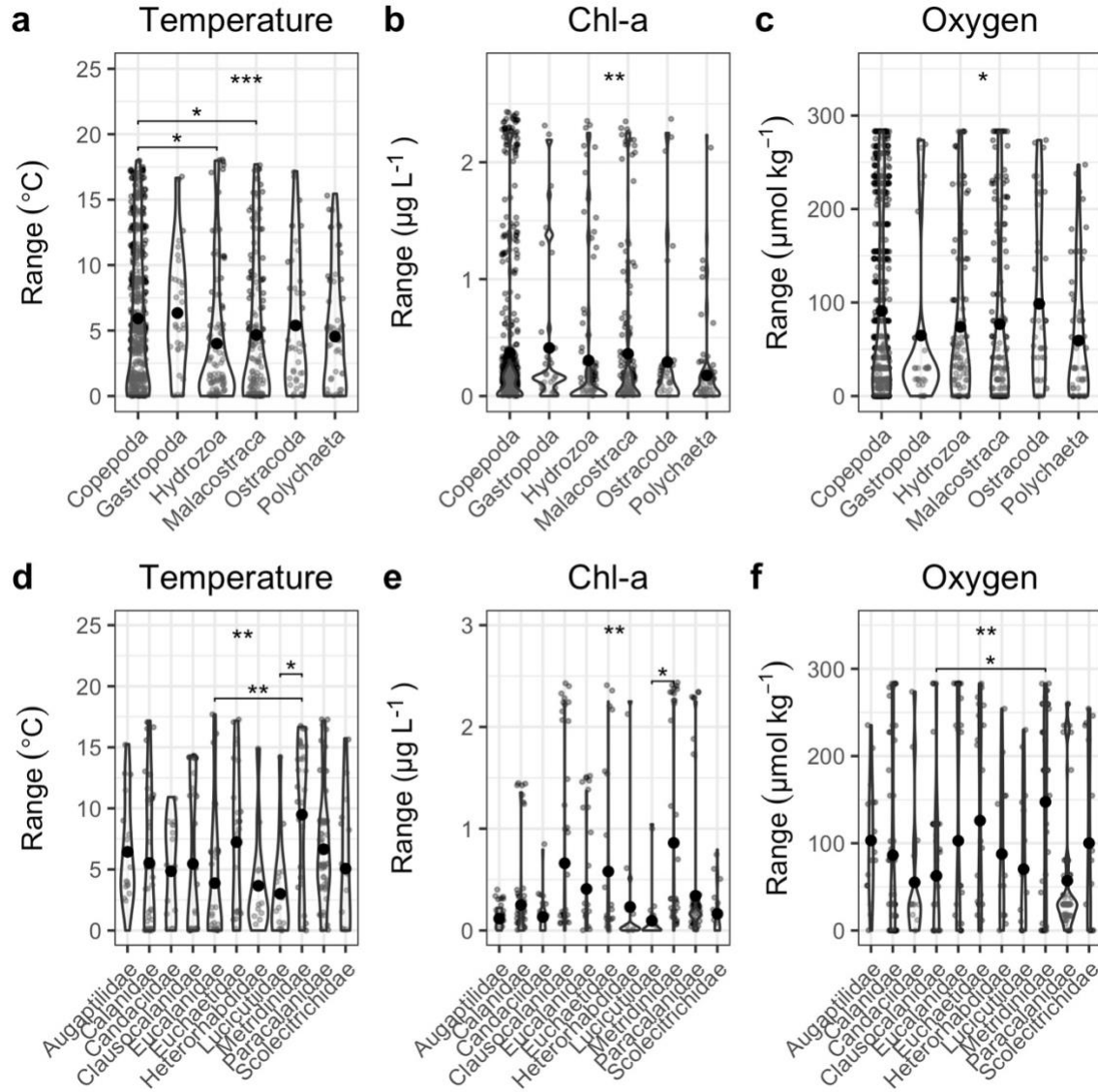


Figure S3.8. For each taxonomic group, OTUs and ASVs are plotted according to the environmental range across which they were observed, for (a) taxonomic class and temperature; (b) taxonomic class and Chl-a; (c) taxonomic class and oxygen; (d) copepod family and temperature; (e) copepod family and Chl-a; and (f) copepod family and oxygen. Violin plots showing the density distribution of points are overlaid on data points jittered within each categorical value. Data points are semi-opaque, variability in the intensity of color are due to overlapping data points. (ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Supplemental tables

Table S3.1. Summary statistics for each biological sample at the two molecular marker regions (COI and 18S), calculated for the raw sequencing data and the zooplankton sequencing remaining after bioinformatic processing, quality filtering, and removal of contaminants.

<u>COI</u>			
<i>Raw Sequences</i>		<i>Zooplankton Sequences</i>	
Total	3,834,095	Total	2,411,212
Average	79,877	Average	50,234
Max	211,782	Max	139,550
Min	23,877	Min	7,430
<u>18S</u>			
<i>Raw Sequences</i>		<i>Zooplankton Sequences</i>	
Total	2,393,381	Total	2,295,818
Average	49,862	Average	47,830
Max	89,999	Max	89,795
Min	12,148	Min	9,514

Table S3.2. Sample metadata and total sequencing depth at each marker for each biological sample after merging technical replicates. Sample ID: Unique sample ID including MOCNESS tow number and net number; Initial COI sequences: Number of raw sequences recovered at the COI marker after *dada2* denoising; COI sequences, zooplankton: Number of zooplankton sequences retained at the COI marker after removal of contaminants, unidentified sequences, and non-zooplankton taxa; Initial 18S sequences: Number of raw sequences recovered at the 18S marker after *dada2* denoising; 18S sequences, zooplankton: Number of zooplankton sequences retained at the 18S marker after removal of contaminants, unidentified sequences, and non-zooplankton taxa; Cruise: Cruise ID where samples were collected (New Horizons, 2012); Station: Cruise station; Tow: MOCNESS tow number; Net: MOCNESS net number; Min. Depth: minimum of the depth range sampled with this MOCNESS net; Max depth: maximum of the depth range sampled with this MOCNESS net; Diel: classification of sampling time as day or night. (continued on next page)

Sample ID	COI sequences, initial	COI sequences, zooplankton	18S sequences, initial	18S sequences, zooplankton	Cruise	Station	Tow	Net	Min. depth	Max. depth	Diel
M7N1	78404	63306	43452	43272	NH1208	7	7	1	796.8	1002	day
M7N2	130072	95042	55059	54337	NH1208	7	7	2	600.9	795.7	day
M7N3	86585	60282	49639	49194	NH1208	7	7	3	400.7	599.9	day
M7N4	60322	37182	72953	72692	NH1208	7	7	4	201.5	400.1	day
M7N5	28289	8118	58157	57803	NH1208	7	7	5	101.4	200.8	day
M7N6	89425	49640	65505	65233	NH1208	7	7	6	51.7	101.1	day
M7N7	81046	54990	62304	62230	NH1208	7	7	7	26.8	51.2	day
M7N8	85635	50858	58640	58382	NH1208	7	7	8	-1.4	26.2	day
M8N1	73809	57919	59865	59674	NH1208	7	8	1	785	999.2	night
M8N2	75733	56991	54330	53940	NH1208	7	8	2	600.9	785.3	night
M8N3	82610	55451	47474	46864	NH1208	7	8	3	494.6	600.3	night
M8N4	49100	34935	47098	47018	NH1208	7	8	4	300.6	493.8	night
M8N5	23877	13544	24787	24202	NH1208	7	8	5	100.2	299.6	night
M8N6	88675	41959	61524	58804	NH1208	7	8	6	51.1	98.7	night
M8N7	71259	29860	74316	73804	NH1208	7	8	7	24.5	51.3	night
M8N8	90926	52816	43006	42698	NH1208	7	8	8	-0.3	24.2	night
M11N1	83997	70131	37802	37288	NH1208	15	11	1	801.2	1000	day
M11N2	74405	50050	45014	44665	NH1208	15	11	2	601.5	800.5	day
M11N3	56402	52559	70972	70699	NH1208	15	11	3	400.8	601.1	day
M11N4	60723	44208	60157	59797	NH1208	15	11	4	201.4	400.4	day
M11N5	34970	24890	55828	55535	NH1208	15	11	5	102.6	200.7	day
M11N6	99115	65140	69003	68754	NH1208	15	11	6	52.2	102.2	day
M11N7	109654	68669	89999	89795	NH1208	15	11	7	25.8	51.8	day
M11N8	98319	59760	13638	9514	NH1208	15	11	8	0.3	25.7	day
M12N1	70032	61799	50236	49848	NH1208	15	12	1	799.3	998.2	night

Table S3.2. (*continued*) Sample metadata and total sequencing depth at each marker for each biological sample after merging technical replicates. Sample ID: Unique sample ID including MOCNESS tow number and net number; Initial COI sequences: Number of raw sequences recovered at the COI marker after *dada2* denoising; COI sequences, zooplankton: Number of zooplankton sequences retained at the COI marker after removal of contaminants, unidentified sequences, and non-zooplankton taxa; Initial 18S sequences: Number of raw sequences recovered at the 18S marker after *dada2* denoising; 18S sequences, zooplankton: Number of zooplankton sequences retained at the 18S marker after removal of contaminants, unidentified sequences, and non-zooplankton taxa; Cruise: Cruise ID where samples were collected (New Horizons, 2012); Station: Cruise station; Tow: MOCNESS tow number; Net: MOCNESS net number; Min. Depth: minimum of the depth range sampled with this MOCNESS net; Max depth: maximum of the depth range sampled with this MOCNESS net; Diel: classification of sampling time as day or night.

Sample ID	COI sequences, initial	COI sequences, zooplankton	18S sequences, initial	18S sequences, zooplankton	Cruise	Station	Tow	Net	Min. depth	Max. depth	Diel
M12N2	79378	48512	46532	45914	NH1208	15	12	2	699.8	798	night
M12N3	54204	47828	56657	56631	NH1208	15	12	3	399.4	699.5	night
M12N4	70079	51991	41981	40752	NH1208	15	12	4	184.4	398.1	night
M12N5	33486	29853	12148	11479	NH1208	15	12	5	101.1	183.9	night
M12N6	107889	101432	27160	25264	NH1208	15	12	6	51.4	101.3	night
M12N7	95744	70590	77828	76901	NH1208	15	12	7	25.5	51.9	night
M12N8	87616	78745	46730	46248	NH1208	15	12	8	-0.6	25.2	night
M26N1	67912	63175	43930	43892	NH1208	34	26	1	800.3	1001	night
M26N2	76452	24085	58590	58423	NH1208	34	26	2	600.4	799.8	night
M26N3	111960	10793	34192	33407	NH1208	34	26	3	401.9	600.1	night
M26N4	75816	43824	42731	30710	NH1208	34	26	4	200.3	401.2	night
M26N5	32263	18655	49436	35181	NH1208	34	26	5	101.6	200.3	night
M26N6	211782	139550	68735	52832	NH1208	34	26	6	51.4	101.4	night
M26N7	106544	58596	52643	48621	NH1208	34	26	7	26.6	51.1	night
M26N8	114495	37242	47161	44696	NH1208	34	26	8	1.4	26.8	night
M25N1	50446	47668	40155	40079	NH1208	34	25	1	785.9	995.3	day
M25N2	85056	71596	40824	40777	NH1208	34	25	2	600.5	784.6	day
M25N3	77961	7430	43944	43762	NH1208	34	25	3	400.7	599.9	day
M25N4	70246	20860	45539	45246	NH1208	34	25	4	201.2	400.2	day
M25N5	29542	18009	23452	21999	NH1208	34	25	5	101.2	201.4	day
M25N6	109332	67582	53841	33908	NH1208	34	25	6	51.8	101.9	day
M25N7	102710	39810	34134	29934	NH1208	34	25	7	23.6	52	day
M25N8	99798	53287	34280	33120	NH1208	34	25	8	0.6	23.6	day
NC	1625	109	0	0	nc	na	na	na	na	na	na

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CHAPTER 4

Distributions of mesopelagic zooplankton across the North Pacific Basin: testing the effects of physical dispersal vs. environmental selection

ABSTRACT

At basin scales, marine zooplankton communities are often associated with major oceanographic circulation features with distinct biogeochemical environments. Slower circulation patterns and increased environmental homogeneity in the deep ocean may lead to differences in biogeographic ranges among zooplankton inhabiting different depths. However, the biogeographic distributions of mesopelagic zooplankton are not well defined. We used metabarcoding of two molecular markers (18S and COI) to assess zooplankton community composition in the upper 1000 m of the water column at 28 locations across the North Pacific Ocean, between 0 – 57 ° N and 138 ° E – 118 ° W. We compared distributional ranges and community similarity across epipelagic and mesopelagic depths to test the relative effects of dispersal potential and environmental selection. Among the epipelagic, upper mesopelagic, and lower mesopelagic depth zones, dispersion of beta diversity was comparable. We found no differences in the biogeographic ranges of taxa associated with each depth zone, but observed larger temperature, salinity, and dissolved oxygen ranges as well as narrower potential food ranges for deeper-dwelling taxa. Our results indicate that epipelagic and mesopelagic zooplankton communities have similar levels of species turnover, despite differences in the magnitude of environmental gradients and dispersal potential. We found significant relationships between community dissimilarity and potential dispersal distance, inferred from a global

circulation model, as well as between community dissimilarity and differences in temperature, salinity, dissolved oxygen concentration, and food flux. Our models explained greater proportions of community dissimilarity in the epipelagic zone than in the mesopelagic zone, demonstrating the complexity of the mechanisms shaping mesopelagic biogeography. Throughout the epipelagic and mesopelagic North Pacific, we find that biogeographic patterns are best explained through a combination of dispersal potential and environmental selection.

4.1. INTRODUCTION

In the pelagic ocean, zooplankton species are often distributed broadly throughout a water mass, but at the boundaries between water masses, biogeographic transitions can be very abrupt (McGowan, 1986). In many cases, distributional patterns associated with major water masses are shared by multiple zooplankton species from different taxonomic groups (Fager & McGowan, 1963). Biogeographic distributions are well-described for many zooplankton species in the Pacific Ocean (e.g. Alvarino, 1965, 1971; Brinton, 1962; McGowan & Walker, 1979). However, most analyses of mesozooplankton biogeography have been restricted to epipelagic taxa. The biogeographic distributions of mesopelagic zooplankton are not well documented, largely due to the technical difficulty and high costs associated with sampling this sparsely inhabited environment (Hidalgo & Browman, 2019; Robinson et al., 2010). The paucity of described biogeographic distributions for midwater zooplankton species leads to uncertainty about the primary mechanisms shaping zooplankton distributions in the mesopelagic zone.

Dispersal, the movement or physical transport of organisms from one location to another, is one of the core ecological mechanisms shaping species distributions and community composition (Vellend, 2016). Limited dispersal can lead to decreasing community similarity as

distance between sampling locations increases, even across a homogenous environment (Morlon et al. 2008). The shape of this relationship, termed the distance-decay relationship, varies among ecosystems and taxa (Chai et al., 2022; Nakaoka et al., 2006; Wetzel et al., 2012). The strength of the distance-decay relationship also depends on dispersal mechanisms, with stronger decay for species with lower dispersal capacity (Astorga et al., 2012; Nekola & White, 1999). In the pelagic ocean, dispersal is driven by movement of water masses, particularly for planktonic species that cannot swim against ocean currents (Bucklin, 1995). Epipelagic circulation patterns can influence both community composition (Lilly et al., 2022) and genetic structure within populations (Goetze, 2011). In comparison to the surface ocean, mesopelagic currents are slower and less defined (Kawabe & Fujio, 2010; Reid et al., 1978; Xie & Zhu, 2008). Slow ocean currents at depth have the potential to limit physical mixing of communities, leading to lower rates of dispersal and increased heterogeneity in community composition. However, the strength and shape of the distance decay relationship for mesopelagic zooplankton communities has not been previously described.

Selection is shaped by the combined effects of competition for resources, inter-individual differences in fitness, and variability in environmental conditions (Vellend, 2010). In the pelagic ocean, environmental tolerance, competition, and selection among zooplankton species are difficult to observe *in situ* (Boltovskoy, 1998). However, spatial changes in zooplankton community composition often align with strong biogeochemical gradients (Reygondeau & Dunn, 2018). Decreased habitat suitability at the edges of species ranges has been detected through analyses of cohort structure (Hirst et al., 2003; Rumrill, 1990); as well as through examination of specimen physiology such as lipid content or carbon:nitrogen ratio (Ikeda, 1974; Roncalli et al., 2019). In the epipelagic zone, zooplankton ranges most commonly covary with temperature,

likely due to the combined effects of decreased food availability and increased metabolic rates at warmer temperatures (Evans et al., 2020; Gregory et al., 2009). In the mesopelagic ocean, temperature and salinity are determined by major water masses, and exhibit less spatial variability than in the epipelagic ocean (Reygondeau et al., 2013, 2018). Food availability in the mesopelagic zone is primarily influenced by export flux from the overlying surface waters and can be decoupled from mesopelagic temperatures (Poteau et al., 2017). Export processes can dampen the small-scale spatial and temporal variability in production rates that are found in the epipelagic zone (Siegel & Deuser, 1997; but see Smith et al., 2018; Stukel et al., 2018). In general, there is less variability in temperature, salinity, and food availability in the mesopelagic zone, compared to the epipelagic zone. The exception to decreased environmental heterogeneity at depth is in concentrations of dissolved oxygen (DO), which exhibit variable pelagic minima within the mesopelagic zone (Paulmier & Ruiz-Pino, 2009). Oxygen minimum zones (OMZs) and other areas of low oxygen can have abrupt edges, and these mesopelagic gradients in DO are often stronger than epipelagic DO variability (Gilly et al., 2013). If mesopelagic taxa have similar tolerances for temperature, salinity, and food availability, they may exhibit broader biogeographic distributions due to larger mesopelagic habitats within a tolerance range. This could lead low levels of spatial heterogeneity in community composition.

