

ZOOSCANNING THE CCZ: A BASELINE STUDY OF ZOOPLANKTON
ASSEMBLAGES IN THE CLARION CLIPPERTON ZONE

A THESIS SUBMITTED FOR PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

BACHELOR OF SCIENCE

IN

GLOBAL ENVIRONMENTAL SCIENCE

MAY 2024

By

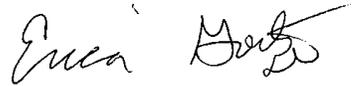
MICHAEL YAMADA

Thesis Advisor

ERICA GOETZE

I certify that I have read this thesis and that, in my opinion, it is satisfactory in scope and quality as a thesis for the degree of Bachelor of Science in Global Environmental Science.

THESIS ADVISOR

A handwritten signature in black ink, appearing to read "Erica Goetze", written in a cursive style.

Erica Goetze
Department of Oceanography

For my family - for supporting me in my education goals. I would not have been able to do it without their support.

ACKNOWLEDGEMENTS

I would like to acknowledge my mentor, Dr. Erica Goetze, for providing me with the support and mentorship that I needed to complete this project. I would not have been able to complete this project without her knowledge and expertise. I would also like to thank Dr. Goetze for taking a chance on me and hiring me into her lab. Since I entered college in the thick of the COVID-19 pandemic, all of my lab experience was virtual, so I am grateful for the opportunity to work with Dr. Goetze in her lab, even with very limited lab experience. I would also like to acknowledge Alexis Cazares for always being there for me whenever I needed advice or help with anything. This project would not have been possible without her assistance. Her patience and knowledge were invaluable when helping me with everything from the ZooScanning process, data analysis, or showing me how to drive the SOEST van to the Waikiki Aquarium to refill our seawater. I would also like to thank the Undergraduate Research Opportunities Program for providing the funding that was needed to allow me to pursue this as a thesis project. Lastly, I would like to thank my family, friends, teachers, and classmates for supporting me throughout my academic career.

Abstract

Zooplankton, small animals that inhabit the water column, are the dominant secondary producers of the global ocean. They serve several important roles in food web dynamics and ecosystem function but may be at risk due to the emerging industry of deep-sea mining. This study examines deep-sea plankton within the Clarion Clipperton Zone (CCZ), a region of the eastern tropical Pacific (ETP) that is rich in polymetallic nodules, as part of a baseline survey of ecosystem function prior to mining impact. Samples were collected over the NORI-D exploration mining claim using a 1m² Multiple Opening and Closing Net and Environmental Sensing System (MOCNESS) on two cruises in 2021, and samples were analyzed using a Hydroptic ZooScan MIV system. Zooplankton abundance was highest in the well-oxygenated upper 100 m of the water column, with lower abundance within the midwater oxygen minimum zone (OMZ) as well as below the OMZ. The expected migratory behavior for zooplankton is to move from the mesopelagic into the upper ocean at night. Most of the samples analyzed from the fall (DG5C cruise) exhibited this trend in all size fractions, but the large size fraction from the spring (DG5B cruise) did not. Seasonal variability in zooplankton abundance was observed in all regions of the water column (Upper, OMZ, Below OMZ) at the preservation reference zone (PRZ) site, with more small (0.2-1.0 mm) and large (1.0-5.0 mm) animals in the spring compared to the fall. At the collector test area (CTA) site, significant seasonality was observed only within the core of the OMZ in the small and large size fractions, with higher zooplankton abundance in spring. These site-specific differences in the strength of seasonality may have been due to higher oxygen concentrations in the upper portion of the OMZ during spring (100-300 m). This study

provides some of the first information on zooplankton seasonality across midwater in the Eastern Tropical Pacific: In providing baseline observations, this work also enables subsequent study of how zooplankton are impacted by commercial mining in this region. The information gathered with the study could be used to help set industry regulations to protect zooplankton communities from deep-sea mining impacts.

Keywords: Zooplankton, ZooScan, Deep-sea mining, eastern tropical Pacific (ETP), Clarion Clipperton Zone (CCZ), oxygen minimum zones (OMZ), seasonality

TABLE OF CONTENTS

Dedication.....	iii
Acknowledgements	iv
Abstract.....	v
List of Tables	viii
List of Figures.....	ix
1.0 Introduction	10
1.1 Deep-sea mining.....	10
1.2 Zooplankton ecology in the Eastern Tropical Pacific	12
1.3 Research rationale and goals	15
2.0 Methods	16
2.1 Collection of zooplankton	16
2.2 ZooScanning the zooplankton	17
2.2 Statistical analyses.....	22
2.4 Classifying the images.....	23
3.0 Results	25
3.1 Zooplankton abundance.....	26
3.2 Diel vertical migration (DVM).....	28
3.3 Seasonal variability	30
3.4 Taxon-specific patterns	35
4.0 Discussion.....	39
4.1 Seasonal Variability.....	39
4.2 Zooplankton abundance.....	41
4.3 Zooplankton DVM	42
4.4 Caveats and future work	44
4.5 Future implications	45
5.0 Conclusion	46
Appendix	47
Literature cited.....	51

LIST OF TABLES

Table 1. Overview of the MOCNESS tows included in this study

Table 2. Typical sample processing volumes for each size fraction and net

Table 3. Total proportion of the sample that was scanned for each size fraction and net

Table 4. Average number of animals (ROIs) scanned for each size fraction and ne

LIST OF FIGURES

Figure 1. Map of the NORI- D claim area and PRZ/CTA sites

Figure 2. Ecotaxa categories for zooplankton classification

Figure 3. Zooplankton abundance in spring 2021 (DG5B cruise)

Figure 4. Zooplankton abundance in fall 2021 (DG5C cruise)

Figure 5. Diel vertical migration of zooplankton in spring 2021 (DG5B cruise)

Figure 6. Diel vertical migration of zooplankton in fall 2021 (DG5C cruise)

Figure 7. Seasonal comparison of zooplankton abundance separated by site

Figure 8. Seasonal comparison of zooplankton abundance integrated 0-1500 m

Figure 9. Seasonal comparison of zooplankton abundance across depth (PRZ site)

Figure 10. Seasonal comparison of zooplankton abundance across depth (CTA site)

Figure 11. Water column oxygen profiles in spring 2021 (DG5B cruise)

Figure 12. Water column oxygen profiles in fall 2021 (DG5C cruise)

Figure 13. Copepod abundance by taxon in the PRZ (100-700m)

Figure 14. Other zooplankton abundances by taxon in the PRZ (100-700m)

Figure 15. Copepod abundance by taxon in the CTA (100-700m)

Figure 16. Other zooplankton abundances by taxon in the CTA (100-700m)

1.0 INTRODUCTION

1.1 Deep-sea mining

Interest in deep-sea mining has been increasing in recent years due to the need for and availability of metals to support green technology (e.g., electric vehicles). Metals, including manganese, nickel, copper, and cobalt, are essential components of electric car batteries. These metals are found on land, but cost and accessibility in the global commercial market has driven greater interest in ocean-based sources (Wedding et al., 2013). Increasing demand is making deep-sea mining more economically viable (Wedding et al., 2015). Polymetallic nodules found on the abyssal seafloor contain these precious metals and are a primary target for the emerging deep-sea mining industry (Washburn et al., 2019). Deep-sea mining may help to support electrification of the US car fleet, as well as other green technology, but environmental impacts of the mining process must be assessed to gain insight into the potential damage it may cause to the environment.

The Clarion Clipperton Zone, or the CCZ, is a region in the abyssal eastern tropical Pacific (ETP) between Hawaii and Mexico that spans about 6 million km² (Washburn et al., 2021). The CCZ is a region of interest for polymetallic nodule mining due to the high density of nodules compared to other areas of the deep seafloor. The 1982 United Nations Convention on the Law of the Sea (UNCLOS) created a set of international guidelines that allow the mineral resources of the deep seafloor to be explored and exploited, with the management of these resources delegated to the International Seabed Authority (ISA) (Cuyvers et al., 2018). As of 2022, the ISA has

granted 19 exploration mining licenses, covering more than 1.3 million km² of the deep seafloor in the CCZ for contractors to conduct baseline geological and ecological surveys in advance of polymetallic nodule mining (Amon et al., 2022a). ISA regulations are intended to prevent ‘serious harm’ and ensure the ‘effective protection of marine habitats’ from mining activities and other potentially harmful events that may occur on the seafloor (Amon et al., 2022b). The ISA requires that mining contractors establish zones that will be impacted by deep-sea mining, as well as preservation reference zones (PRZ) that will not be impacted by mining activity (set aside zones; Amon et al., 2022b). Our study examines animal communities within the NORI-D mining claim (southeastern CCZ), with samples collected at both the PRZ site and the Collector Test Area (CTA), a region impacted by test mining operations in the fall of 2022.

Deep-sea mining may release toxic pollutants, destroy marine habitat, cause biodiversity loss, reduce ecosystem services that the deep ocean provides, and create sediment plumes that smother animals inhabiting either the water column and/or the abyssal benthos (Drazen et al., 2020; Spearman et al., 2020). The deep-sea mining process breaks up polymetallic nodules, and toxic concentrations of metals may be released into the water column where it could be absorbed through permeable tissue, such as the gills or guts of marine animals (Hauton et al., 2017). Metal uptake could cause long-term bioaccumulation of toxic metals as predators feed on contaminated prey. Sediment plumes will be created in the vicinity of mining vehicles on the seafloor as well as in midwater due to sediment discharge following nodule removal and processing on a surface vessel (Sharma, 2015). The midwater discharge depth was initially proposed to be

at 1200 m, with creation of a large sediment plume expected at and below this depth horizon in the water column. Mining operations may impact the zooplankton community by reducing zooplankton abundance and biodiversity, creating irregular diel vertical migratory patterns, and impacting seasonal variability. The preservation of biodiversity and ecosystem integrity is essential to sustain the ecosystem functions that the deep ocean provides (Ramirez-Llodra et al., 2019). Deep-sea mining could have large, unintended consequences to the zooplankton community and the ecosystem services that they provide.

1.2 Zooplankton ecology in the Eastern Tropical Pacific

Zooplankton, small animals that inhabit the water column, are the dominant secondary producers of the global ocean, and they serve several important roles in ecosystem function. They inhabit the water column, with many species largely avoiding the oxygen minimum zone (OMZ) in midwaters. The OMZ region in the eastern tropical Pacific has oxygen concentrations at $< 0.1 \text{ ml L}^{-1}$ and spans from ~ 100 meters to 700 meters depth (Saltzman and Wishner, 1997). Many animals reside above or below the OMZ in areas where the oxygen concentration is higher. Animals that live within the OMZ often have modified metabolic systems that allow them to survive low oxygen availability (Saltzman and Wishner, 1997), for example by curtailing energy expenditure during the day. In the ETP, zooplankton can reduce their aerobic respiration by over 50% to conserve energy while exposed to hypoxic conditions (Seibel et al., 2016). The OMZ can vary in size and intensity depending on the location and ocean conditions, and in the

future may be influenced by climate change (Wishner et al., 2013). In the ETP, zooplankton communities are variable in their vertical distribution and depth of maximum abundance, as well as their diel vertical migration patterns based on the intensity and vertical extent of the OMZ (Wishner et al., 2020). These OMZ-influenced distributions and abundance of zooplankton can impact ecosystem function and influence fisheries (Wishner et al., 2020). The zone below the OMZ has higher oxygen content than the OMZ core but is still lower in oxygen concentration than the well-oxygenated upper water column above the OMZ (Loescher et al., 2016). The zone from the sea surface to the top of the OMZ has the highest zooplankton abundance, while the OMZ core typically has the lowest concentration of animals (Loescher et al., 2016). The zone below the OMZ may have slightly higher zooplankton abundance than the OMZ core, but it is still significantly less than near the sea surface (Loescher et al., 2016).

Diel vertical migration also impacts zooplankton abundance across depth in the water column. Zooplankton typically migrate to the surface at night and inhabit deeper zones during the daytime. Food availability is higher at the surface, so zooplankton typically migrate up in the water column to feed at night when predation risk is lower (Fernández-Álamo & Färber-Lorda, 2006). The OMZ in the ETP is expanding as a result of climate change, which may cause a change in biological distributions and ecosystem function (Wishner et al., 2013). Peak zooplankton biomass has consistently been found at the thermocline, regardless of OMZ thickness (Wishner et al., 2013). Macrozooplankton and micronekton of the upper ocean and upper portion of the OMZ are dominated by euphausiids and myctophid fish, which descend to the core of the OMZ during the day

(Maas et al., 2014). The region below the OMZ core has very distinctly different resident communities, and the lower oxycline was dominated by *Cyclothone* spp. fish as well as a diverse population of other taxa (Maas et al., 2014).