The relative effects of dispersal and environmental selection due to temperature, salinity, food availability, or DO concentrations on the biogeographic distributions and community composition of mesopelagic zooplankton are unknown. In the mesopelagic zone of the North Pacific, there is greater similarity among mesopelagic copepod communities than among epipelagic copepod communities (Hirai et al., 2020). These authors also found significant effects of temperature on community composition, indicating dominance of selective processes. In

contrast, a comparison of mesozooplankton collected across the Pacific, Indian, and Atlantic Oceans found greater dissimilarity among zooplankton communities in the mesopelagic zone than communities in the epipelagic zone (Canals et al., 2023). This greater dissimilarity at depth was observed both within and among ocean basins and was associated with stronger effects of dispersal limitation in deep-water communities with weaker circulation. However, this study primarily sampled the tropical and subtropical North Pacific, not capturing the strong latitudinal gradients observed by Hirai et al. (2020). At global scales, mesopelagic zooplankton community composition assessed by the relative abundance of nineteen major taxonomic groups was best associated with DO and suspended particle concentrations (Soviadan et al., 2022). The coarse taxonomic resolution of this analysis limits comparisons with molecular analyses but suggests important influences of vertical connectivity on mesopelagic zooplankton community structure. These contradictory results from the few studies examining spatial patterns of beta diversity in the mesopelagic zone highlight the need for a mechanistic understanding of the processes shaping mesopelagic biogeography.

Here, we examine the relative importance of dispersal and selection in shaping the biogeographic distributions of mesopelagic zooplankton, and thus community composition in the mesopelagic zone. We use metabarcoding at two molecular marker regions to analyze zooplankton samples collected between the surface and 1000m at 28 locations across the North Pacific basin. We tested 1) whether there is greater similarity among zooplankton communities at mesopelagic depths than communities in the epipelagic zone, 2) whether biogeographic ranges are larger for mesopelagic zooplankton species than for epipelagic species, and 3) whether horizontal turnover in epipelagic and mesopelagic zooplankton community composition is

predicted by variability in temperature, salinity, DO concentrations, food availability, or dispersal potential.

4.2. MATERIALS & METHODS

4.2.1. Zooplankton collections

Depth-stratified zooplankton net samples were collected at 32 locations across the North Pacific Basin between 2011 and 2021 (Table S4.1, Fig. 4.1). Sampling locations extended across six biogeographic regions, defined by geographic location, water masses, and oxygen and chlorophyll values (Figs. 4.1, S4.1). Sampling methods included vertically towed VMPS (Terazaki and Tomatsu 1997), vertically towed MultiNet (Hydro-Bios 2020), and obliquely towed MOCNESS (Wiebe et al. 1985), with net mesh sizes ranging from 100 μm to 202 μm , all samples from 1,000 m to the surface. Upon net recovery, samples were either split quantitatively, size-fractionated, or entirely preserved in 70-95% non-denatured EtOH. Full descriptions of sample collection, processing, and preservation have been previously published for all locations (Blanco-Bercial, 2020; Hirai et al., 2020; Questel et al., 2021) except 24-26, which follow the methods described for previous studies in the California Current Ecosystem Long Term Ecological Research program (Matthews et al., 2021).

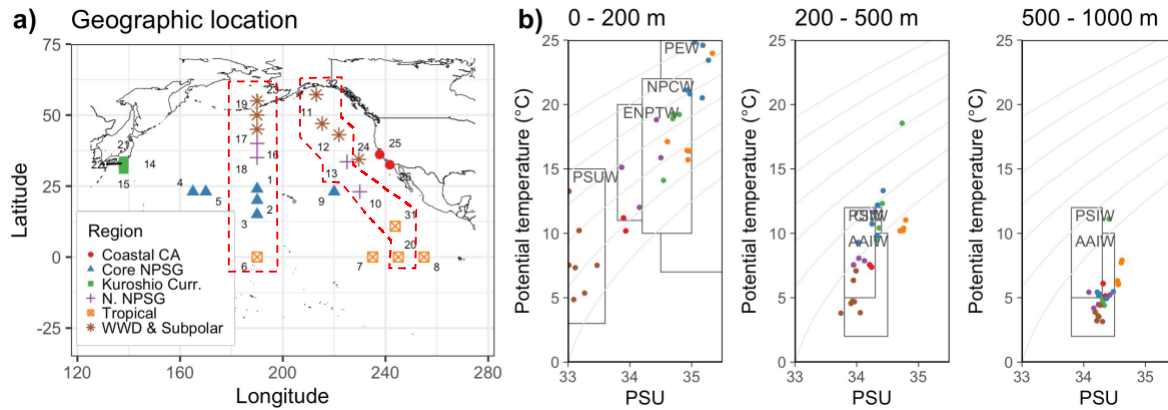


Figure 4.1. Zooplankton sampling locations included in this study; a) geographic locations across the North Pacific color coded by biogeographic region and with latitudinal transects highlighted in red; b) in temperature-salinity space, superimposed on the major water masses of the Pacific Ocean. 0 – 200 m: PSUW, Pacific Subarctic Upper Water; NPCW, North Pacific Central Water; ENPTW, Eastern North Pacific Transition Water; PEW, Pacific Equatorial Water. 200 – 500 m and 500 – 1000 m: PSIW, Pacific Subarctic Intermediate Water; CIW, California Intermediate Water; AAIW, Antarctic Intermediate Water. Colors and shapes denote biogeographic regions, assigned based on sample location and environmental similarity (Fig S4.1).

4.2.2. DNA extraction, amplification, and sequencing

DNA extraction methods varied among samples. Samples from locations 1-10 and 14-23 were extracted using a Gentra Puregene Cell and Tissue Kit (QIAGEN), as described previously (Hirai et al., 2020). Samples from locations 11-13 were extracted using a modified protocol for the E.Z.N.A.® Mollusc DNA Kit (Blanco-Bercial, 2020). For samples from locations 24-26 and 31, DNA was extracted using modified E.Z.N.A Blood and Tissue Maxi Kits (OMEGA), or for samples processed after Blood and Tissue Maxi Kits were discontinued, E.Z.N.A Blood Maxi Kits modified to include overnight incubations and chloroform:isoamyl phase extraction (Matthews et al., 2021, Appendix C). Samples from location 32 were extracted with the E.Z.N.A.® Mollusc DNA Kit, modified to include mechanical lysis and overnight incubation (Questel et al., 2021). For locations 1-26 and 31, the DNA concentration of each sample was quantified with PicoGreen or Qubit assays, then normalized to 4 ng/μl. Normalized DNA was amplified with

two-step PCR and library preparation with duplicate dual-indexed primers, as described previously (Matthews et al., 2021). Briefly, for each biological sample, triplicate technical replicates with unique indices were prepared at the nuclear 18S-V4 (Zhan et al., 2013) and mitochondrial COI (Leray et al., 2013) marker regions. These two marker regions provide complementary representations of the zooplankton community, with broader taxonomic coverage at the more conserved 18S gene and better resolution between closely related species at the more rapidly evolving COI gene (Bucklin et al. 2021). In addition to complementary resolution for overlapping target taxa, there are some major zooplankton groups primarily resolved by one or the other marker (Matthews et al. 2021). To minimize stochastic variability, the first PCR reaction for each replication was performed twice, with the two reactions pooled and diluted 10x for template in the subsequent PCR reaction. The second PCR reaction appended an independent pair of indexes and the Illumina sequencing regions. Final PCR products were cleaned with AMPure magnetic beads, quantified with PicoGreen or Qubit assays, and pooled in equimolar concentrations for sequencing on an Illumina MiSeq with 300bp PE V3 chemistry. Negative controls from extraction kits and each PCR plate were included in the pooled library at the maximum volumes possible, to increase detection sensitivity to contamination. Mock communities with two compositions (even and staggered) were included as unique biological samples in the libraries for sample locations 1-25 and 31 (see Appendix D for details). Location 32 was amplified and sequenced in triplicate with modified iTru partial adapter sequences (Questel et al. *in prep*).

4.2.3. Bioinformatic processing

Sequences were demultiplexed using *cutadapt* or custom demultiplexing scripts (Martin, 2011; Questel et al., 2021). Only sequences containing all expected indexes were retained. Sequences were truncated where quality scores for the 25th percentile of sequences dropped below Q18, typically between 230 – 270 bp and 160 – 220 bp for forward and reverse reads, respectively. Bioinformatic filtering, denoising, and clustering were performed in QIIME2, using the *dada2* plugin (Bolyen et al., 2019; Callahan et al., 2016). At each marker, sequences were denoised into ASVs with pseudopooling among samples and pooled chimera detection, and all other default settings. ASVs at the 18S marker were treated as unique taxa, due to low levels of variability at this marker region (Wu et al., 2015). ASVs at the COI marker were clustered into OTUs at 97% similarity using the *vsearch* algorithm implemented in QIIME2, with all default settings (Bucklin et al., 2010; Rognes et al., 2016).

4.2.4. Sample and amplicon filtering and normalization

Replicate technical samples at each marker (n=3) were visually assessed to identify outliers using Non Metric Multidimensional Scaling (NMDS) visualizations of Bray-Curtis distance. Outlier replicates were removed, including all samples without high similarity between at least two replicates. After removal of outlying samples, independent sequencing runs were compared by visual examination of mock communities, including examination of taxon relative abundances, NMDS visualization of Bray-Curtis distance, and comparison of total richness, diversity, and evenness. Outlier libraries were identified through visual analysis of mock community dissimilarity and were removed from all subsequent analyses (Fig S4.2). ASVs/OTUs occurring in only a single replicate were removed, with retention of ASVs/OTUs

occurring in more than one replicate even if the replicates were from different biological samples. ASVs/OTUs were classified using the RDP Naive Bayesian Classifier implemented in *dada2* (Callahan et al., 2016; Caporaso et al., 2010). Classification was based on sequences in the MetaZooGene databases for 18S and COI (v.2023-m01-12) (Bucklin et al., 2021) trimmed to the target region for each marker. Sequences unidentified at class were removed, as were all non-zooplankton sequences.

Technical replicates were summed, followed by unweighted averaging of discrete samples within the three target depth zones, which were 0 – 200 m (epipelagic), 200 – 500 m (upper mesopelagic), and 500 – 1000 m (lower mesopelagic). As significant variability exists in our sampling methods, including in net mouth size ($0.5 \text{ m}^2 - 1.0 \text{ m}^2$), mesh size (100 μm , 150 μm , and 202 μm), and tow type (oblique and vertical), we did not attempt to further normalize vertical sampling effort by weighting the number of reads summed from samples within each depth zone. We tested two methods to normalize variability in the net mesh used for original sampling: normalized amplicon abundances with sample-weighted Partial Least-Squares Discriminant Analysis (swPLSDA) (Wang & Lê Cao, 2023), and Conditional Quantile Regression (ConQuR) (Ling et al., 2022). For swPLSDA, we tested for batch effects of mesh size using depth zone and biogeographic region as control variables. As swPLSDA analysis requires amplicon abundances greater than 10 and variances below than 0.05%, mesh-size corrected community composition with this method was restricted to the most abundant 43% of ASVs and 46% OTUs. For ConQuR, mesh-size corrected community composition was calculated for 100% of zooplankton ASVs/OTUs. Visual analysis of raw and corrected OTU tables and PCA density distributions, as well as PERMANOVA tests comparing the amount of variability explained by mesh size in raw and corrected showed that neither swPLSDA nor ConQuR normalization

decreased the variability associated with mesh size (Fig. S4.3). Thus, we chose to use non-normalized datasets for all analyses.

4.2.5. Geographic distance

For geographic distance between sampling locations, we used the latitude and longitude of each location to calculate pairwise Great Circle distances based on the WGS84 ellipsoid (Karney, 2013).

4.2.6. Ocean circulation

To test for dispersal potential due to physical circulation, we modeled particle connectivity between each pair of stations at the midpoint of each vertical sampling range. We used climatological mean hydrodynamic fields of ocean circulation modeled by the OGCM For the Earth Simulator (OFES) (JAMSTEC, 2009; Masumoto et al., 2004). The OFES model is an eddy-resolving model with a horizontal resolution of $1/10^{\text{th}}^{\circ}$, and includes 54 depth levels of varying resolution, with 20 layers in the epipelagic (0 – 200 m depth), 9 in the upper mesopelagic (200 – 500 m depth), and 6 in the lower mesopelagic (500 – 1000 m depth). The model incorporates monthly mean wind stress (1950 - 1999), and surface heat flux from the National Centers for Environmental Prediction/National Center for Atmospheric Research (NCEP/NCAR) reanalysis outputs. In addition to advection fields, the full model includes viscosity and diffusivity calculated proportionally to differences between cells, and vertical boundary layer mixing. At boundary regions (continental and at the artificial 75°S and 75°N limits of the model), temperature and salinity are restored to monthly mean climatological values from the World Ocean Atlas (Talley et al., 2007).

We used velocity vectors from the one-month climatological means of the 41st year of the OFES model. These monthly means, with global 0.5 x 0.5 ° resolution, are provided through the Japan Agency for Marine-Earth Science and Technology for the promotion of the OFES research project (JAMSTEC, 2009). This approach does not account for interannual variability in circulation due to oscillations such as ENSO or the PDO. These interannual fluctuations can alter ocean transport volumes by up to 25% (Douglass et al., 2006). However, the velocities we use here capture the major basin-scale ocean currents and are sufficient for an initial investigation at large space and time scales. Monthly climatological mean velocities were interpolated for 30.437 days to build a yearly climatology, and the yearly climatology was extrapolated for 21 years. We implemented x-y velocity fields through Parcels (Probably A Really Computationally Efficient Lagrangian Simulator), a freely available Python project for particle tracking simulations (Delandmeter & van Sebille, 2019). Hydrographic fields were initiated from 30°S – 62°N and 100°E – 55°W. At each zooplankton sampling location, particles were seeded at each of 75 m, 350 m, and 750 m, with particles released every 12 hours for 365 days. A total of 731 particle trajectories were initiated per depth at each location and 2,193 particles total per location. We spread particle release across a full year to account for seasonal variability in ocean currents.

The simulation was run for 21 years with 72-hour timesteps (see Appendix E). Particles were tracked for a maximum of 20 years, and distances between each particle and all sampling locations was recorded at 30 day time intervals. Particles that were advected out of the defined hydrographic fields were removed from the experiment. Particles that passed within 350 km of a sampling location were designated as “arrived particles”. We used a radius of 350 km to include 200 km for physical mixing on time scales of approximately 1 month (the maximum temporal resolution of our environmental datasets, see Sections 4.2.7 – 4.2.8), plus the distance travelled

between saved time stamps. Arriving particles at each location were allowed to continue their trajectories for the duration of the experiment.

We calculated the proportion of particles released at each station that arrived at all other stations (percent arrival), the proportion of particles that did not arrive (percent lost), and the average age of arriving particles (dispersal time). We multiplied dispersal time by the percent of particles lost to obtain total dispersal distance for each pair of stations. This metric accounts both for the likelihood of physical transport between locations (percent arrival) and the time for potential loss due to processes such as predation (dispersal time).

We seeded particles at 75m, 350m, and 750m and calculated dispersal distance independently for each depth. As connectivity between locations is directional, we used the maximum calculated dispersal distance for each pair. This allows one location to serve as a source population for another, without requiring consideration of life history stage or age structure.

4.2.7. Modeled climatology

At each sampling location, we used monthly mean temperature and salinity values from the 41st year of the OFES model to calculate the environmental climatology. Values were averaged across a 200 km radius around each sampling location, to account for mixing of zooplankton communities on time scales of approximately 3-4 weeks. Depth-stratified means were subsequently averaged to obtain a mean value for each depth range sampled. For each sampling location, we calculated the mean temperature and salinity across the climatological year as well as the annual amplitude of variability.

4.2.8. Satellite-observed climatology

We estimated food availability, including net primary production (NPP), surface chlorophyll (Chl-*a*), and export flux (EF), from satellite-observed ocean color as follows. Five-day composites of Chl-*a* (mg m^{-2}) were calculated from the ocean color sensors SeaWiFS, MERIS, MODIS-Aqua, VIIRS-SNPP, and OLCI-A. Composites were gridded at 4.5 km resolution (Sathyendranath et al., 2019); missing pixels were linearly interpolated from previous and subsequent 5-day compositions. NPP ($\text{mg C m}^{-2} \text{ day}^{-1}$) was calculated with a modified VGPM algorithm (Behrenfeld & Falkowski, 1997; Kahru et al., 2009) from Chl-*a*, daily photosynthetically active radiation from NASA's Ocean Biology Processing Group (Frouin et al., 1989) and daily sea surface temperature from the 4 km resolution Advanced Very High Resolution Radiometer (Reynolds et al., 2007). Finally, EF ($\text{mg C m}^{-2} \text{ day}^{-1}$) was calculated according to the CCE-fitted algorithm with York Type II regression (Kahru et al., 2020; Kelly et al., 2018).