Seasonality is present in tropical oceans, but it is not as strong as in temperate and polar oceans (Kessler, 2006). While ecosystem change may not be as noticeable in the ETP, ocean conditions that are present in different seasons still affect zooplankton communities. Seasonality in the ETP is observed in the velocity and extent of the Equatorial Countercurrent (Longhurst and Pauly, 1987). This current only extends to about 120°W in the winter but extends to the east coast of North America and deflects towards the poles from May to November (Kessler, 2006). Zooplankton abundance in the ETP varies synchronously with chlorophyll concentration, so when upwelling is strong and ocean temperatures are cool, zooplankton abundance is at its peak (March-May; Fernández-Álamo & Färber-Lorda, 2006). Seasonality of the zooplankton is influenced by many factors, such as currents, upwelling, and ocean temperature.

Deep-sea mining may cause sediment plumes that disturb zooplankton communities in the CCZ (Drazen et al., 2020). Many large, commercially important fish rely on zooplankton as food resources, and if zooplankton are reduced in abundance, biomass, or their spatial distribution is altered, these effects could alter trophic interactions in the food web (Drazen et al., 2020). The amount of CO₂ that is being sequestered in the ocean's deep midwaters may also be impacted by reductions in zooplankton abundance or altered migration behavior due to mining. If the ecosystem function of the deep ocean is altered, there may be large consequences, spanning beyond

the midwater zone. This is important because deep midwater ecosystems are home to 100 times more fish biomass than the global annual fish catch, connect deep and shallow ocean ecosystems, and regenerate nutrients that are responsible for supporting fish stocks (Drazen et al., 2020).

1.3 Research rationale and goals

The overarching goal of this project is to conduct a baseline survey of zooplankton communities within the Clarion Clipperton Zone (CCZ) prior to deep-sea mining impacts. Such a baseline will allow us to assess ecosystem function and zooplankton community structure throughout the water column before mining perturbation to the ecosystem. Our aims are to: (1) characterize zooplankton community structure, vertical distribution, and obtain taxon-specific estimates of abundance and biomass in the upper 1500 m of the water column over the NORI-D exploration mining claim area, a region at near-term risk of mining impacts; (2) assess seasonal variation in these parameters through comparison of fall and spring cruises, in order to understand how seasonal cycles may interact with mining disturbance in this ecosystem; and (3) assess whether the zooplankton communities in the PRZ and CTA are comparable or if there are systematic differences between them that may impact our ability to successfully monitor ongoing mining impact. Prior work on zooplankton in the eastern tropical Pacific has not examined community structure across all taxonomic groups, and there is limited prior information available about seasonality across the mesopelagic in this ecosystem. This study is the first to address these important gaps in knowledge.

2.0 METHODS

2.1 Collection of zooplankton

Zooplankton was collected over the NORI-D exploration mining claim in the southeastern Clarion Clipperton Zone (10.25-11.0 °N/116.14-117.10°W). Sampling was conducted in two regions: (1) a Preservation Reference Zone (PRZ), which is designated as a protected no-mining area, and (2) the Collector Test Area (CTA), a region impacted by test mining operations in the fall of 2022. A map showing the location of the NORI-D claim and the regions of interest within the CCZ (PRZ and CTA) are shown in figure 1.

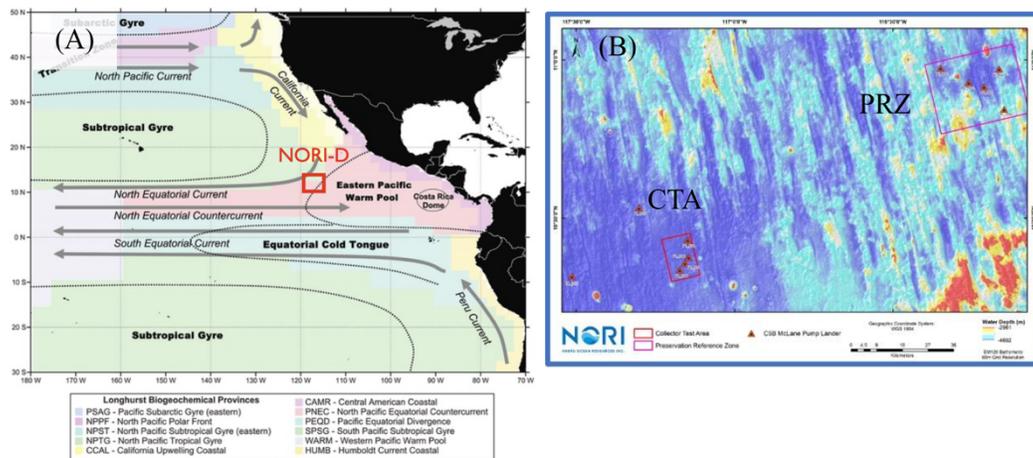


Figure 1. (A) Overview of the major oceanographic currents in the eastern tropical Pacific (from Fiedler et al. 2013), with the approximate location of the NORI-D claim indicated by a red box. (B) Overview map of the bathymetry of the NORI-D claim area, with sites CTA and PRZ marked by red boxes. MOCNESS tows were conducted broadly over these two areas of the NORI-D.

Zooplankton were collected using a 1m² Multiple Opening/Closing Net and Environmental Sensing System (MOCNESS) on 12 oblique tows conducted during each of two cruises in spring (DG5B cruise, 3/21-4/7) and fall (DG5C cruise, 10/6-10/21) of 2021 (24 tows total). Three tows were conducted in daytime and three tows were conducted at night in each of the PRZ and CTA (in each cruise), in order to examine diel vertical migration (DVM) behavior and changing animal distributions. The MOCNESS

system sampled across 9 discrete depth strata, with target depths 1500-1250 m, 1250-1000 m, 1000-800 m, 800-700 m, 700-500 m, 500-300 m, 300-100 m, 100-50, and 50 m to the surface. Plankton from each net was split quantitatively at sea using a Folsom plankton splitter, with 50% preserved immediately at sea using 5% buffered formalin. Buffered formalin was replaced within 4 months of initial preservation.

2.2 ZooScanning the zooplankton

Zooplankton samples were analyzed using a Hydroptic ZooScan MIV system (Gorsky et al. 2010), including 4 MOCNESS tows on each of the spring (DG5B) and fall (DG5C) cruises (8 tows total; Table 1). Preserved samples were digitized by ZooScan and processed using ZooProcess software. The ZooScan system coupled with ecotaxa for web-based machine learning classification of images is able to detect, enumerate, measure, and classify digitized objects (or regions of interest, ROIs).

Table 1. Overview of the MOCNESS tows included in this study. Latitude and Longitude are recorded at the start of each tow, dates and times are local. PRZ = Preservation reference zone, CTA = Collector test area.

Event No.	Tow #	Site	Latitude	Longitude	Date	Start Time	Day/Night
DG5B_1MOC2	2	PRZ	10 ° 55.5479 N	116 ° 19.268 W	3/21/21	20:34	Night
DG5B_1MOC5	5	PRZ	10 ° 48.479 N	116 ° 14.372 W	3/26/21	6:40	Day
DG5B_1MOC8	8	CTA	10 ° 26.8541 N	117 ° 10.3205 W	4/3/21	8:11	Day
DG5B_1MOC10	10	CTA	10 ° 25.937 N	117 ° 13.157 W	4/7/21	20:55	Night
DG5C_1MOC2	2	PRZ	10 ° 58.227 N	116 ° 20.9 W	10/6/21	9:31	Day
DG5C_1MOC5	5	PRZ	11 ° 00.143 N	116 ° 18.1189 W	10/10/21	19:36	Night
DG5C_1MOC8	8	CTA	10 ° 28.1634 N	117 ° 08.6963 W	10/15/21	19:05	Night
DG5C_1MOC12	12	CTA	10 ° 23.5291 N	117 ° 00.8386 W	10/21/21	7:07	Day

Plankton samples were subsampled in order to obtain quantitative data based on 1000s of animals in each size fraction without scanning all animals in the bulk sample. Preserved plankton samples were transferred to dH₂O, size fractionated into small (0.2 – 1.0 mm, SM), large (1.0 – 5.0 mm, LG), and extra-large (> 5.0 mm, XL) size fractions, with animals resuspended into a known volume of dH₂O. All animals retained in the extra-large size fraction were scanned. Replicate subsamples from the small and large size fractions were scanned (typically 6 scans) to ensure that > 1000 animals in each size fraction from each sample were imaged (Gorsky et al. 2010). Plankton was mixed by inversion > 3 times to ensure an even resuspension of animals, and a subsample taken immediately using a volumetric pipette with wide-bore tip. This subsample volume was

determined empirically to obtain the maximum number of animals that could be placed on the ZooScan without any of the animals touching one another. Animals suspended in dH₂O inside the ZooScan frame were manually separated with wooden probes, to facilitate scanned animals being separated into distinct ROIs by ZooProcess. Table 2 reports the typical volume of the suspension for each size fraction and net, the median volume of the subsample taken for each scan, and the number of scans for each net. Table 3 reports the fraction of each original sample that was scanned.

Background scans were run at the start of each day of laboratory analysis. Each set of background scans consisted of two scans with at least 40 seconds between each scan. Scans were processed using VueScan. This process was repeated until the difference between mean gray values, which was generated by ZooScan, was < 30 prior to running samples. Background scans are important to creating high quality images of each animal, with automated removal of background gray from each scan. Initial scans were processed using ZooProcess under default settings, creating separate images of each animal for visual analysis and a data table with 40 measured properties for each animal in the scan.

Table 2. Example of the typical processing volumes and number of scans taken for each size fraction. Small, Large, X-Large) and each Net. Average Vol is the average subsample volume taken for each scan. Data from DG5B_PRZ_1MOC5.

Depth (m)	Small (0.2-1 mm)			Large (1-5 mm)			X-Large (>5 mm)
	Suspension volume (mL)	Average vol (mL)	# scans	Suspension volume (mL)	Average vol (mL)	# scans	# scans
1500-1250	200	10	4	200	10	7	2
1250-1000	200	6.67	6	200	15	6	2
1000-800	200	7	6	200	10	6	4
800-700	200	4	6	200	4.167	6	4
700-500	200	5	6	200	10	6	1
500-300	200	4	6	200	4.33	6	4
300-100	200	2	6	200	2.1667	6	4
100-50	200	1.667	6	400	2	6	4
50-0	200	1.667	6	400	2	12	4

Table 3. Total proportion of the formalin-preserved sample that was scanned during ZooScan processing, for (A) the small size fraction (0.2 - 1.0 mm), and (B) the large size fraction (1.0-5.0 mm). 100% of the extra-large size fraction was scanned for all samples.

(A) Small	1500-1250 m	1250-1000 m	1000-800 m	800-700 m	700-500 m	500-300 m	300-100 m	100-50 m	50-0 m
DG5B_PRZ_1MOC2	33%	15%	13%	10%	15%	10%	8%	6%	2%
DG5B_PRZ_1MOC5	20%	20%	21%	12%	15%	12%	6%	4%	4%
DG5B_CTA_1MOC8	30%	30%	30%	30%	24%	9%	3%	6%	3%
DG5B_CTA_1MOC10	30%	26%	21%	21%	23%	10%	7%	6%	4%
DG5C_PRZ_1MOC5	30%	21%	30%	21%	15%	15%	10%	6%	18%
DG5C_PRZ_1MOC2	30%	30%	28%	18%	24%	30%	21%	3%	3%
DG5C_CTA_1MOC8	25%	18%	15%	15%	15%	18%	15%	3%	3%
DG5C_CTA_1MOC12	30%	21%	18%	12%	9%	21%	4%	3%	3%