At each zooplankton sampling location, satellite values were averaged across a 200 km radius. Spatial averages were used to calculate monthly averages of each variable from 2010 – 2021. As for the modeled climatology, a 200 km radius was selected to account for lateral transport of primary production occurring on time scales of up to approximately 3 – 4 weeks, assuming an average horizontal velocity of 10cm/s in the epipelagic ocean (Fossette et al., 2012). In the mesopelagic ocean, the influence of surface productivity follows a statistical funnel, and the 200 km radius accounts for effects of surface communities on mesopelagic communities at time scales of up to 5 – 10 days, assuming particle sinking speeds of 50 – 100 m/d (Siegel & Deuser, 1997). Monthly averages were used to calculate the climatological mean, maximum, and minimum yearly values for each location, as well as mean, maximum, and minimum amplitude

of annual variability. NPP was used as a proxy for food production within the epipelagic, and EF as a proxy for food input to the system within the mesopelagic (both the 200 – 500 m and 500 – 1000 m depth zones).

4.2.9. *In situ* environment

Maximum, minimum, and mean temperature and salinity values for each depth range (0-200m, 200-500m, 500-1000m) were calculated for each location. For sampling locations 1-23 and 32, values were calculated from all CTD values within each depth bin, including day and night tows where applicable. For sampling location 31, values were calculated from all values within each depth bin from the net-associated sensors on the nighttime MOCNESS tow, as the pressure sensor was compromised on the daytime tow. For sampling locations 24-26, values were calculated from Cycle-averaged CTD values (available through CCE DataZoo). Oxygen data were converted from $\mu\text{mol kg}^{-1}$ to $\mu\text{mol L}^{-1}$ where necessary using the R package “respR” (Harianto et al., 2019). Data collected with SBE oxygen sensors in units of ml L^{-1} were converted to $\mu\text{mol L}^{-1}$ by multiplying by 44.6596, as described in the recommended best practices from SCOR WG 142 (Bittig et al., 2015). Moderate and severe hypoxia thresholds were defined as $122 \mu\text{mol L}^{-1}$ and $61 \mu\text{mol L}^{-1}$ ($\sim 4 \text{ mg L}^{-1}$ and $\sim 2 \text{ mg L}^{-1}$, respectively) (Chu & Tunnicliffe, 2015; Reygondeau et al., 2018). We calculated the shallowest depths at which oxygen concentrations dropped below these thresholds, the total depth range (m) within each net that was below each threshold, and the average mixed layer oxygen concentration (between 13-17 m, to account for variability in the minimum depths sampled and missing 1 m bins). We compared *in situ* mean temperature and salinity values to modeled monthly mean temperature and salinity values from the OFES dataset (Figs. S4.4, S4.5). As there was satisfactory

agreement between the modeled and *in situ* data, we used modeled temperature and salinity for all subsequent analyses, to better represent the climatological environment.

4.2.10. Statistical analyses

All statistical analyses were performed in R version 4.2.1 (2022-06-23) (R Development Core Team, 2009). Sampling locations were grouped into regions based on visual analysis of temperature and salinity, as well as geographic proximity. Zooplankton ranges were measured by presence at unique sampling stations (number of locations) and presence within biogeographic regions (number of regions), after rarefaction of read abundances with replacement. The preferred depth zone for each zooplankton taxon was calculated from weighted mean vertical depth of occurrence across all observations of the taxon. Richness was estimated from abundance and incidence of non-rarefied ASV and OTU counts using a bias-corrected Chao equation to account for absence of singletons (Chiu et al., 2014).

Environmental ranges (temperature, salinity, DO, NPP, Chl-*a*, EF) were calculated as the difference between the maximum and minimum of the mean environment observed within depth bins at each sampling location. To test for differences in biogeographic and environmental ranges, we used Kruskal-Wallis rank comparison, which is a non-parametric analog to a one-way ANOVA. Significant Kruskal-Wallis results were further investigated with post-hoc Dunn's tests to identify significant pairwise comparisons.

For all analyses of zooplankton community distance, Bray-Curtis dissimilarity (BCD) was calculated from relative abundance. Beta diversity within depth zones were compared using distance-based tests for homogeneity of dispersion around centroids, which is a multivariate extension of Levene's test (Anderson et al., 2006). To define environmental distance between

sampling locations, we calculated the differences between the mean values obtained from *in situ* CTD sampling and satellite data products. Environmental distance between samples was calculated independently for each environmental variable. Correlations of BCD against environmental and dispersal distances were tested with non-parametric Spearman rank order correlations, to allow for non-linear relationships and to account for non-normal distributions of BCD.

4.2.11. Generalized dissimilarity modeling

We used generalized dissimilarity models (GDMs) to test the predictive power of dispersal potential and biogeochemical environmental variability on beta diversity, measured by Bray-Curtis dissimilarity (Mokany et al., 2022). We fit GDMs independently for each depth zone, using temperature, salinity, DO, food availability, particle connectivity, and geographic distance as potential predictors. We tested climatological mean values for temperature and salinity, but used *in situ* measurements of DO. To account for potential food input to the system, we used the mean annual total NPP for samples collected in the upper 200 m, and the mean annual total EF for samples collected from 200 – 1000 m. We calculated overall model significance and the importance of each variable by comparing model fit of the real data against randomized permutations of the data ($n = 1000$) for the full model and for each variable independently. Sequential removal of the least important variable was repeated until all remaining predictors were significant ($p < 0.05$).

4.3. RESULTS

4.3.1. *Community composition and richness*

The zooplankton community resolved by both the 18S and COI markers was dominated by arthropod reads, across all depth zones and all biogeographic regions (Fig. 4.2). In the 18S resolved zooplankton community, molluscs and annelids were the next most abundant phyla (Fig. 4.2a). Mollusc reads were most abundant in epipelagic depth zone of the Core NPSG, as well as the West Wind Drift and Subpolar region, and unevenly distributed across samples (Fig. S4.6a). Annelid reads were most abundant in the epipelagic depth zone of the Northern NPSG. In the COI resolved zooplankton community, cnidarians were the second most abundant group by relative read abundance, followed by chaetognaths (Fig 4.2b). Cnidarian reads were relatively more abundant in the lower mesopelagic depth zone, particularly in the Coastal CA, Kuroshio Current, Northern NPSG, and Tropical biogeographic regions (Fig. 4.2b). In the Core NPSG and WWD and Subpolar regions, cnidarians were also more abundant in the epipelagic depth zone. Both cnidarian and chaetognath reads were distributed across multiple unique samples within each biogeographic region (Fig. S4.6b). Within the phylum Arthropoda, reads associated with calanoid copepods were the most abundant, followed by cyclopoid copepods (including Ergasilida, previously Poecilostomatoida) at the 18S marker and ostracods (Halocyprida) at the COI marker, then by euphausiids at both markers (Fig. 4.2c, d). Cyclopoid copepods were relatively more abundant in the epipelagic and upper mesopelagic depth zones, while ostracods were primarily observed at upper and lower mesopelagic depths (Fig. 4.2c, d, S4.6c, d). Euphausiids were also more abundant in the epipelagic and upper mesopelagic and were most abundant within the Coastal CA and WWD & Subpolar biogeographic regions. In the mesopelagic depth zones, decapods and lophogastrids were relatively more abundant.

Patterns of zooplankton diversity also revealed the importance of crustacean taxa in our samples. Arthropods comprised 6 of the 13 most diverse taxonomic orders (Calanoida, Cyclopoida, Euphausiacea, Decapoda, Halocyprida, and Amphipoda) (Fig. 4.3). At both markers, the highest diversity was within Calanoida, followed by Cyclopoida at the 18S marker and Halocyprida at the COI marker (Fig. 4.3a, b). Worms (Phyllodocida, Spionida), cnidarians (Coronatae, Trachymedusae), pteropods (Pteropoda), and siphonophores (Siphonophorae) also exhibited moderate levels of diversity. Total richness at COI primarily reflects calanoid copepod and ostracod richness patterns, while 18S richness primarily reflects the diversity of calanoid and cyclopoid copepods.

Among depth zones and transects, latitudinal richness peaks for the full zooplankton community were most commonly observed in the subtropical region (Fig. 4.3, S4.7). However, local richness maxima were also observed at the northernmost temperate latitudes in the Eastern Pacific transect, across all depth zones at the 18S marker (Fig 4.3a, right column) and in the upper mesopelagic depth zone at the COI marker (Fig 4.3b, middle right panel). In the Central Pacific transect, the 18S marker also detected temperate peaks in richness, although the magnitude and location of these peaks varied across depth, occurring at 50 ° N in the epipelagic depth zone, at 45 ° N in the upper mesopelagic depth zone, and at 55 ° N in the lower mesopelagic depth zone (Fig 4.3a, left column). We also observed equatorial richness peaks in the Central Pacific within the COI-resolved zooplankton community, and in the lower mesopelagic depth zone equatorial richness was higher than the subtropical peak (Fig. 4.3b, left column). When summed across depth, latitudinal richness maxima were found at subtropical latitudes in both the Central and Eastern Pacific (Fig. S4.7).

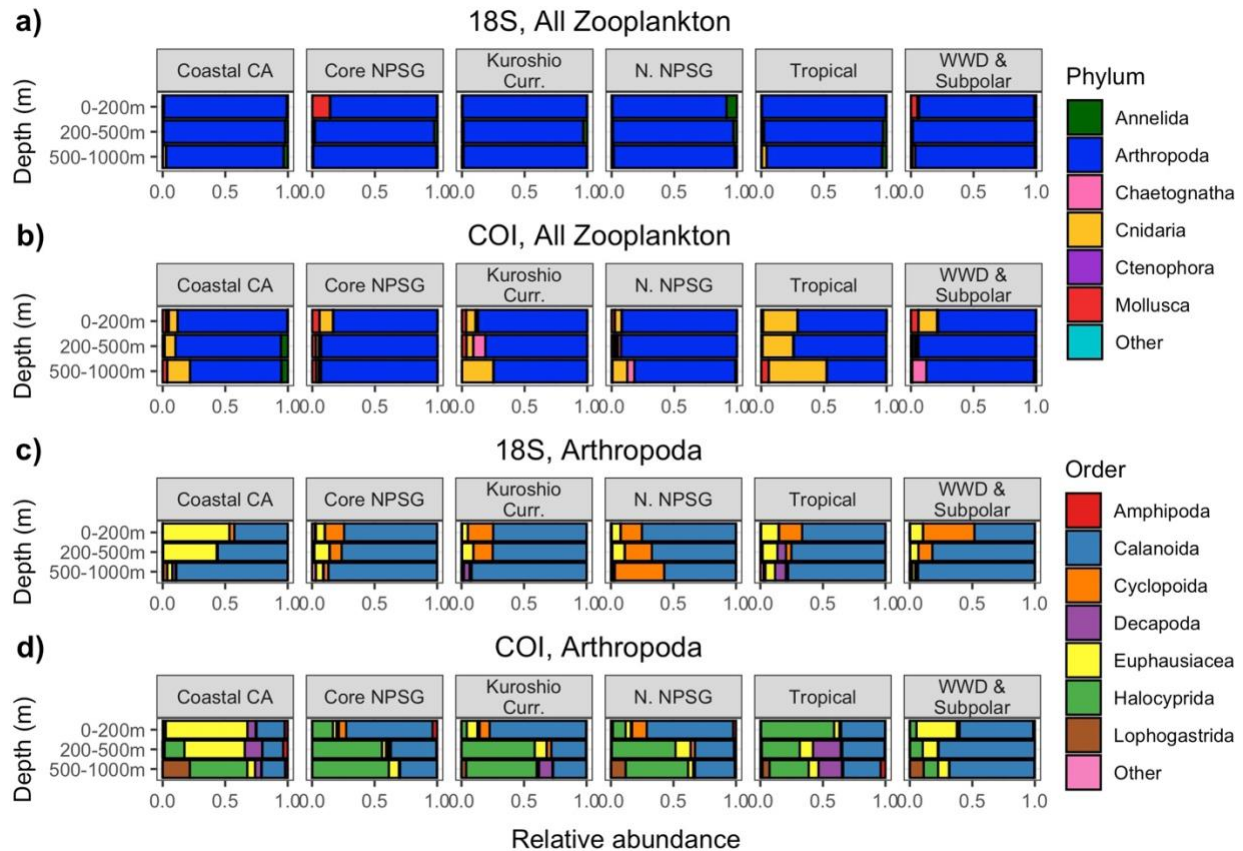


Figure 4.2. Zooplankton community composition across biogeographic regions from relative read abundance; classified by phylum for a) the 18S marker region, and b) the COI marker region; and classified by order within Arthropoda for c) 18S, and d) COI. For each depth zone, all sampling events within each biogeographic region have been combined. Phyla denoted as “Other” include Bryozoa, Nemertea, Platyhelminthes, and Sipuncula. Arthropod orders denoted as “Other” include Harpacticoida, Mormonilloida, Siphonostomatoida, Myodocopida, and Ctenopoda.

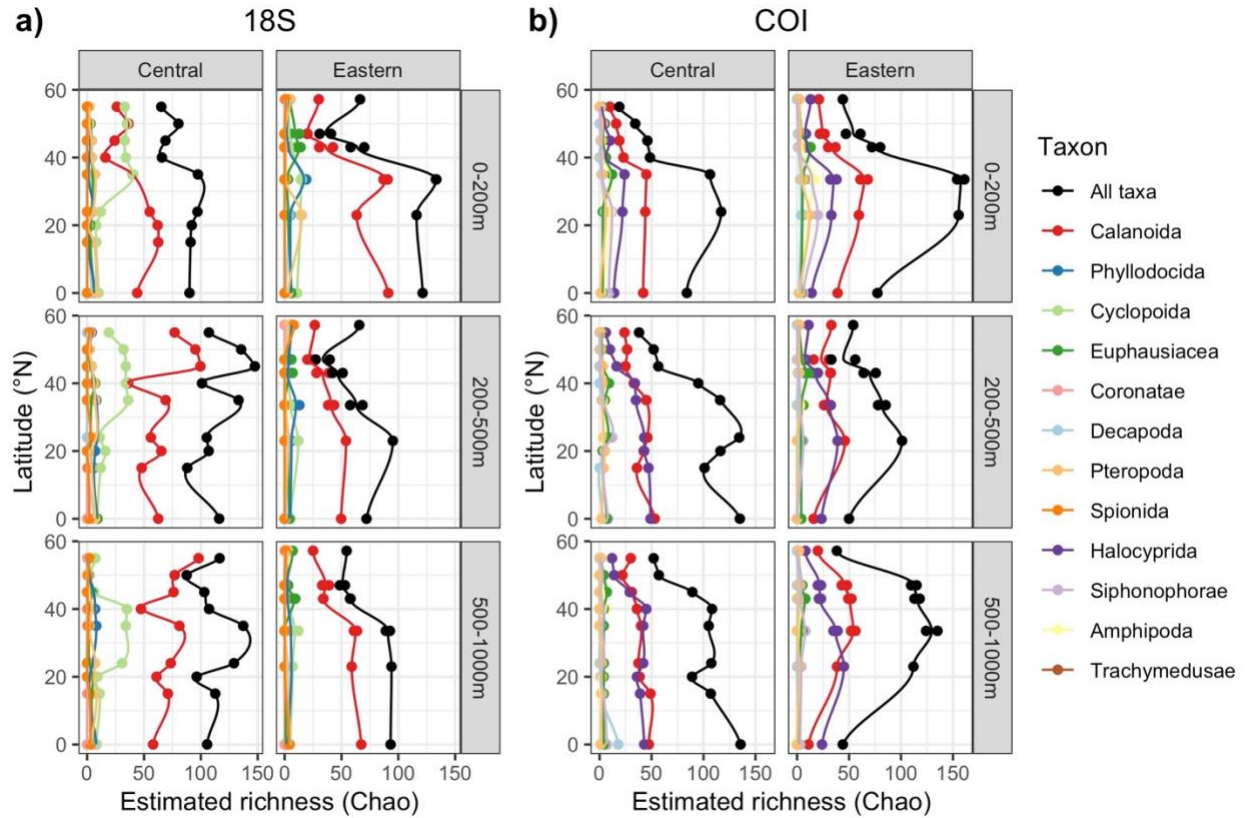


Figure 4.3. Total zooplankton richness and richness within each depth zone for the 12 most diverse orders observed in a) the 18S community and b) the COI community. The Central Pacific transect follows the 170°E meridian; the Eastern Pacific transect parallels the North American coastline, between 140°W and 115°W, as shown in Figure 4.1. Richness is shown independently for each depth zone within transects. Each point is a unique sampling event; day/night pairs, where present, are plotted independently. Lines are the loess best-fit, averaging paired tows at a single latitude.