(B) Large	1500-1250 m	1250-1000 m	1000-800 m	800-700 m	700-500 m	500-300 m	300-100 m	100-50 m	50-0 m
DG5B_PRZ_1MOC2	45%	30%	25%	18%	NA	14%	13%	5%	7%
DG5B_PRZ_1MOC5	40%	45%	30%	13%	30%	13%	7%	3%	6%
DG5B_CTA_1MOC8	30%	35%	45%	44%	24%	9%	5%	4%	2%
DG5B_CTA_1MOC10	50%	30%	29%	21%	15%	15%	6%	6%	3%
DG5C_PRZ_1MOC5	30%	21%	30%	35%	15%	15%	9%	6%	18%
DG5C_PRZ_1MOC2	60%	30%	23%	18%	12%	60%	21%	3%	2%
DG5C_CTA_1MOC8	30%	30%	30%	30%	13%	30%	21%	5%	2%
DG5C_CTA_1MOC12	30%	30%	30%	30%	12%	30%	11%	3%	2%

2.3 Statistical analyses

Zooplankton abundance was visualized using histogram bar plots of the number of zooplankton per m^3 of seawater from each net and each tow, including the proportions for each zooplankton size fraction. Calculations were based on the total # of animals and percent of each sample scanned, the volume of seawater filtered by each net, and the 50% quantitative split executed prior to preservation, for final estimates that reflect zooplankton abundance per m^3 based on the total seawater filtered by each net. These calculations were conducted for all tows of both cruises. This approach allows for the entire sample to be accounted for, while only processing a subsample. Diel vertical migration was visualized as the difference in abundance at each depth between the day and night pairs of MOCNESS tows from each site. Positive numbers indicate higher zooplankton abundance at night, while negative numbers show higher zooplankton abundance in the day. DVM plots by size fraction were created for both cruises.

To test for seasonal differences in integrated zooplankton abundance (0-1500 m) between fall and spring, we combined the data from both sites, tested for normality using the Shapiro-Wilks test, and then applied a Wilcoxon rank sum test. We also examined differences in seasonality with data separated by sites (PRZ/CTA), also testing for normality and using both t-tests and non-parametric Mann Whitney U tests. Seasonality in integrated abundance for each size fraction at different depth horizons (Upper 0-100 m; OMZ 100-700 m; Below OMZ 700-1500 m) was also examined for both sites, using approaches as described above. Oxygen profiles (0-1500 m) of the water column are also

reported from each season at both sites since this oceanographic gradient may have an impact on seasonality.

2.4 Classifying images

Images from the MOCNESS tow DG5C_PRZ_1MOC5 were uploaded to ecotaxa as 3 image sets representing different pelagic habitats (Upper 0-100m, OMZ 100-700m, Lower 700-1500m) and used to create an image learning set for machine learning classification into morphotaxonomic groups. Of the 18,909 images from OMZ depths of this tow (100-700 m), 23.81% were manually classified (by EG) into 40 categories. Of the 56,420 images from the upper ocean of this tow (0-100 m), 21.04% were classified (by AC/EG) into 44 categories (Fig. 2). The OMZ learning set was then used to train a machine learning classifier in ecotaxa for automated classification of DG5C_1MOC2_OMZ (3 nets; 100-700m). Images classified in this set (OMZ depths, 3 nets) were manually validated for accuracy (99.96% validated; 16,385 images). The initial OMZ learning set (DG5C_PRZ_1MOC5) and this fully validated OMZ set (DG5C_1MOC2_OMZ) were used together to train ecotaxa classifiers for the OMZ depths of the 6 remaining MOCNESS tows. Once classified using ecotaxa, OMZ images from the 6 remaining tows were manually validated to >20% (23.81-99.96% all tows). In total, 51 taxonomic categories were created for organisms from the OMZ depths of these tows. Initial validation focused on calanoid copepod taxa and larger organisms, and considerable errors may remain in the smaller-bodied categories, including nauplii, oncaeids, and oithonids, which are not yet well validated. Calanoid groups are well

resolved at this time. Ongoing work will apply similar methods to the upper (0-100m) and deep (700-1500m) image sets in order to accurately classify images from all remaining tows.

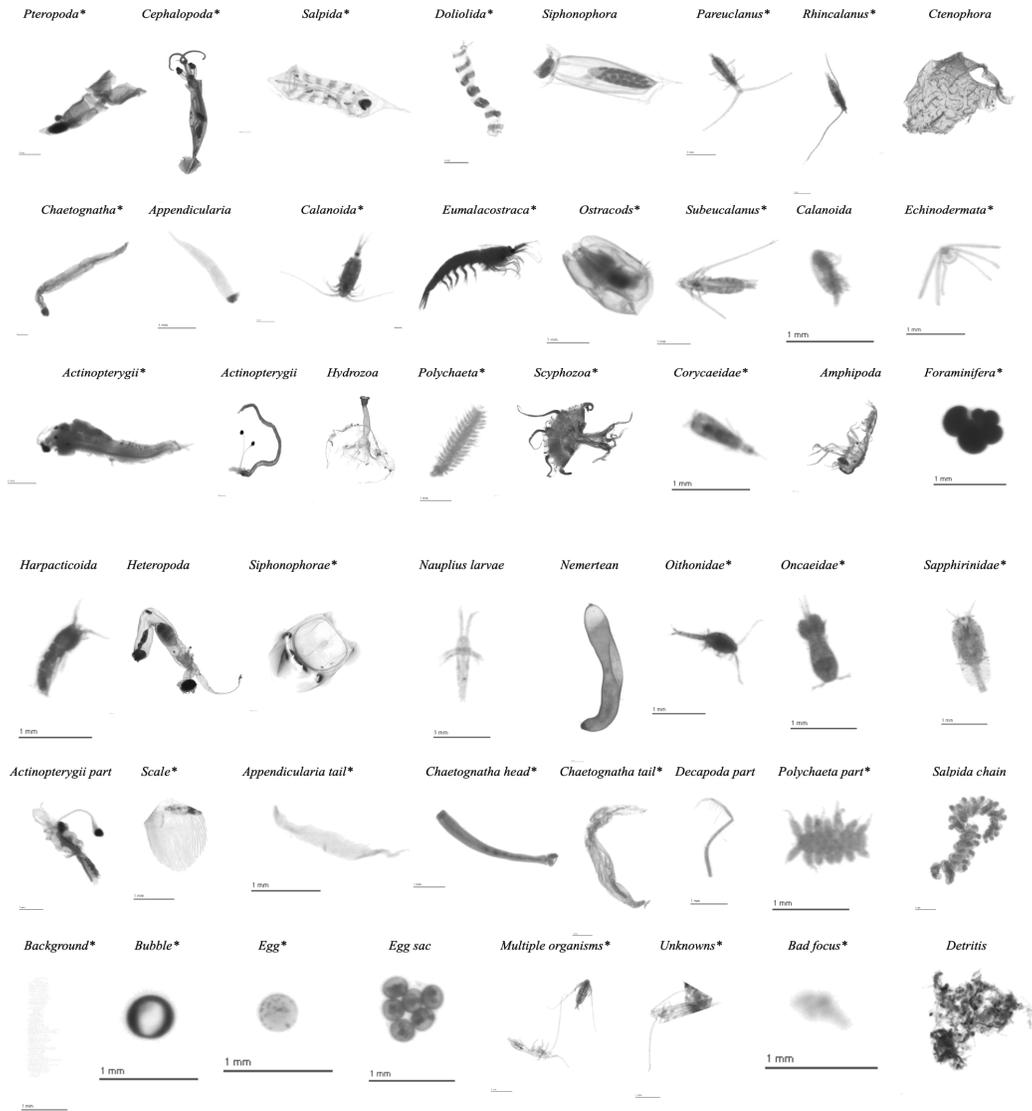


Figure 2. Categories for image classification in ecotaxa from the MOCNESS tow DG5C_PRZ_1MOC5.

Taxonomic groups found in the upper ocean (0-100m) are the focus, with * indicating categories also found within the OMZ (100-700m).

3.0 RESULTS

A total of 1,267 plankton scans were generated across eight MOCNESS tows and nine depth strata. For the DG5C cruise, a total of 440,663 animals were captured in 602 scans, and from the DG5B cruise 588,821 animals were imaged over 661 scans. Table 4 reports the average number of animals (ROIs) scanned for each net across all tows. In all cases > 1000 ROIs were scanned for each size fraction and net, well above the minimum recommendation in most cases (Gorsky et al., 2010).

Table 4. Mean number and standard deviation of ROIs scanned for each net and size fraction across all MOCNESS tows. In all cases, we scanned on average well over the recommended minimum of 1000 ROIs (Gorsky et al., 2010)

Depth (m)	Small		Large		X-Large	
	mean	st dev	mean	st dev	mean	st dev
1500-1250	6010	1880	1481.38	407	1028.75	591
1250-1000	6883.88	1946	1871.5	515	1212.5	527
1000-800	6089.88	1593	2463.38	848	2138.25	863
800-700	5547.63	2491	2134.63	681	2779.75	1190
700-500	2937	1189	1574.38	562	1477.50	1495
500-300	5776.5	2610	2160	1096	1563.75	2655
300-100	6178.25	1988	5224.63	4040	3185.75	3142
100-50	11424.63	5499	8529.75	3890	10490	5829
50-0	10992.38	3309	8784.25	4741	8745.25	5004

3.1 Zooplankton abundance

Highest abundance was found from 100-0 m, in the well oxygenated upper ocean. In general, biomass declined with depth; however, 700-300 m often had fewer animals than the depths below because this material was collected within the core of the OMZ (Figs. 3, 4). Across all depths, smaller animals were more abundant than larger animals, and large animals were more common at depths above the OMZ than below or within the OMZ. Figure 3 shows zooplankton abundance across depth in the spring of 2021 (DG5B cruise). From 0-50 m, there were 4358 small, 475 large, and 50 extra-large animals per m^3 seawater in the PRZ at night. During the day, the same depth horizon had lower abundance of both small and large animals with 3128 small, 2497 large, and 54 extra-large animals per m^3 seawater. In the CTA at this depth during the day, there were 1070 small, 2065 large, and 22 extra-large animals per m^3 seawater, a bit lower abundance in comparison to the PRZ. The nighttime CTA tow had 1015 small, 1007 large, and 30 extra-large animals per m^3 seawater. Surprisingly, there was a higher abundance of large animals than small animals in the daytime tow of the PRZ. Another unexpected observation was that in the CTA at night, the region with highest abundance was 50-100 m (3070 animals per m^3 seawater), rather than 0-50 m (2054 per m^3) as observed in all other tows.

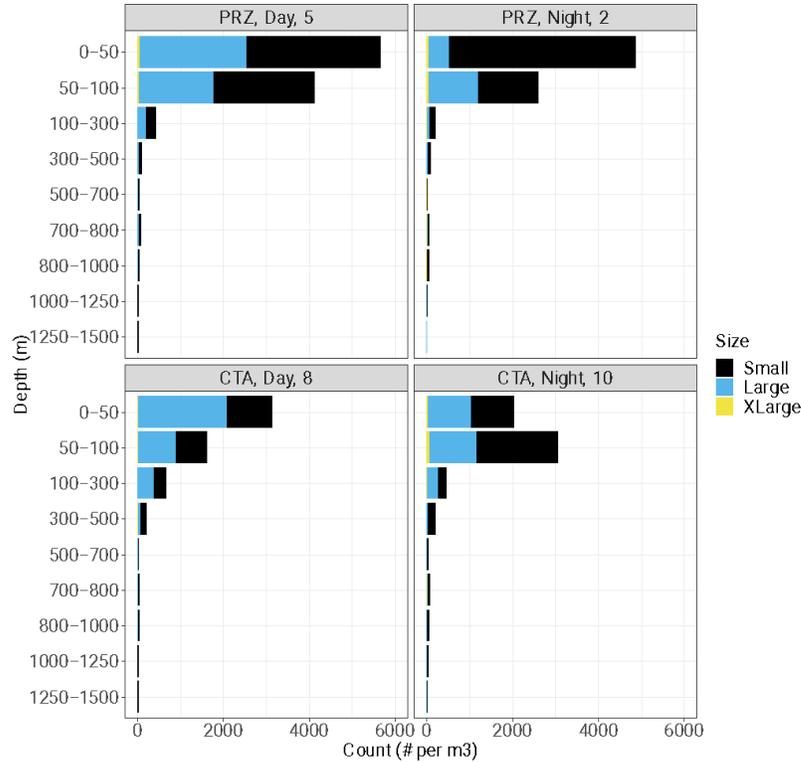


Figure 3. Zooplankton abundance per m^3 seawater for three size fractions from each tow and net on the spring 2021 (DG5B) cruise.