4.3.2. Zooplankton community similarity within depth zones

To test whether there is greater similarity among mesopelagic zooplankton communities than epipelagic ones, we compared beta dispersal of Bray-Curtis Dissimilarity (BCD) among samples collected within each depth zone. We observed greater differentiation among depth zones for the 18S-resolved community than for the COI-resolved community (Fig. 4.4a, c, overlap between colors). Across all samples, we observed no difference in beta diversity dispersal among depth zones at either marker region (Fig. 4.4b, d; 18S: $F(2, 96) = 0.802$, $p = 0.45$; COI: $F(2, 100) = 0.233$, $p = 0.79$). Within each biogeographic region, we then summed all

sampling locations within each depth zone and compared beta diversity across regions, among depth zones. We also found no significant differences in beta diversity dispersal between depth zones (not shown; 18S: $F(2, 15) = 0.442$, $p = 0.65$; COI: $F(2, 15) = 0.012$, $p = 0.98$). As previous studies at sub-basin spatial scales have found greater similarity among mesopelagic communities than among epipelagic communities, we then tested whether there were differences in dispersal at smaller scales, examining samples in each depth zone within each biogeographic region. We found no differences in dispersal among depth zones, within any region or at either molecular marker (Fig. 4.5; $p > 0.05$, all tests).

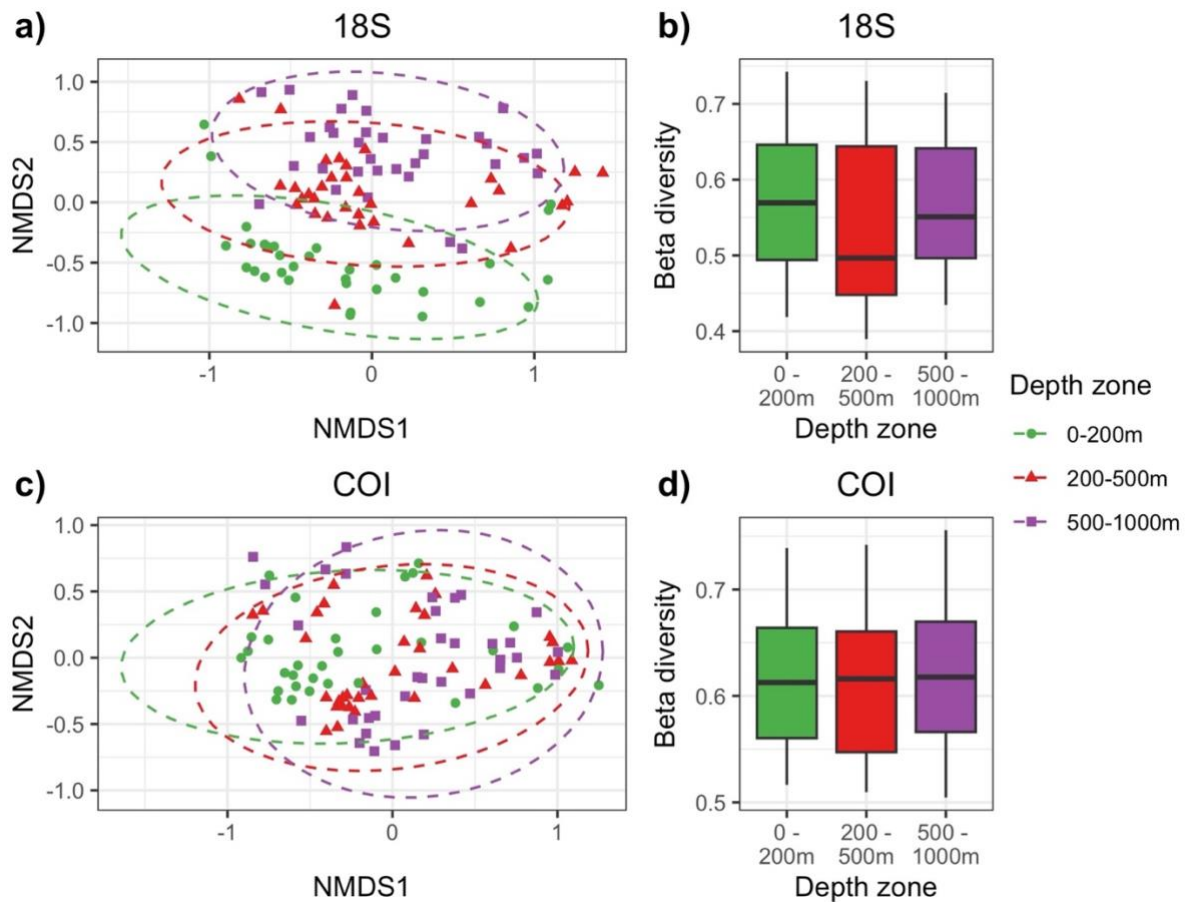


Figure 4.4. Community dissimilarity within depth zones, detected by the two molecular marker regions. a) and c): non-metric multidimensional scaling of Bray-Curtis dissimilarity, shown with the 95% confidence interval; b) and d) boxplots of distances between each unique sample and the group centroid.

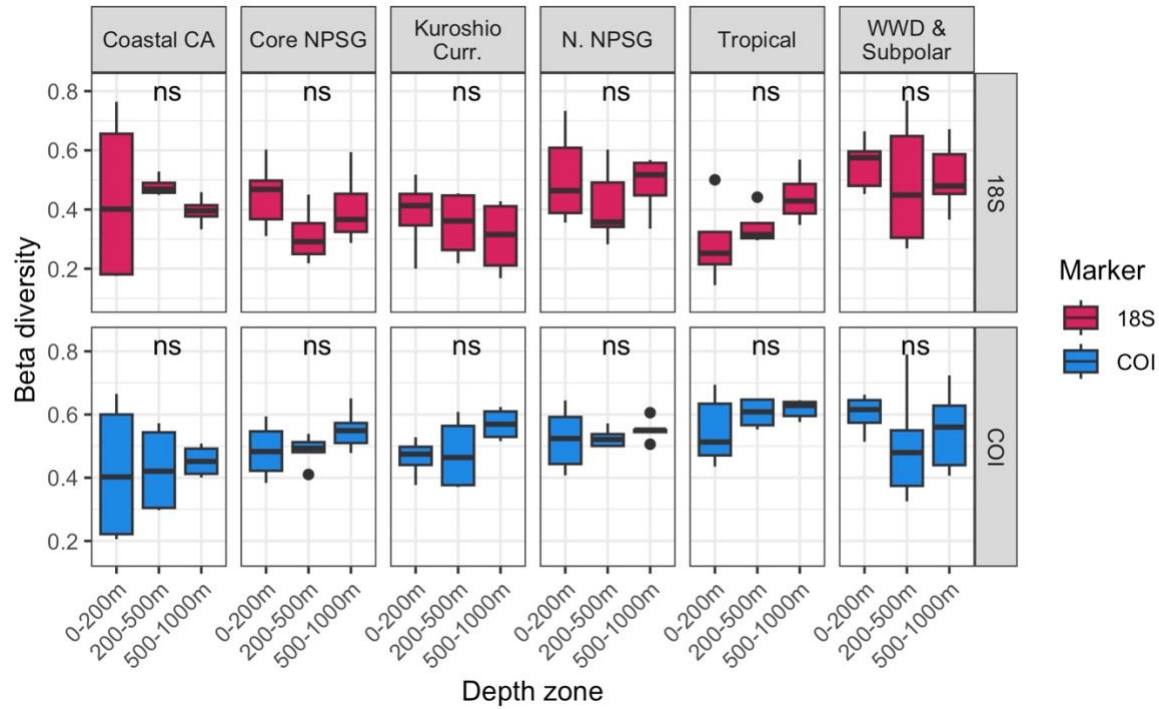


Figure 4.5. Beta-diversity dispersal distances within each biogeographic region, for all samples within each depth zone in each biogeographic region. Boxplots show the distances between each unique sample and the group centroid. Colors denote molecular markers. (Kruskal-Wallis: ns $p > 0.05$).

4.3.3. Biogeographic distributions

To test whether taxa in the epipelagic, upper mesopelagic, or lower mesopelagic had larger biogeographic distributions, we compared the number of sampling locations in which taxa were found, treating each depth zone independently. Across depths, we found no significant differences in the number of sampling locations or the number of biogeographic regions taxa occurred across, for either marker region (Fig. S4.8; 18S locations: $\chi^2 = 2.063$, 2 df, $p = 0.357$; COI locations: $\chi^2 = 0.067$, 2 df, $p = 0.967$; 18S regions: $\chi^2 = 1.018$, 2 df, $p = 0.601$; COI regions: $\chi^2 = 0.047$, 2 df, $p = 0.976$). We then tested whether there were differences in distributional ranges among taxa preferentially occurring within each depth zone. We again found no differences in the number of locations or number of regions that taxa occurred across, at either

molecular marker (18S, Fig. 4.6a; regions: $\chi^2 = 5.658$, 2 df, $p = 0.059$; locations: $\chi^2 = 0.746$, 2 df, $p = 0.689$; COI, Fig. 4.6b; regions: $\chi^2 = 4.510$, 2 df, $p = 0.105$; locations: $\chi^2 = 3.88$, 2 df, $p = 0.144$).

4.3.4. Environmental ranges

To assess whether there are differential effects of environmental variables on zooplankton across depth, we examined the environmental ranges of zooplankton taxa that preferentially inhabited each depth zone. For all six environmental variables tested, we found significant differences in environmental range widths among taxa inhabiting different depths zones (Fig. 4.7). Ranges of temperature, salinity, and DO concentrations were larger for taxa preferentially inhabiting deeper depth zones (Fig. 4.7a, b). For the 18S-resolved zooplankton community, deeper taxa had broader temperature and salinity ranges for all pairwise comparisons (18S temperature: $\chi^2 = 61.302$, 2 df, $p < 0.001$; 18S salinity: $\chi^2 = 74.018$, 2 df, $p < 0.001$). Taxa in either the upper or lower mesopelagic depth zones were distributed across wider ranges of DO than taxa preferentially inhabiting the epipelagic zone, but there were no differences between taxa preferentially occurring in the two mesopelagic depth zones (18S oxygen: $\chi^2 = 18.2$, 2 df, $p < 0.001$). For the COI-resolved community, taxa preferring either mesopelagic depth zone exhibited larger temperature and salinity ranges than taxa preferring the epipelagic zone (COI temperature: $\chi^2 = 50.029$, 2 df, $p < 0.001$; COI salinity: $\chi^2 = 40.27$, 2 df, $p < 0.001$). All pairwise comparisons of DO concentration ranges were significant, with the widest ranges observed for taxa preferentially inhabiting the upper mesopelagic depth zone (COI oxygen: $\chi^2 = 29.934$, 2 df, $p < 0.001$). In contrast, taxa preferentially inhabiting the lower mesopelagic depth zone were distributed across narrower ranges of mean sea-surface NPP and

mean EF, when compared against taxa preferentially occupying the epipelagic or upper mesopelagic depth zones (Fig. 4.7a, b; 18S NPP: $\chi^2 = 12.073$, 2 df, $p < 0.01$; 18S EF: $\chi^2 = 12.073$, 2 df, $p < 0.01$; COI NPP: $\chi^2 = 18.909$, 2 df, $p < 0.001$; COI EF: $\chi^2 = 18.909$, 2 df, $p < 0.001$).

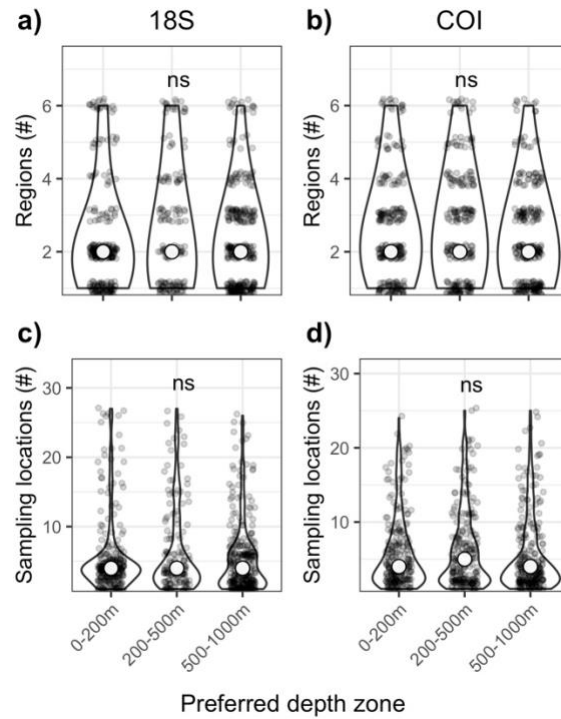


Figure 4.6. Biogeographic ranges of individual taxa preferentially inhabiting each depth zone, across unique sampling locations for a) 18S taxa and b) COI taxa, and across biogeographic regions for c) 18S taxa and d) COI taxa. Violin plots show density distribution, data points jittered within each categorical value. (Kruskal-Wallis: ns = $p > 0.05$).

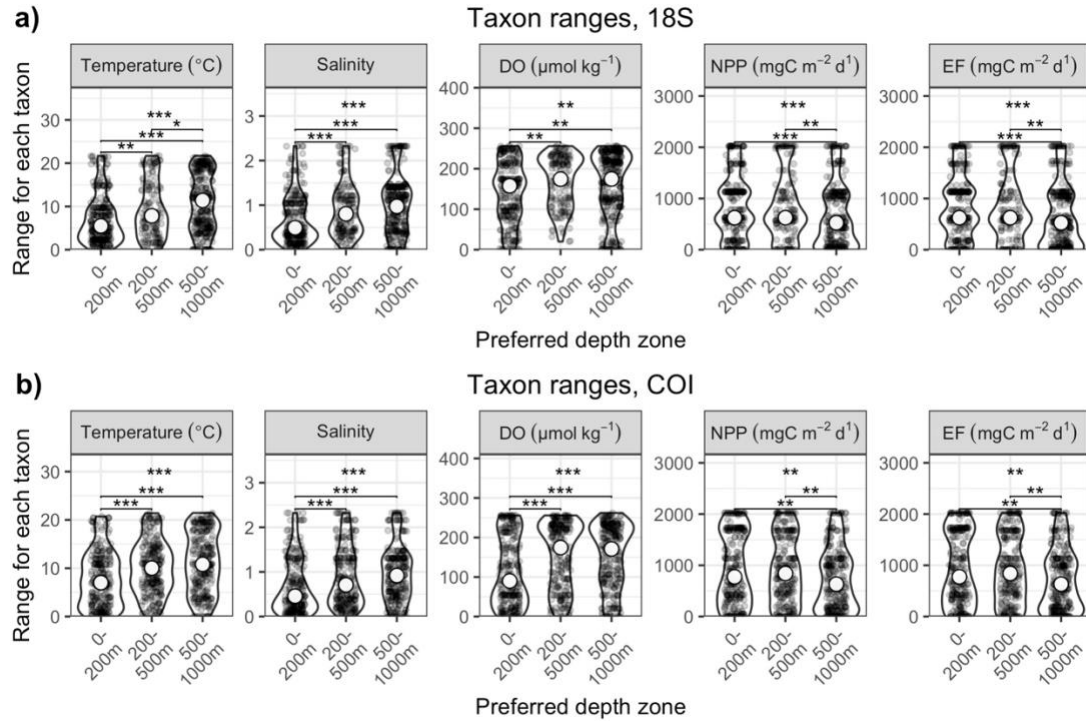


Figure 4.7. Environmental ranges of taxa primarily occurring within each depth zone, for a) 18S ASVs and b) COI OTUs. Violin plots show density distribution, data points jittered within each categorical value. (Kruskal-Wallis, adjusted Dunn's post-hoc: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

4.3.5. Zooplankton communities and environmental conditions

To test whether turnover in zooplankton community composition covaried with the changes in the biogeochemical environment, we tested whether pairwise Bray-Curtis Dissimilarity (BCD) was correlated with differences in annual average temperature, annual average salinity, average *in situ* DO concentration, and annual total food input (yearly total NPP and EF) for each pair of samples. Visual analysis of the data revealed non-linear relationships for all environmental variables (Fig. 4.8). At the 18S marker, we found stronger correlations between BCD and temperature or salinity differences in the surface ocean, with decreasing correlation coefficients at depth (18S temperature: epipelagic $\rho = 0.5$, upper mesopelagic $\rho = 0.43$, lower mesopelagic $\rho = 0.41$, $p < 0.001$, all; 18S salinity: epipelagic $\rho = 0.34$, upper

mesopelagic $\rho = 0.21$, lower mesopelagic $\rho = 0.19$, $p < 0.001$, all). For the COI community, correlations between BCD and temperature and salinity differences were overall stronger and also decreased with depth (COI temperature epipelagic $\rho = 0.8$, upper mesopelagic $\rho = 0.62$, lower mesopelagic $\rho = 0.52$, $p < 0.001$, all; COI salinity: epipelagic $\rho = 0.66$, upper mesopelagic $\rho = 0.49$, lower mesopelagic $\rho = 0.26$, $p < 0.001$, all). On the other hand, correlations between BCD and differences in DO concentrations did not exhibit a monotonic decrease with depth. At the 18S marker, the strength of the correlation between BCD and DO concentration differences was greatest in the epipelagic depth zone ($\rho = 0.19$, $p < 0.001$), followed by the lower mesopelagic depth zone ($\rho = 0.14$, $p < 0.001$), but there was no significant relationship in the upper mesopelagic depth zone. At the COI marker, BCD showed very similar correlations with DO concentrations in the epipelagic, upper mesopelagic, and lower mesopelagic depth zones. The relationship between BCD and food input to the environment (measured as Net Primary Production (NPP) in the epipelagic depth zone and Export Flux (EF) in the two mesopelagic depth zones) was stronger at the surface and decreased with depth, at both marker regions. For the 18S community, potential food input was better correlated with BCD than was oxygen (18S NPP epipelagic: $\rho = 0.31$, $p < 0.001$; EF upper mesopelagic: $\rho = 0.28$, $p < 0.001$; EF lower mesopelagic: $\rho = 0.14$, $p < 0.001$). The COI community showed slightly weaker relationships between BCD and food input than between BCD and oxygen (COI NPP epipelagic: $\rho = 0.20$, $p < 0.001$; EF upper mesopelagic: $\rho = 0.22$, $p < 0.001$; EF lower mesopelagic: $\rho = 0.1$, $p < 0.001$). We performed sensitivity analyses to test potential effects of rarefaction or relative abundance normalization and found the same patterns of correlation strength and significance across depths and marker regions (not shown). Correlations between BCD and difference in *in situ* temperature and salinity were similarly strongest in the epipelagic depth zone and decreased with depth, and

were stronger for the COI-resolved zooplankton community than for the 18S-resolved zooplankton community (Fig. S4.9).