Most of the abundance trends in the fall follow those from the spring (DG5B cruise) with higher abundance in the upper ocean above the OMZ (0-100 m; Figure 4), fewer animals within and below the OMZ, and with smaller animals being more abundant than other size fractions. The tow conducted in the PRZ during the day had an unexpectedly low abundance of animals compared to the other tows from both cruises. There was a very low abundance of animals in all depth zones. The top 50 m of the water column only had 139 small, 38 large, and 7 extra-large animals per m^3 seawater, which is much lower than the same depth horizon in the spring as well as other tows in the fall. Additional verification of the recorded subsample volumes scanned and seawater filtered

is warranted to ensure that these numbers are correct. The fall tows had an average of 229 small, 226 large, and 10 extra-large animals per m³ and the spring tows averaged 502 small, 347 large, and 12 extra-large animals per m³. There was a lower abundance than the spring tows across most of the tows, except for the nighttime CTA tow.

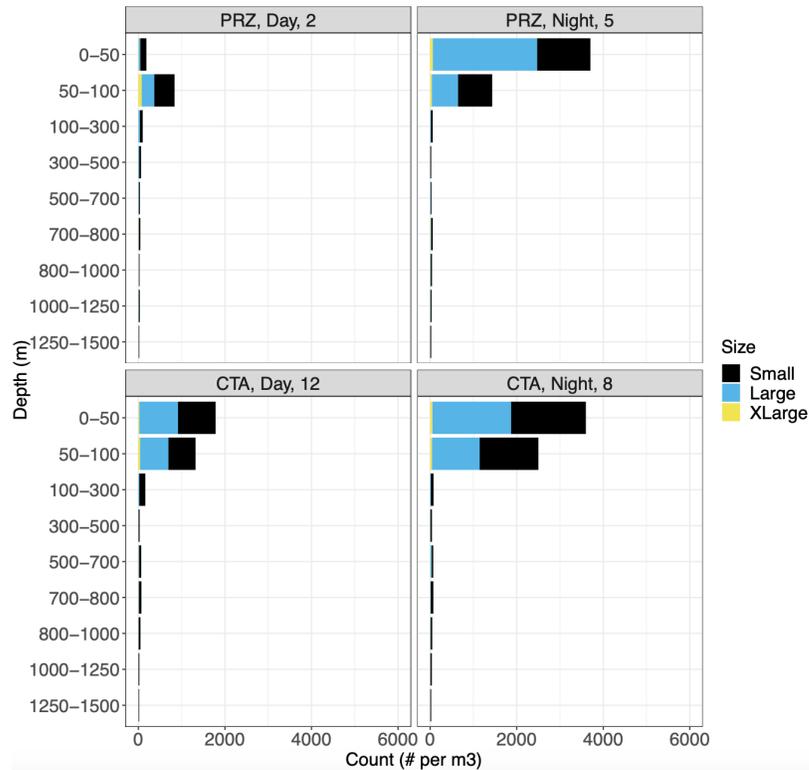


Figure 4. Zooplankton abundance per m³ seawater for three size fractions from each tow and net on the fall 2021 (DG5C cruise). The x-axis range in Fig 4 is held the same as in Fig 3 for comparison.

3.2 Diel vertical migration (DVM)

The difference in abundance of zooplankton between the day and night paired tows at each site is due in part to diel vertical migration (DVM) of the zooplankton (Fig. 5, spring). During spring, small and x-large animals exhibited a migration into shallower depths at night. There was some variability in whether animals were arriving in the 50-

100 m layer or in the 0-50 m layer. The large size fraction had fewer animals at night compared to the day in the upper 100 m (less 2,610 PRZ; 846 CTA).

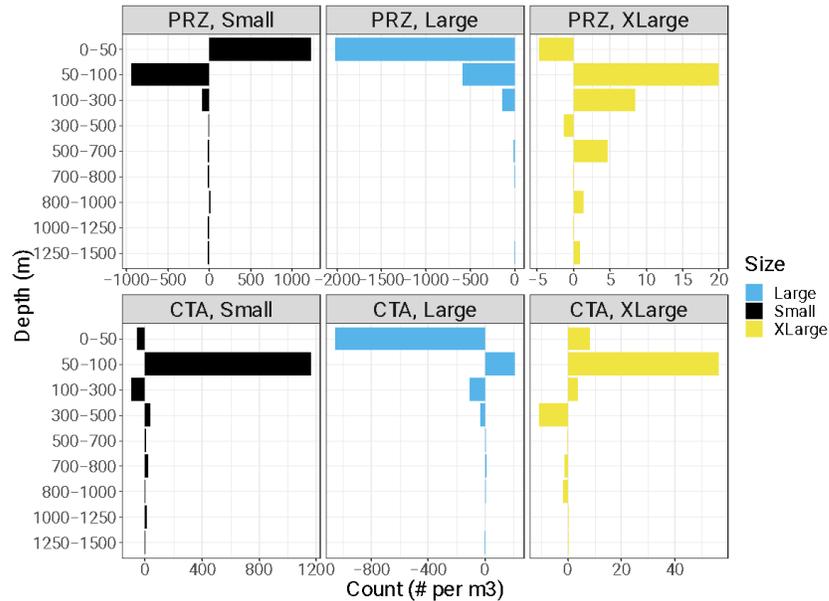


Figure 5. Zooplankton diel vertical migration in three size fractions at the PRZ and CTA sites in spring 2021 (DG5B cruise). Plotted as the difference in zooplankton abundance per m^{-3} seawater between day and night tows in each size fraction; Positive values indicate higher abundance at night, negative values are higher abundance during day.

In fall, animal DVM largely followed the expected trend with more animals in the near surface at night for every size fraction (Fig. 6). In the PRZ, there was a nighttime increase of 1,412 animals in the small size fraction, 2,686 large animals, and 15 extra-large animals in depths above the OMZ. In the CTA, there was a nighttime increase of 1,588 animals in the small size fraction, 1,371 large animals, and 32 animals in the extra-large size fraction in the upper ocean per m^3 seawater. All tows from the fall exhibited

the expected trend of having more animals in the surface at night compared to the day. However, abundance in the total depth integrated values in most cases were not balanced between day and night, with a lack of clear signal indicating resting depths during daytime.