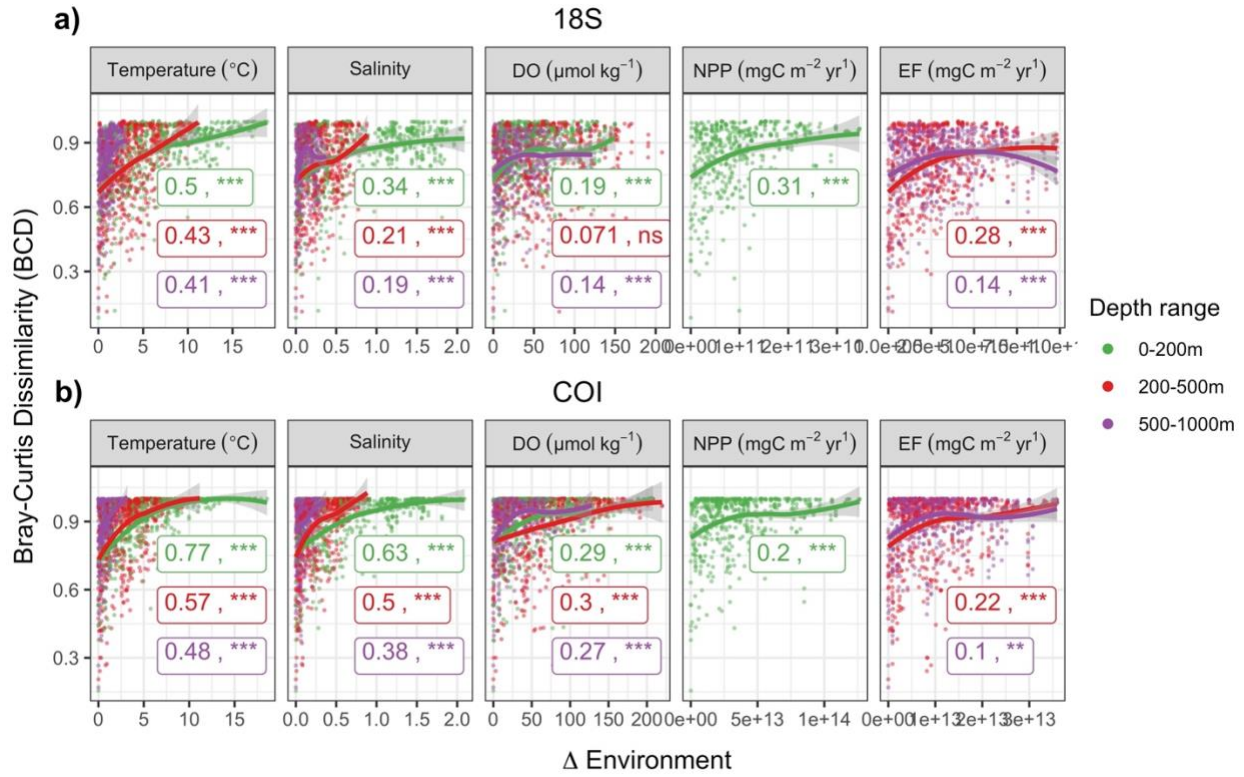


Figure 4.8. Non-parametric Spearman rank correlation (ρ) between Bray-Curtis dissimilarity and environmental dissimilarity for a) 18S and b) COI. Loess best-fit lines are shown for significant relationships. Shading surrounding lines represents the 95% CI. Colors represent depth zones. (Spearman's rank: ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

4.3.6. Zooplankton communities and dispersal

To test the role of dispersal potential on community similarity, we tested whether BCD was correlated with potential dispersal distance, calculated from dispersal time and percent of particles lost between station pairs in the OFES ocean circulation model. We found positive, nonlinear correlations between BCD and potential dispersal distance at all three depth zones, for both markers (Fig. 4.9a, b). The strongest relationship was observed in the epipelagic depth zone at both marker regions (18S: $\rho = 0.37$, $p < 0.001$; COI: $\rho = 0.53$, $p < 0.001$). Correlations in the

upper and lower mesopelagic depth zones were slightly lower at both molecular markers (18S: $\rho = 0.21$, $p < 0.001$, both mesopelagic depth zones; COI $\rho = 0.48$, $p < 0.001$, both mesopelagic depth zones). Sensitivity analyses testing rarefied or normalized read abundances and different particle arrival radii (ranging from 200 – 750 km) revealed that correlations were consistently stronger in the epipelagic zone than in the mesopelagic zone, as well as stronger for the COI marker than the 18S marker (not shown). Relationships between BCD in the upper and lower mesopelagic depth zones were also tested against dispersal distance within the epipelagic depth zone, due to the potential influence of diel and ontogenetic vertical migration, but the strongest relationships were observed for dispersal potential within each community's depth zone (not shown). In contrast, geographic distance was more strongly correlated with BCD at depth than in the epipelagic at the COI marker, and at the 18S marker was only significant in the deepest depth zone (Fig. S4.10).

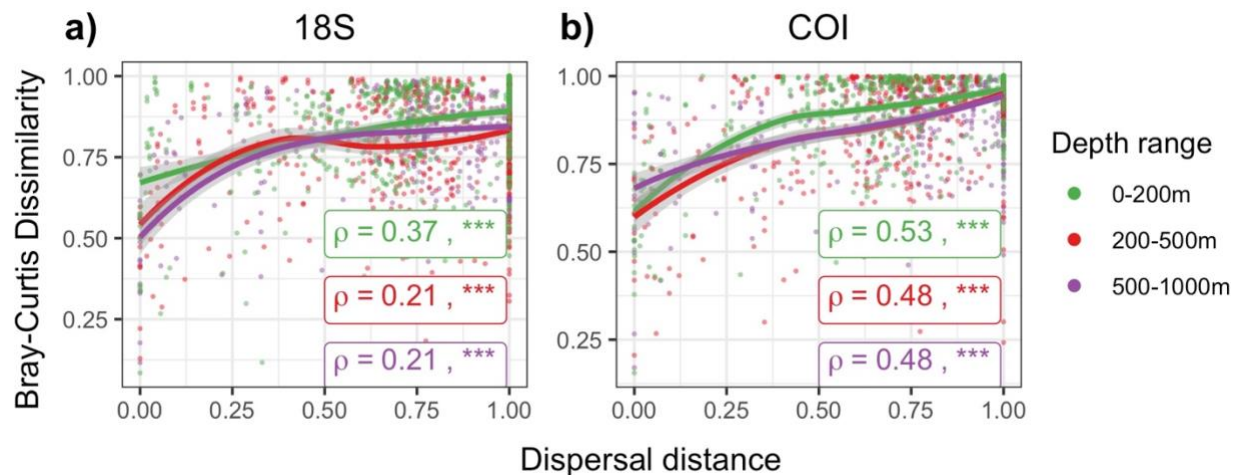


Figure 4.9. Non-parametric Spearman rank correlation between Bray-Curtis dissimilarity and potential dispersal distance (see Methods) for a) the 18S community and b) the COI community. Loess best-fit lines are shown for significant relationships. Shading surrounding lines represents the 95% CI. Colors represent depth zones. (Spearman's rank: ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

4.3.7. Relative importance of environment vs. dispersal

We used generalized dissimilarity models (GDMs) to integrate the effects of environmental differences and dispersal potential on community composition. GDM predictor importance analysis revealed that temperature, salinity, DO, and food input to systems explained 43 – 71 % of variation in community composition within each depth zone (Table 4.1, $p < 0.001$, all models). For both marker regions, dissimilarity within the lower mesopelagic zone was least predictable with the predictors we tested. In the epipelagic depth zone, NPP and temperature were significant predictors at both markers, as well as DO concentrations at the COI marker. In the upper mesopelagic depth zone, EF and salinity were significant predictors at both markers, as well as temperature at the COI marker. Finally, temperature was a significant predictor in the lower mesopelagic depth zone at both markers, and at 18S, EF and salinity were also significant in this depth zone. Dispersal distance was not identified as a significant predictor by GDMs, for the zooplankton community resolved by either marker. For each predictor, the domains across which the strongest effects on partial dissimilarity were observed were largely conserved across models for each depth zone and marker region (Fig. S4.11).

Table 4.1. Explanatory power and significance of generalized dissimilarity models for BCD within each depth zone. Full model: deviance and significance for models including only significant predictors, identified through sequential removal of non-significant predictors after model permutation. Predictor significance: significance levels for all potential predictors tested: NPP in the epipelagic zone and EF in the mesopelagic zone (food input to the system), mean DO concentration, modeled mean temperature, modeled mean salinity, and calculated dispersal distance. Bootstrapped p-values from 1000 permutations: ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	18S			COI		
	0 –	200 –	500 –	0 –	200 –	500 –
	200 m	500 m	1000m	200 m	500 m	1000m
Full model						
Deviance explained, significance	47%, ***	53%, ***	43%, ***	71%, ***	63%, ***	49%, ***
Predictor significance						
NPP (Net Primary Production)	*	-	-	*	-	-
EF (Export Flux)	-	*	***	-	*	ns
DO (Dissolved Oxygen)	ns	ns	ns	**	ns	ns
Salinity	ns	***	*	ns	**	ns
Temperature	**	ns	***	***	***	**
Dispersal distance	ns	ns	ns	ns	ns	ns

4.4. DISCUSSION

We assessed zooplankton community composition and species distributions in the epipelagic (0-200 m), upper mesopelagic (200-500 m) and lower mesopelagic (500-1000 m) depth zones at 28 locations across the North Pacific Ocean. We found no differences in the degree of beta-diversity dispersal among zooplankton communities inhabiting the epipelagic, upper mesopelagic, and lower mesopelagic depth zones. We also found no evidence for larger biogeographic ranges in mesopelagic zooplankton species. In comparison with epipelagic taxa,

mesopelagic taxa were distributed across wider ranges of temperature, salinity, and DO, but narrower ranges of potential food. Across all depth zones, community dissimilarity was better predicted by environmental conditions such as temperature, salinity, DO, and food availability than by calculated dispersal potential. Models explained epipelagic community dissimilarity better than mesopelagic community dissimilarity, highlighting the ambiguity and potential complexity of the mechanisms shaping mesopelagic biogeography.

4.4.1. Zooplankton community composition and richness

Compared across biogeographic regions, relative read abundances of major zooplankton phyla were comparable, but variability in community composition became more apparent with increasing taxonomic resolution. Such changes at lower taxonomic levels are exemplified by shifts in arthropod community composition among regions. The fine taxonomic resolution of metabarcoding increases sensitivity to species-specific changes in community composition. Our two molecular markers resolve partially overlapping suites of zooplankton taxa (Matthews et al., 2021). Dual marker regions allow for increased confidence in observed patterns shared between markers (e.g., calanoid copepods, euphausiids) as well as detection of a wider array of taxon-specific patterns (e.g., cyclopoid copepods, ostracods). At both marker regions, we observed high proportions of calanoid copepods, reflecting the high relative abundance of this group within the zooplankton community (Lavaniegos & Ohman 2007; Steinberg et al., 2008). The higher abundances of cyclopoid copepod reads (18S) in the epipelagic zone and ostracod reads (COI) at mesopelagic depths align with previously described vertical distributions (Angel, 1979; Yamaguchi et al., 2002).

The subtropical richness maxima observed in our study align with expected patterns for pelagic zooplankton, as well as for specific taxa (Angel, 1993; Brinton et al., 1999; van der Spoel et al., 1997). While metabarcoding-detected richness can be difficult to compare among methodologies, we note some expected patterns within our study that could be further investigated with morphological analyses. Across depth zones, we found unexpected differences in the latitudinal location and extent of the cyclopoid copepod richness maxima. High cyclopoid richness in the epipelagic and upper mesopelagic depth zones extends throughout the temperate and subpolar latitudes. In the lower mesopelagic cyclopoid community, species richness reaches a maximum at slightly lower latitudes (approximately 20°N), and the maximum only extends to 40°N. This result could be due to vertical displacement of a continuous cyclopoid community at the southern limit of species ranges, or may represent a distinct deep-water cyclopoid community. Cyclopoid distributions and community structure are notably understudied in the pelagic ocean due to their small size relative to commonly used zooplankton net mesh sizes (Gallienne & Robins, 2001). Significant patchiness has been reported for the free-living cyclopoids (Boxshall, 1977), as well as uncertainty about the mechanisms shaping distance-based patterns of dissimilarity (Dahms et al., 2015). Our results suggest that the cyclopoid communities in surface and deep ocean may be structured differently.

4.4.2. Species ranges

In contrast to expectations of larger biogeographic distributions for mesopelagic taxa (e.g., Sutton et al., 2017), we found no significant differences in the ranges of taxa within each depth zone or the ranges of taxa preferentially associated with each depth zone. The vertical limits of the mesopelagic zone when defined by environmental properties can vary by up to

600 m (Reygondeau et al., 2018), and zooplankton can follow their preferred environment within the water column through active selection of vertical habitats (Chapter 2 of this dissertation). Standardized depth bins are commonly employed for vertically stratified zooplankton tows, particularly in large-scale sampling efforts and in the absence of *a priori* expectations regarding vertical structure. These depth bins do not account for vertical variations in community distribution. Discrete communities and fine-scale patchiness are averaged within the depth range sampled by each net (Whitmore et al., 2019), and the distributional tails may be represented in an adjacent depth range, leading to additional noise when interpreting community composition. However, our identical results for static depth zones and for taxa primarily associated with each depth indicate that distributional ranges within discrete depth zones may accurately portray biogeographic ranges.

Differences in the ranges of environmental characteristics across which epipelagic and mesopelagic taxa are distributed suggest that environmental tolerances may vary with depth. Given the greater homogeneity of the midwater environment, the wider temperature, salinity, and oxygen ranges of mesopelagic taxa contrasts with the narrower environmental niches we expected (Kassen, 2002). If biogeographic ranges were limited by selection due to temperature, salinity, or oxygen, wider environmental tolerances should lead to larger biogeographic ranges. In contrast, we did not observe any evidence for larger biogeographic ranges in deep-dwelling taxa. Changes in mesopelagic zooplankton community composition have previously been correlated with temperature and salinity (Canals et al., 2023; Hirai et al., 2020), and we also observed significant correlations between BCD and temperature (see 4.4.3.). However, our analysis of taxon-specific biogeographic ranges indicate that temperature and salinity niches are unlikely to shape biogeographic ranges.

In contrast, we found significantly narrower potential food ranges for lower mesopelagic taxa. These narrower ranges were also not associated with smaller biogeographic ranges, but less spatial heterogeneity of food flux in the deep ocean relative to the epipelagic zone (Siegel et al., 2014) could lead to equivalent biogeographic ranges across depth. Mesopelagic taxa have previously been found to exhibit narrow potential food niches (Hirai et al. 2020, Soviadan et al. 2022). Our results corroborate these findings, but we find that this pattern is restricted to taxa that preferentially inhabit the deep mesopelagic, while taxa inhabiting the upper mesopelagic zone have potential food ranges that more closely mirror those of epipelagic taxa.

4.4.3. Community dissimilarity and potential mechanisms

The zooplankton community within the epipelagic, upper mesopelagic, and lower mesopelagic depth zones exhibited similar levels of beta diversity. Across marine communities, our results neither support previous studies reporting greater dissimilarity among mesopelagic communities (Canals et al., 2023; Villarino et al., 2022), nor previous studies reporting greater similarity among mesopelagic communities (Hirai et al., 2020; Matthews & Blanco-Bercial, *in review*; Soviadan et al., 2022). The similar degrees of dissimilarity we observed among depth zones suggests balanced relative effects of dispersal and environmental selection across depth, despite differences in the absolute magnitude of each. Greater environmental homogeneity that could lead to larger distributions may be counteracted by decreased dispersal rates, if both processes influence the emergent biogeographic distributions. Similarities among depth zones could also result from strong effects of vertical connectivity rather than independent processes shaping each layer of the ocean. Since the epipelagic and mesopelagic zones of the ocean are connected by vertical migrations and sinking export flux, as well as physical mixing of water

masses, epipelagic and mesopelagic zooplankton distributions are unlikely to be entirely independent.

We found the strongest correlations of zooplankton community BCD with temperature, followed by dispersal distance and salinity, and finally by DO and potential food. However, the observed correlations do not indicate mechanistic explanations. We attempted to partition the influence of environmental selection and dispersal potential on BCD using Generalized Dissimilarity Models (GDMs), incorporating both dispersal distance and environmental dissimilarity. We interpret the observed correlations between community dissimilarity and temperature or salinity differences to be generated by conservation of zooplankton communities within water masses, reflecting dispersal processes rather than environmental selection. Although the most parsimonious GDMs failed to identify dispersal potential as a significant predictor, GDMs assume predictor independence, and may have assigned predictive power to temperature when it in fact reflects dispersal potential. If temperature and salinity are correlated primarily because they are conserved water mass tracers, then greater effects of dispersal potential also align with our previous observation that temperature and salinity do not limit the biogeographic ranges of mesopelagic zooplankton (see 4.4.2.).

Correlations between temperature and zooplankton community composition are most often explained by fluctuations in food concentrations and prey composition covarying with temperature (Huntley & Boyd, 1984; Venkataramana et al., 2019), with some influence of metabolic rate (Ikeda, 1985; Ikeda et al., 2006). Potential food availability in the deep ocean is primarily predicted by export flux from the overlying surface waters, which is more closely coupled to sea-surface temperature than to midwater temperatures (Baker et al., 2017; Stukel et al., 2014, 2017). Narrower ranges of potential food for mesopelagic zooplankton taxa agree with

the GDM identification of EF as a significant predictor for midwater community dissimilarity. Mesopelagic zooplankton communities include carnivorous taxa in addition to detritivorous particle feeding taxa. Food availability for carnivores is, at minimum, one trophic step further removed from EF. Additionally, predation pressure by carnivores may be an important process shaping the distributions of potential prey items (e.g., Robison et al., 2020). Our data are limited in the ability to address this question, due both to biases in sampling (Hetherington et al., 2022) and absence of data trophic levels for observed taxa (but see Silva et al., 2022), but future analyses should consider including predation processes.

Previous work has found that dispersal can help explain species distributions when considered in combination with biological traits, but is insufficient to fully explain population structure and seascape genetics in the absence of ecological variables (Jahnke & Jonsson, 2022). In coastal systems, distributional shifts associated with climate change are influenced by ocean currents, but only when ocean currents align with temperature gradients, indicating that both dispersal and environmental characteristics influence species distributions (García Molinos et al., 2017). In our pelagic, basin-scale system, dispersal time scales between sampling locations are much longer than the generation of time most zooplankton species. Community similarity at basin scales must reflect population-level connectivity, rather than transport of a single generation. Combined effects of environmental selection and dispersal potential make sense on these scales, as zooplankton must be able to survive their dispersal journey. Our results suggest that in addition to dispersal potential, dissimilarity is shaped by food input either in the form of export flux or primary productivity, as well as concentrations of dissolved oxygen. Decreased explanatory power in the mesopelagic zone may reflect a transition toward top-down processes, at least for a portion of the zooplankton community.

4.5. CONCLUSION

Overall, we found significant relationships between community dissimilarity and temperature, salinity, DO, potential food, and dispersal distance. The relative importance of each potential predictor varied among marker regions and methods, but all predictors were significantly correlated with community dissimilarity. We propose stronger influences of potential dispersal distance on mesopelagic zooplankton community dissimilarity, but acknowledge the difficulty of disentangling dispersal processes from covarying environmental differences. The processes of dispersal potential and environmental selection, perhaps primarily through differences in food flux and food web structure, appear to work together to shape biogeographic distributions throughout the epipelagic and mesopelagic zones. Similar levels of beta-diversity across depth are likely achieved through similar balances of dispersal and selection mechanisms in the epipelagic and mesopelagic depth zones, perhaps with some influence of vertical connectivity.

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Chapter 4, in full, is currently being prepared for submission for publication of the material. Matthews, Stephanie A.; Kaminsky, Katarina; Cazares, Alexis E.; Questel, Jennifer M.; Blanco-Bercial, Leocadio; Hirai, Junya; Ohman, Mark D. The dissertation author was the primary researcher and author of this paper.

Data accessibility

Raw sequences will be made available through GenBank upon submission for publication. Scripts for QIIME2 analyses, Parcels particle circulation models, and all statistical analyses will be made available at

https://github.com/samatthews/NorthPacificBasin_Dispenv/Code upon submission for

publication. CTD profiles and processed satellite data products will be made available at

https://github.com/samatthews/NorthPacificBasin_Dispenv/Metadata upon submission for publication.

Author contributions

Conceptualization: SAM, MDO; *Formal analysis:* SAM; *Funding acquisition:* JMQ, LBB, JH, MDO; *Investigation:* SAM, KK, AEC, JMQ, LBB, JH.; *Methodology:* SAM; *Project administration:* SAM, MDO; *Resources:* SAM, AEC, JMQ, LBB, JH, MDO; *Supervision:* MDO; *Visualization:* SAM; *Writing - original draft:* SAM; *Writing - review & editing:* SAM, MDO.

Supplemental figures

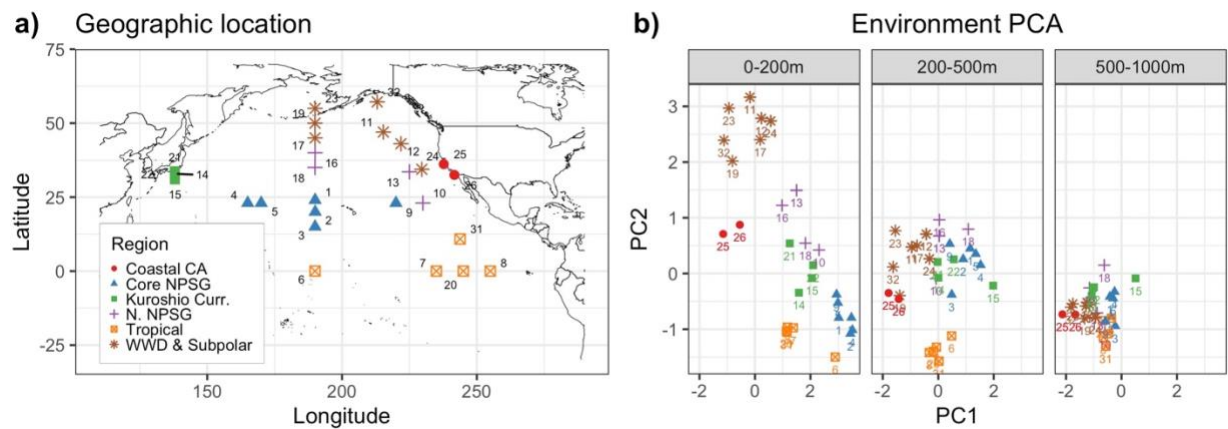


Figure S4.1. Zooplankton sampling locations included in this study; a) geographic locations of net tows across the North Pacific Basin, and b) Principal Component clustering of samples based on mean temperature, salinity, dissolved oxygen, and surface Chl-a. Colors and shapes denote biogeographic regions, assigned based on sample location and environmental similarity.

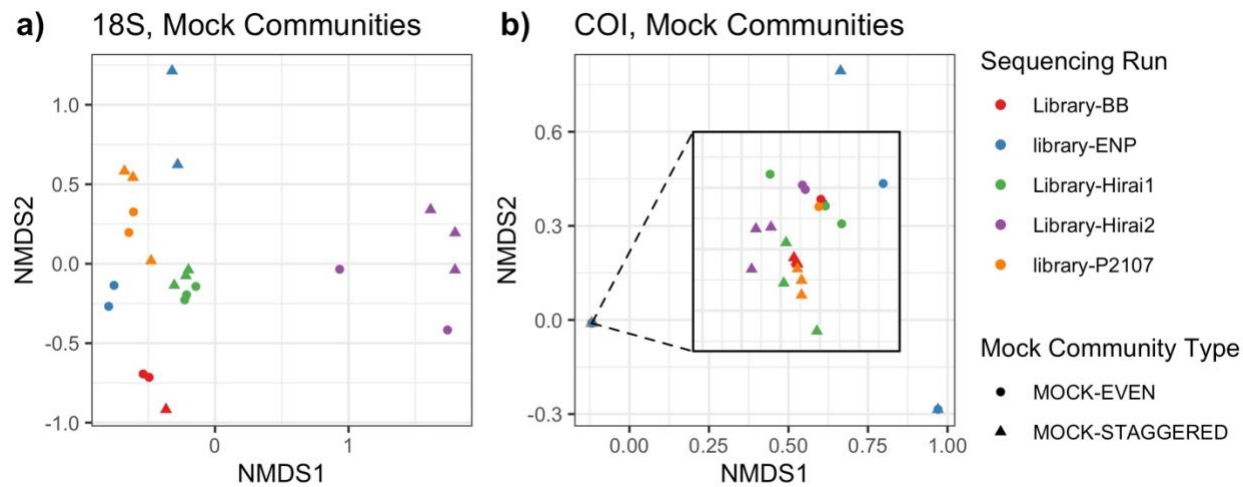


Figure S4.2. NMDS plots of Bray-Curtis dissimilarity among mock community samples from each MiSeq Illumina sequencing run. Sequencing runs correspond to library names in Table S1. Three replicates of each mock community were sequenced in each library, all mock community samples with >1000 reads are shown.

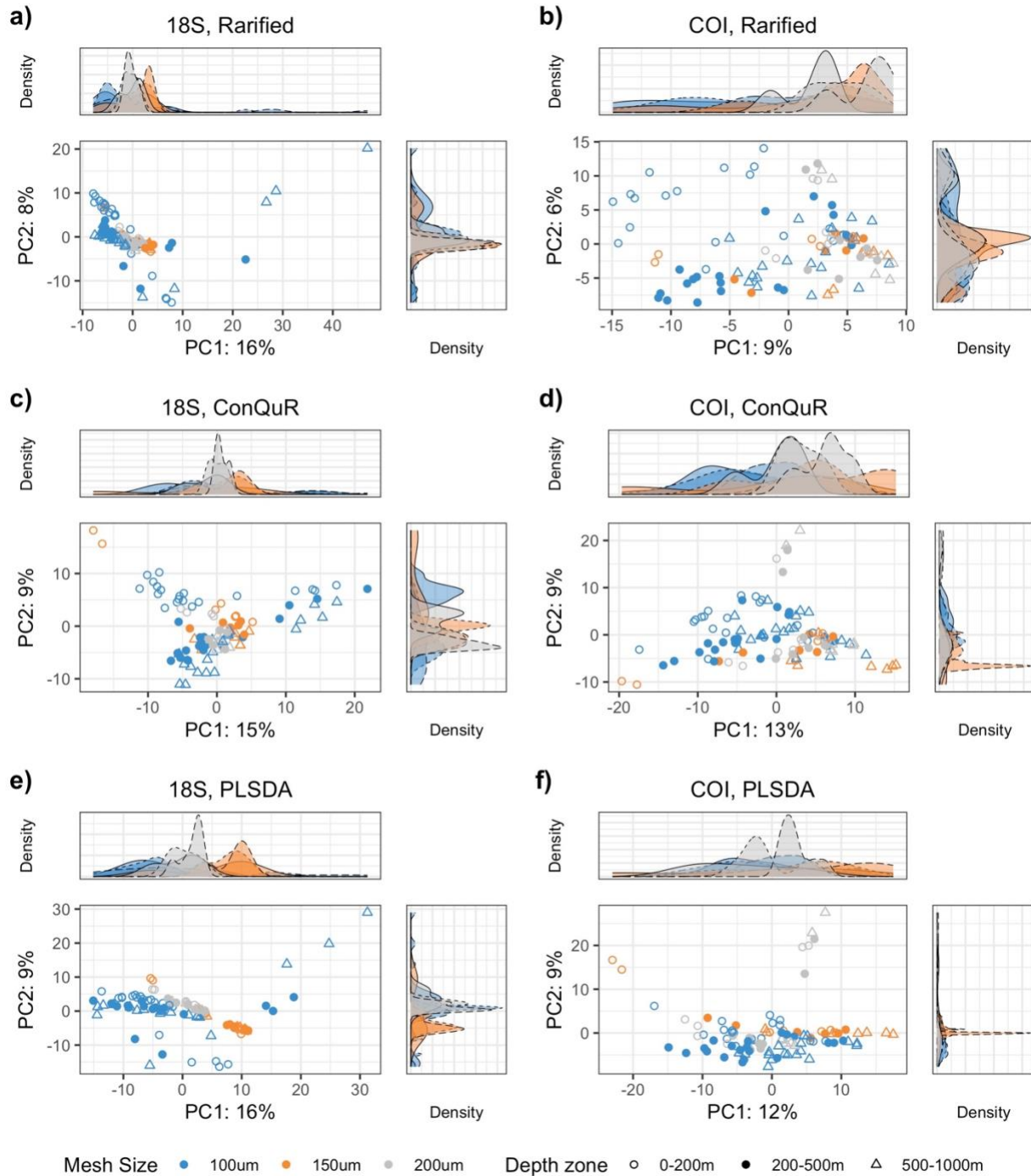


Figure S4.3. Potential methods for normalizing sampling net mesh sizes, visualized through Principal Component Analysis of Euclidean distances among zooplankton communities with and without transformations to normalize differences in net mesh size. ASV (18S) and OTU (COI) abundances were visualized a) and b) after rarefaction and without normalization; c) and d) with normalization through quantile regression with ConQuR, and e) and f) with normalization through Partial Least-Squares Discriminant Analysis. Colors denote sampling net mesh sizes, shapes and line types denote depth zones. For each panel, subplots at the top and right of the PCA show the density distribution of samples of each mesh size and depth zone.

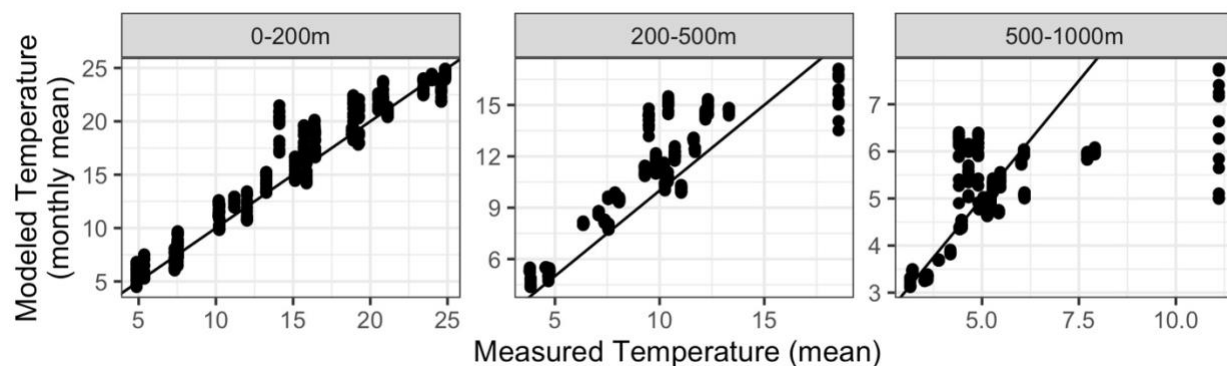


Figure S4.4. Depth-bin averages of modelled monthly mean temperatures at each sampling location plotted against mean in situ temperatures for each sampling event. Model data are shown for each month of the year. The solid black line shows the 1:1 relationship.

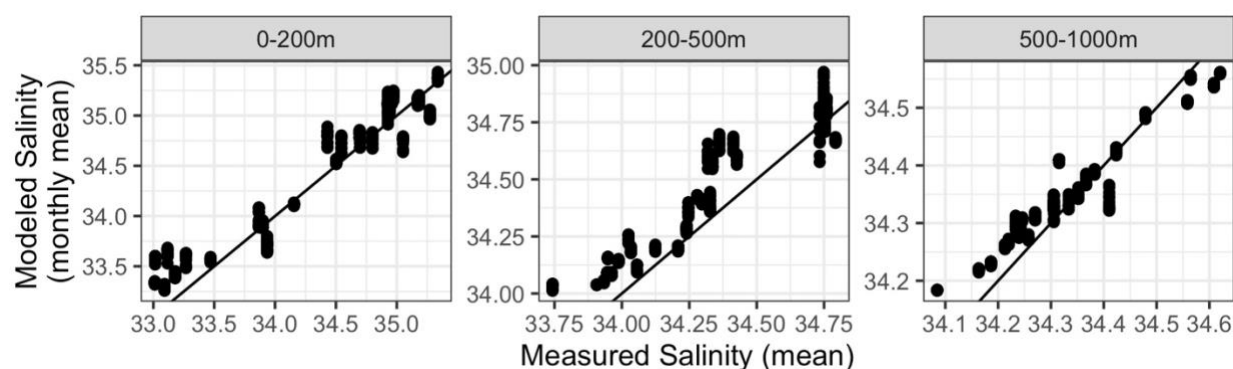


Figure S4.5. Depth-bin averages of modelled monthly mean salinity for each sampling location plotted against mean in situ salinity for each sampling event. Model data are shown for each month of the year. The solid black line shows the 1:1 relationship.

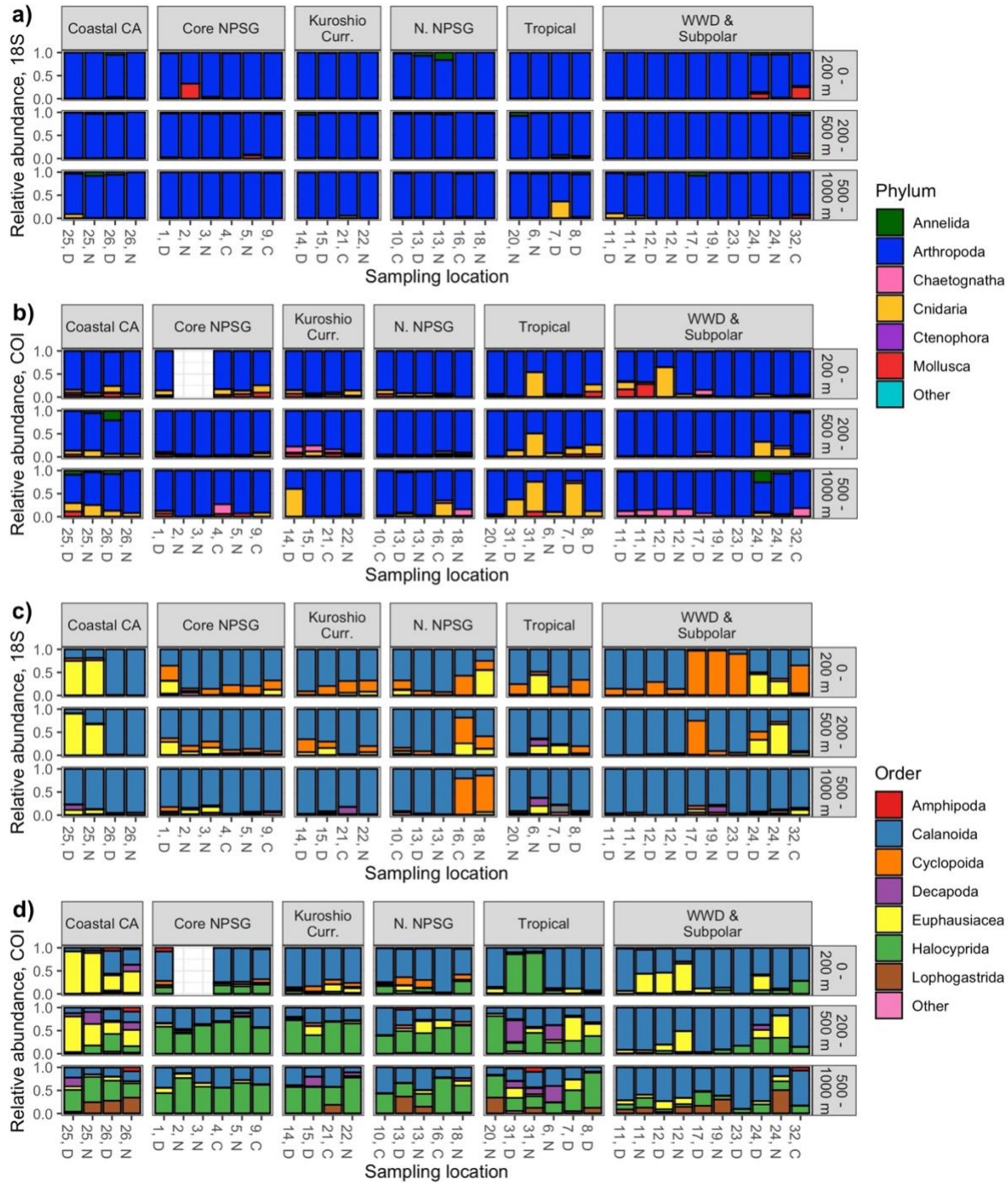


Figure S4.6. Relative read abundance of phyla within the zooplankton community resolved by a) the 18S marker and b) the COI marker, and relative read abundance of arthropod orders (relative to all arthropod reads) for c) the 18S marker and d) the COI marker. Each sampling event is shown as a unique column within biogeographic regions. Sampling location numbers correspond to the geographic locations in Figs. 1, S1. D, N, and C denote daytime, nighttime, and crepuscular sampling times. Phyla denoted as “Other” include Bryozoa, Nemertea, Platyhelminthes, and Sipuncula. Orders denoted as “Other” include Harpacticoida, Mormonilloidea, Siphonostomatoida, Myodocopida, and Ctenopoda.

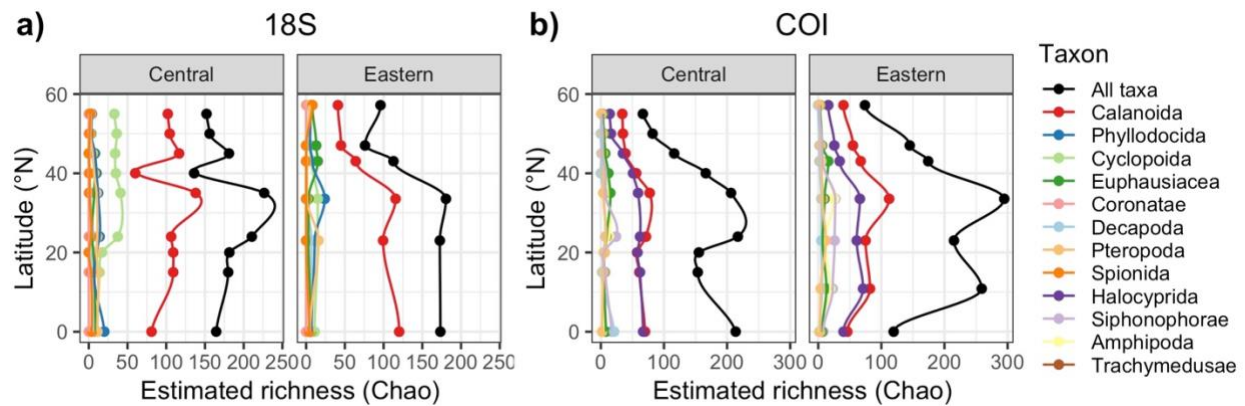


Figure S4.7. Total zooplankton richness and richness within the 12 most diverse orders for a) the 18S community and b) the COI community, summed across the upper 1000m of the water column. Transect locations are as annotated in Figure S1. Each point is a unique sampling location; day/night pairs, where present, have been summed. Lines are the loess best fit.

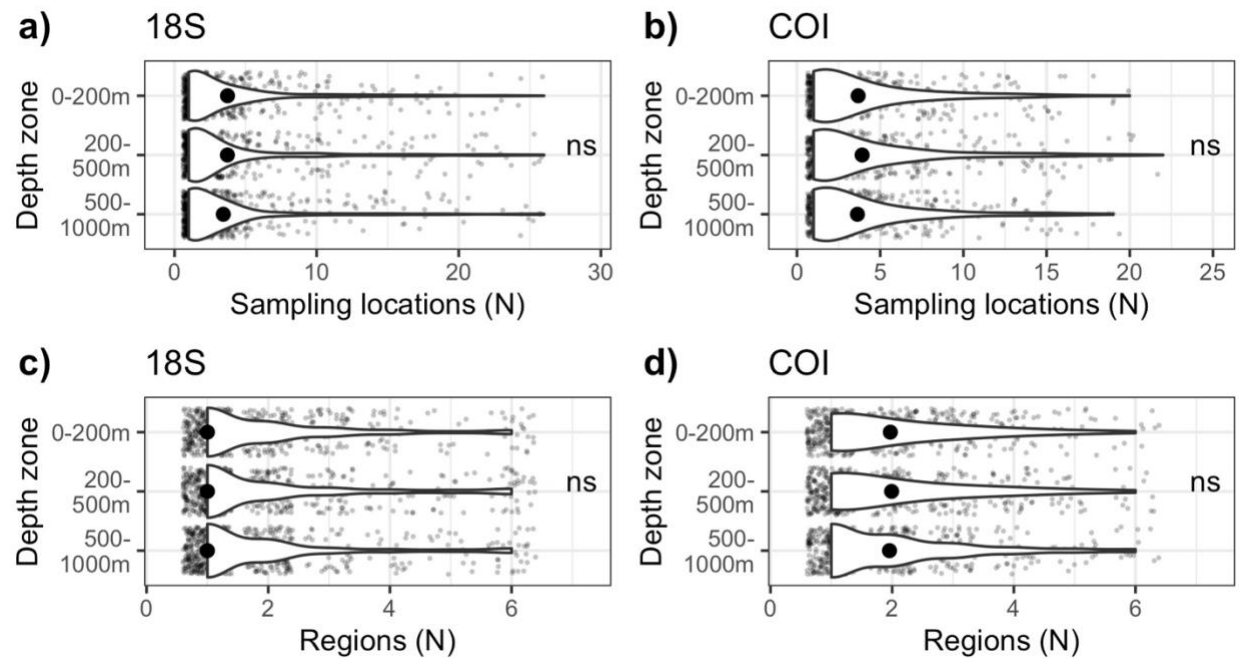


Figure S4.8. Biogeographic ranges of taxa within each discrete depth zone, across unique sampling locations for a) 18S taxa and b) COI taxa, and across biogeographic regions for c) 18S taxa and d) COI taxa. Violin plots show density distribution, data points jittered within each categorical value. (Kruskal-Wallis: ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

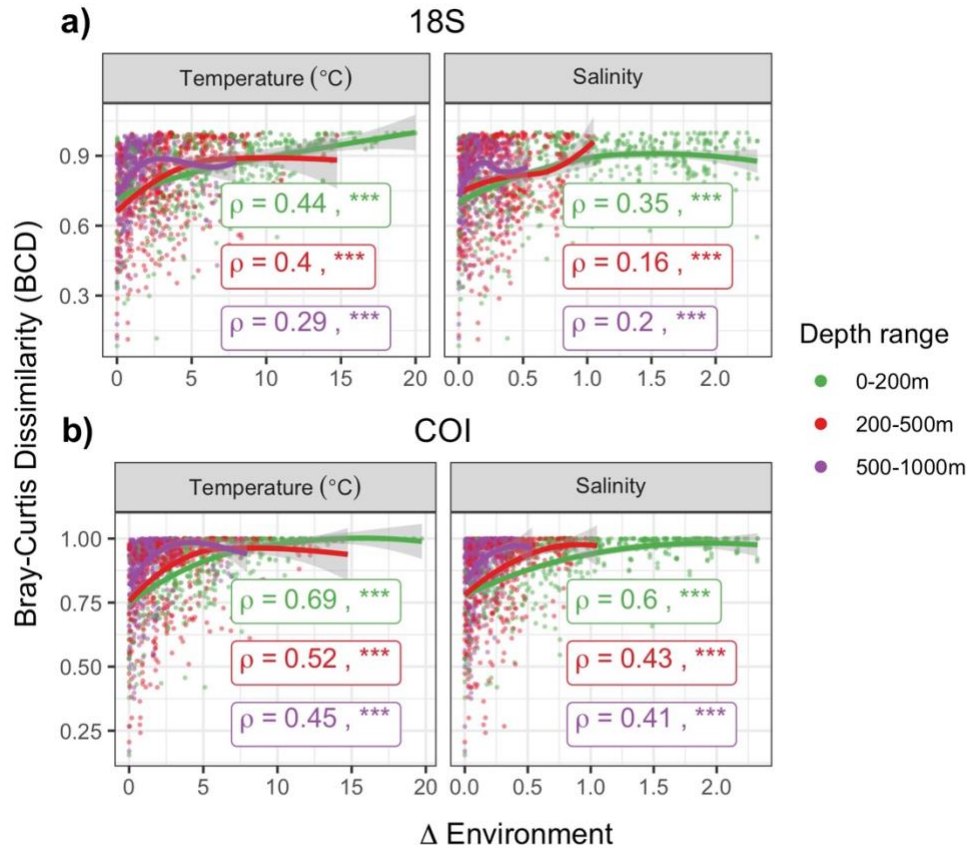


Figure S4.9. Spearman rank correlation between pairwise Bray-Curtis dissimilarity and *in situ* environmental difference between samples for a) 18S-resolved zooplankton communities and b) COI-resolved zooplankton communities.

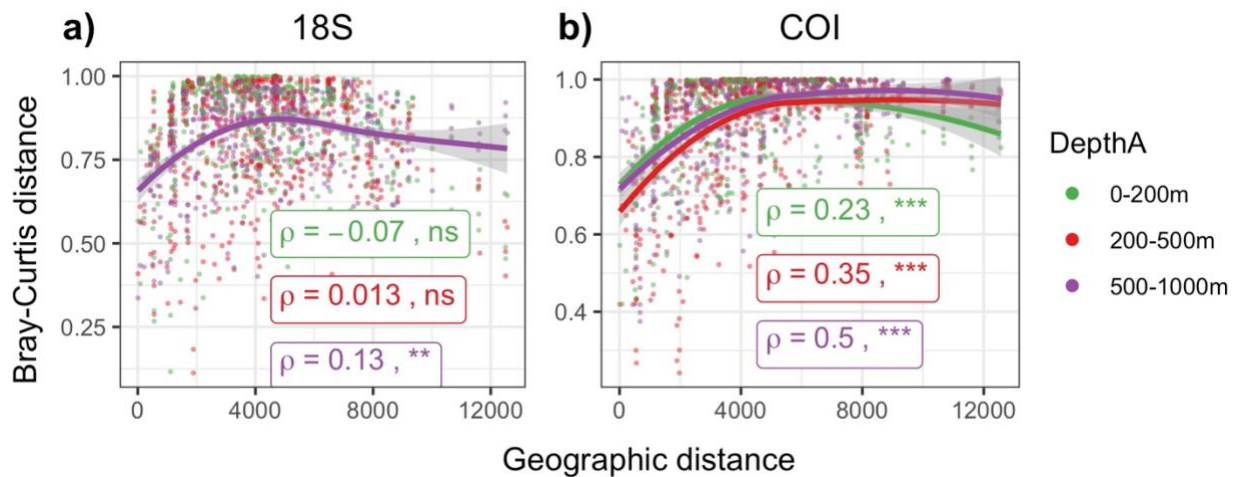


Figure S4.10. Non-parametric Spearman rank correlation (ρ) between Bray-Curtis dissimilarity and geographic distance for a) the 18S community and b) the COI community. Loess best-fit lines are shown for significant relationships. Shading surrounding lines represents the 95% CI. Colors represent depth zones. (Spearman's rank: ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

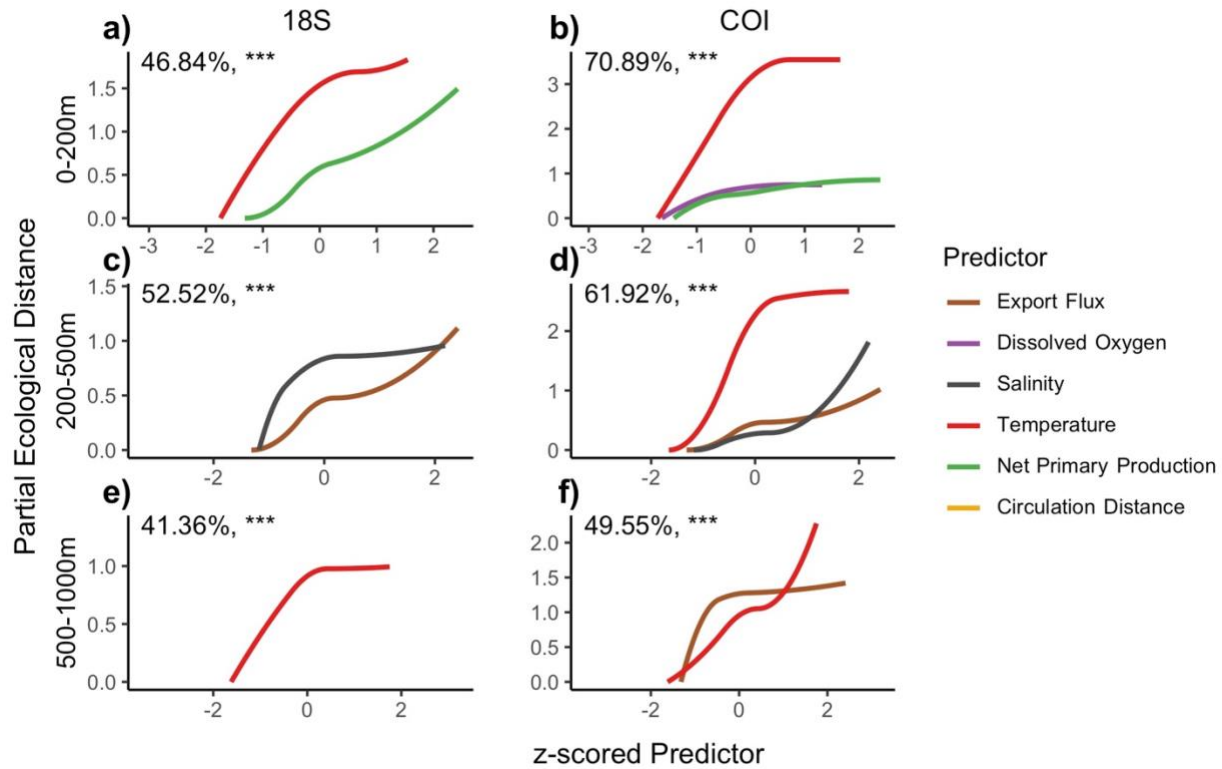


Figure S4.11. Significant predictors of ecological distance identified through generalized dissimilarity modeling, for the zooplankton community within each depth zone (rows) as captured by the 18S marker (left column) and the COI marker (right column). Predictors are z-score normalized. Higher values of partial ecological distance indicate stronger effects; for each predictor, domains of steeper slopes indicated increased community variability associated with that predictor range. Percent deviance explained, in the upper left of each panel, is the sum deviance of all significant predictors. Boot-strapped p-values for the full model are from 1000 permutations: ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Supplemental tables

Table S4.1. Sample collection locations, times, and type of sampling, as well as laboratory locations of sample processing. Loc.: Location number in this study; Lat. (N) and Lon. (E): geographic location of zooplankton sampling, averaged among tows for locations with multiple tows; Region: assigned biogeographic region, as in Figs. 1 and S1; Max depth: maximum depth sampled; Diel: time(s) of day that samples were collected, D – day, N – night, I – intermediate, B – both day and night; Col. by: initials of the author who collected zooplankton samples at sea; Cruise: unique identifier for the oceanographic cruise on which samples were collected; Station: station identifier, unique within cruises; Sampling date: local date of sample collection; Mesh size: zooplankton net mesh size use for collection; Tow type: Equipment type used for sampling and type of tow performed; DNA: initials of the author who performed DNA extractions; 18S PCRs: initials of the coauthor who performed 18S amplifications; COI PCRs: initials of the coauthor who performed COI amplifications. *(continued on next page)*

Loc.	Lat. (N)	Lon. (E)	Region	Max. depth (m)	Diel	Col. by	Cruise	Station	Sampling date	Mesh size (µm)	Tow type	DNA	18S PCRs	COI PCRs
1	24	190	Core NPSG	1000	D	JH	KH-14-3	st6	6/10/14	100	VMPS, vertical	JH	KK	KK
2	20	190	Core NPSG	1000	N	JH	KH-14-3	st5	7/9/14	100	VMPS, vertical	JH	KK	KK
3	15	190	Core NPSG	1000	N	JH	KH-14-3	st4	7/22/14	100	VMPS, vertical	JH	KK	KK
4	23	165	Core NPSG	1000	I	JH	KH-11-10	st3	12/9/11	100	VMPS, vertical	JH	KK	KK
5	23	170	Core NPSG	1000	N	JH	KH-11-10	st4A	12/10/11	100	VMPS, vertical	JH	KK	KK
6	0	190	Tropical	1000	N	JH	KH-13-7	st1	12/23/13	100	VMPS, vertical	JH	KK	KK
7	0	235	Tropical	1000	D	JH	KH-12-01	st7	2/9/12	100	VMPS, vertical	JH	KK	KK
8	0	255	Tropical	1000	D	JH	KH-12-01	st3	2/4/12	100	VMPS, vertical	JH	KK	KK
9	23	220	Core NPSG	1000	I	JH	KH-17-4	st8	8/28/17	100	VMPS, vertical	JH	KK	KK
10	23	230	N. NPSG	1000	I	JH	KH-17-4	st7	8/25/17	100	VMPS, vertical	JH	KK	KK
11	47	215.3	WWD & Subpolar	1000	B	LBB	NH1208	7	9/2/12	150	MOCNESS, Oblique	LBB	SAM	SAM
12	43	221.9	WWD & Subpolar	1000	B	LBB	NH1208	15	9/5/12	150	MOCNESS, Oblique	LBB	SAM	SAM
13	33.5	225	N. NPSG	1000	B	LBB	NH1208	34	9/14/12	150	MOCNESS, Oblique	LBB	SAM	SAM

Table S4.1. (continued from previous page) Sample collection locations, times, and type of sampling, as well as laboratory locations of sample processing. Loc.: Location number in this study; Lat. (N) and Lon. (E): geographic location of zooplankton sampling, averaged among tows for locations with multiple tows; Region: assigned biogeographic region, as in Figs. 1 and S1; Max depth: maximum depth sampled; Diel: time(s) of day that samples were collected, D – day, N – night, I – intermediate, B – both day and night; Col. by: initials of the author who collected zooplankton samples at sea; Cruise: unique identifier for the oceanographic cruise on which samples were collected; Station: station identifier, unique within cruises; Sampling date: local date of sample collection; Mesh size: zooplankton net mesh size use for collection; Tow type: Equipment type used for sampling and type of tow performed; DNA: initials of the author who performed DNA extractions; 18S PCRs: initials of the coauthor who performed 18S amplifications; COI PCRs: initials of the coauthor who performed COI amplifications.

Loc.	Lat. (N)	Lon. (E)	Region	Max. depth (m)	Diel	Col. by	Cruise	Station	Sampling date	Mesh size (µm)	Tow type	DNA	18S PCRs	COI PCRs
14	32.9	138	Kuroshio Curr.	1000	D	JH	SY-11-05	K01	5/17/11	100	VMPS, vertical	JH	KK	KK
15	31	138	Kuroshio Curr.	1000	D	JH	SY-11-05	C3100	5/9/11	100	VMPS, vertical	JH	KK	KK
16	40	190	N. NPSG	1000	I	JH	KH-14-3	st9	7/23/14	100	VMPS, vertical	JH	KK	KK
17	45	190	WWD & Subpolar	1000	D	JH	KH-14-3	st10	7/24/14	100	VMPS, vertical	JH	KK	KK
18	35	190	N. NPSG	1000	N	JH	KH-14-3	st8	7/22/14	100	VMPS, vertical	JH	KK	KK
19	50	190	WWD & Subpolar	1000	N	JH	KH-14-3	st11	7/26/14	100	VMPS, vertical	JH	KK	KK
20	0	245	Tropical	1000	N	JH	KH-12-01	st5	2/7/17	100	VMPS, vertical	JH	KK	KK
21	33.8	138	Kuroshio Curr.	1000	I	JH	SY-15-01	C3345	1/27/15	100	VMPS, vertical	JH	KK	KK
22	33	138	Kuroshio Curr.	1000	N	JH	SY-15-01	C3300	1/26/15	100	VMPS, vertical	JH	KK	KK
23	55	190	WWD & Subpolar	1000	D	JH	KH-14-3	st12	6/27/14	100	VMPS, vertical	JH	KK	KK
24	34.4	229.6	WWD & Subpolar	1000	B	SAM	P2107	C3	8/6/21	200	MOCNESS, Oblique	SAM	SAM	SAM
25	36.1	237.7	Coastal CA	1000	B	SAM	P2107	C1	7/23/21	200	MOCNESS, Oblique	SAM	SAM	SAM
26	32.5	241.7	Coastal CA	1000	B	SAM	CCE-2007	SCB	7/1/20	200	MOCNESS, Oblique	SAM	SAM	SAM
31	10.9	243.8	Tropical	1000	B	AEC	DG5B_PRZ		3/21/21	200	MOCNESS, Oblique	AEC	KK	AEC
32	57.2	213	WWD & Subpolar	1200	I	JMQ	SKQ2019	GAK-19	7/25/19	150	MultiNet, vertical	JMQ	JMQ	JMQ

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CHAPTER 5

Conclusions

This dissertation reports on the biogeographic distributions of zooplankton communities in the mesopelagic zone of the North Pacific Ocean. It assesses the influence of dispersal and selection on zooplankton species ranges and examines the differential effects of these two processes on zooplankton with different traits and inhabiting different depth zones. The biogeographic ranges of zooplankton have been studied by biogeographers and oceanographers for at least a century. The work presented here expands this body of work into the mesopelagic zone of the ocean and is a small step toward understanding the dynamics of midwater ecosystems.

I begin by refining and validating the metabarcoding approach used throughout the dissertation. In Chapter 1, I compare metabarcoding-resolved zooplankton community composition against carbon biomass values calculated from digital Zooscan imaging. I find good overall correspondence between relative read abundance and relative carbon biomass for the dominant zooplankton groups that are resolved by both methods. This chapter also establishes the minimum sample fraction needed to fully represent zooplankton community diversity. I show that technical replication of PCR reactions is fundamental for detection of rare taxa. This chapter illustrates the value of using multiple approaches to characterize zooplankton community composition, including metabarcoding marker regions. It also demonstrates the importance of *a priori* understanding of biases in different methods. This work is an important contribution to the development of the emerging field of zooplankton metabarcoding.

Chapter 2 tests whether there are changes in zooplankton community composition and vertical habitat use along a cross-shore environmental gradient within one biogeographic region. Using paired metabarcoding and ZooScan imaging, I observe substantial changes in community composition and find that zooplankton shift their vertical distributions in association with their optimal habitat. Vertical shifts to deeper depths in the offshore are strongest among carnivorous zooplankton. Together with increasing relative abundance of specialized feeding behaviors and egg-brooding behavior, this change suggests a transition toward top-down dynamics in less productive waters. Through the lens of a space-for-time substitution, I find that zooplankton communities in future, more stratified oceans may have increased rates of carnivory, egg brooding, and asexual reproduction. My results also suggest that changes in community composition co-occur with reorganization of vertical habitat use patterns. Deepening vertical distributions can introduce primarily epipelagic taxa into mesopelagic depths, resulting in either vertical habitat overlaps with deeper-dwelling species, or deepening vertical distributions for mesopelagic taxa as well. Active selection of vertical habitat can alter the community observed within fixed depth bins. This chapter highlights the importance of considering spatial patterns of zooplankton communities within the context of the surrounding water column.

Chapter 3 begins to test whether there are broader biogeographic ranges and greater community similarity among mesopelagic zooplankton than among epipelagic zooplankton. This chapter focuses on a major latitudinal biogeographic transition across the Subarctic Pacific, Transition Zone, and North Pacific Subtropical Gyre. Across these regions, I find weaker differentiation among mesopelagic communities than among epipelagic communities and broader distributional ranges for mesopelagic zooplankton. I compare environmental and distributional ranges sizes for epipelagic and mesopelagic taxa and reveal stronger associations

of temperature and Chl-*a* with distributional ranges among epipelagic taxa, and stronger association of dissolved oxygen concentrations with distributional ranges for mesopelagic taxa. I examine distributional and environmental ranges for taxa with different traits and find the broadest ranges for taxa that exhibit Diel Vertical Migration. Epipelagic taxa with the capacity for asexual reproduction exhibited larger Chl-*a* and distributional ranges than taxa without. Mesopelagic omnivores were distributed across larger Chl-*a* and dissolved oxygen ranges, but omnivory was not associated with larger distributional ranges. Suspension feeding was similarly associated with larger ranges of dissolved oxygen but was also associated with larger distributional ranges. These results highlight the importance of food to mesopelagic taxa, but indicate that environmental range width does not always correspond with the size of distributional ranges. The larger distributional ranges and greater community similarity in the mesopelagic zone reveals greater porosity of biogeographic boundaries for mesopelagic zooplankton. Variability in environmental and distributional ranges among taxa from different depth zones suggests greater influence of temperature and Chl-*a* on epipelagic distributions, and greater influence of oxygen concentrations on mesopelagic distributions.

In Chapter 4, I compare epipelagic and mesopelagic zooplankton communities across the North Pacific basin, testing whether mesopelagic zooplankton exhibit larger biogeographic ranges or greater community similarity. At the basin scale, I explicitly test whether turnover in community composition is associated with variability in environmental conditions or dispersal potential due to modeled ocean circulation. I find no differences in the size of biogeographic ranges or the degree of community similarity across depth zones. Dissimilarity among communities is positively and non-linearly correlated with potential dispersal distance as well as with environmental dissimilarity. For all environmental predictors tested, correlation with

community dissimilarity was stronger in the epipelagic zone than in the mesopelagic zone, and generalized dissimilarity models including environmental variables and dispersal had more explanatory power in the epipelagic zone than in the mesopelagic zone. In this chapter, I find that the cumulative effects of dispersal and environmental dissimilarity in the mesopelagic ocean result in similar levels of community dissimilarity as found in the epipelagic ocean.

Environmental variability and dispersal potential together predict approximately half of zooplankton community dissimilarity in the mesopelagic ocean. This chapter suggests that at basin scales, mesopelagic zooplankton communities are strongly associated with water masses, and finds somewhat smaller influences of dissolved oxygen concentrations and potential food influx.

In this dissertation, I set out to define the degree to which dispersal and selection influence mesopelagic zooplankton biogeography and community composition. Although substantial uncertainty remains, this dissertation provides insight into the fundamental processes shaping community ecology. The mechanisms of community assembly and the processes shaping biogeographic distributions are complex but are strongly influenced by both dispersal and selection.

Chapters 2 and 3 find significant differences in trait composition of communities and environmental ranges of zooplankton taxa with different traits. These results suggest that the relative importance of dispersal and selection varies among taxa. Species with different morphologies, life history strategies, feeding behaviors, trophic levels, and vertical habitat use patterns are influenced differently by circulation regimes and have different environmental tolerance range widths.

Together, Chapters 3 and 4 demonstrate the scale-dependence of ecological processes. Greater porosity of biogeographic boundaries in the mesopelagic does not necessarily translate to larger biogeographic distributions at basin scales, perhaps due to dispersal limitation. If this is due to dispersal limitation, there is potential for trait-based differences, as dispersal limitations should be stronger for mesopelagic residents than for vertically migrating taxa. In combination, these two chapters highlight the need for strategic field sampling in order to disentangle the spatial and temporal scales across which dispersal or selection processes dominate.

In Chapters 2 and 4, I identify two distinct pathways for vertical connectivity and surface influence on deep communities. With finer vertical sampling resolution in the California Current Ecosystem, I detect vertical shifts in habitat use that can result in intrusions of primarily epipelagic taxa into mesopelagic depths. At the basin scale, I find significant, if noisy, associations of community turnover with differences in export flux.

Overall, my results indicate that the dominant processes shaping biogeographic distributions in the epipelagic ocean also influence mesopelagic zooplankton communities. The processes of dispersal and environmental selection appear to differentially affect mesopelagic zooplankton taxa with different feeding behaviors and dietary strategies. Stronger parallels between depth zones across the North Pacific Basin than between or within biogeographic regions suggest scale-dependence of these ecological processes.

Limitations and future priorities

While this dissertation provides important insights into ecological processes in the mesopelagic zone, it also emphasizes the importance of well-supported research programs. Contradictory results at basin scales and across regional biogeographic transitions indicate that

the processes of dispersal and selection may have different effects at different scales. Spatial and temporal scales are closely related (Haury et al., 1978). Extensive analyses at multiple spatial and temporal scales are required to more fully define the ecological processes that influence mesopelagic zooplankton and to disentangle the effects of scale on our understanding of these processes. The work presented here was only possible through the combined generosity of many collaborators who conducted sampling of the mesopelagic ocean as a part of their independent research programs. Additional studies of the mesopelagic ocean will require significant time, effort, and support. In some cases, observation and analysis can be facilitated through technical advances, including engineering developments, molecular approaches, or machine learning techniques. For example, advances in technical platforms and data processing of have increased the accessibility of midwater exploration (Ohman et al., 2019; Reisenbichler et al., 2016). In other cases, laborious net-sampling and time-consuming taxonomic identifications may still be necessary.

The work of describing and understanding the mesopelagic ocean can only be accomplished through the combined efforts of many researchers. The insights that will prove most influential are impossible to predict, as they frequently arise from novel questions and methods made possible by interdisciplinary collaborations. However, I note three areas of particular potential. First, I highlight the importance of relating biogeochemical budgets to organismal ecology, and vice versa. Several syntheses of mesopelagic biogeochemistry and ecology have been published in the past few decades (Buesseler et al., 2007; Burd et al., 2010; Robinson et al., 2010). Current efforts to constrain midwater carbon cycling include ecological processes such as respiration and excretion at depth by diel vertically migrating zooplankton communities (e.g., Maas et al., 2018). As the budgets of marine biogeochemical cycles become

better constrained, these types of analyses may identify ecological processes or fractions of the zooplankton community that play an outside role in marine carbon cycles. Second, differences in the ecology of individual species must be considered. Many ecological analyses of midwater zooplankton have relatively coarse taxonomic resolution (e.g., Steinberg et al., 2008; Wilson et al., 2010). With increased taxonomic resolution, there is increased capacity for detection of species-specific differences in community composition. Species-specific differences in ecological processes such as feeding strategy (e.g., Matsuura et al., 2010) can lead to variable effects on biogeochemical cycles as well as fine-scale complexity in community structure and ecological relationships. Decreased explanatory power of environmental conditions in the deeper ocean may reflect a transition toward top-down processes. Trophic interactions are difficult to study in the absence of species-specific data, whether in the form of ecological observations, of trophic level measurements such as from amino acid or fatty acid analyses, or of gut-content studies. Third, comparative studies and trait-based analyses have the potential to provide emergent insights into general ecological principles (e.g., Ohman & Romagnan, 2016). Trait-based approaches can be an alternative to studying individual species, provided sufficient trait information is available for all taxa within a region or group of interest. Given the technical difficulty of conducting extensive ecological studies on midwater zooplankton species, trait-based analyses may be a promising method for testing hypotheses about processes controlling ecological boundaries in the mesopelagic ocean.

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