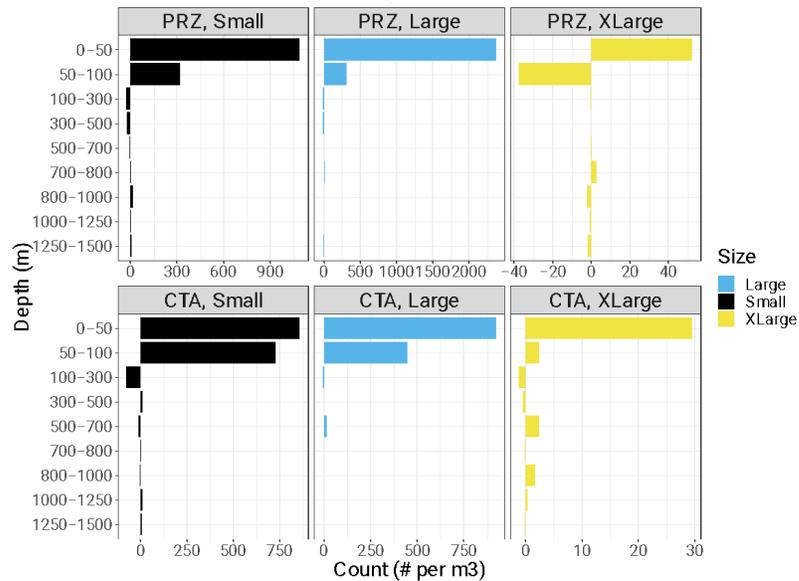


Figure 6. Zooplankton diel vertical migration in three size fractions at the PRZ and CTA sites in fall 2021 (DG5C cruise). Plotted as the difference in zooplankton abundance per m³ seawater between day and night tows in each size fraction; Positive values indicate higher abundance at night, negative values are higher abundance during day.

3.3 Seasonal variability

Higher springtime abundances were apparent at both sites (PRZ/CTA; Fig 7), but with some consistent differences in the strength of the pattern observed and in the depth horizons involved. The seasonality of zooplankton abundance in the PRZ was more pronounced than in the CTA (Fig. 7). In the CTA, there was on average 128,567 animals per m² in the spring and 81,324 animals per m² in the fall (158% higher in the spring). In

the PRZ there were 145,513 animals per m² in the spring and 57,374 animals per m² in the fall (averages; 254% higher in the spring). However, the relatively low number of MOCNESS tows analyzed impedes our finding statistical significance, despite broadly non-overlapping observed values in several cases. For example, when data from all tows were combined, abundance of animals in the spring was not significantly different from the fall (Wilcoxon rank sum test, $p = 0.11$; Fig. 8).

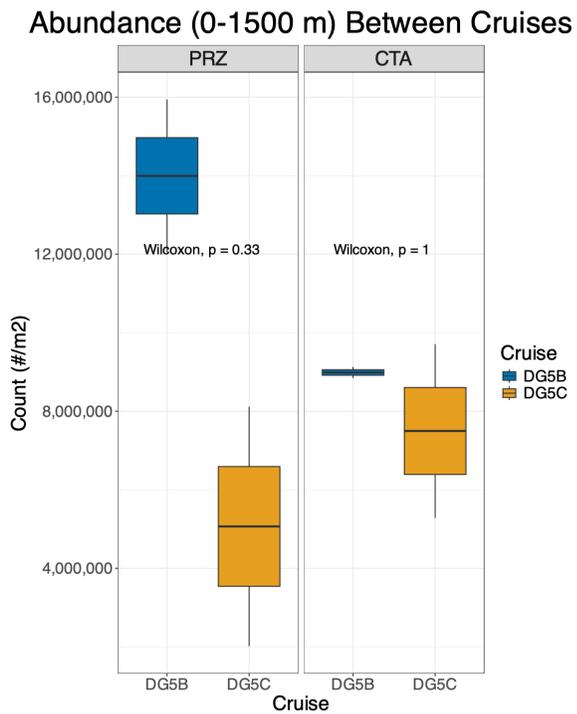


Figure 7. Boxplots of integrated zooplankton abundance (# m⁻²) in the spring cruise (DG5B, blue) and fall cruise (DG5C, orange) across the entire water column (0-1500 m), separated by sites (PRZ left; CTA right).

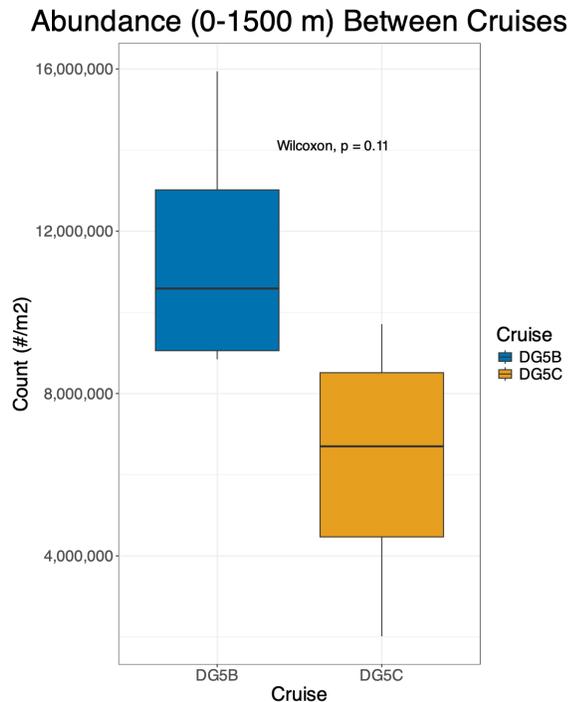


Figure 8. Boxplots of integrated zooplankton abundance ($\# \text{ m}^{-2}$) in the spring cruise (DG5B, blue) and fall cruise (DG5C, orange) across the entire water column (0-1500 m). Data from all tows (both sites) in each season combined.

Across three depth zones of the water column at the PRZ site, we find generally higher integrated abundance ($\# \text{ m}^{-2}$) of animals in the spring than the fall in both the small and large size fractions (Fig. 9). However, these seasonal differences were not statistically significant (Wilcoxon rank sum test, $p > 0.05$), with the comparison between 2 fall MOC tows and 2 spring MOC tows when data are parsed this way. In the upper ocean, there were 432,081 more small animals per m^2 (429% increase) and 125,850 more large animals per m^2 on average (175% increase) in spring. In contrast, the extra-large animal size fraction exhibited little variation in abundance between the seasons (Fig. 9).

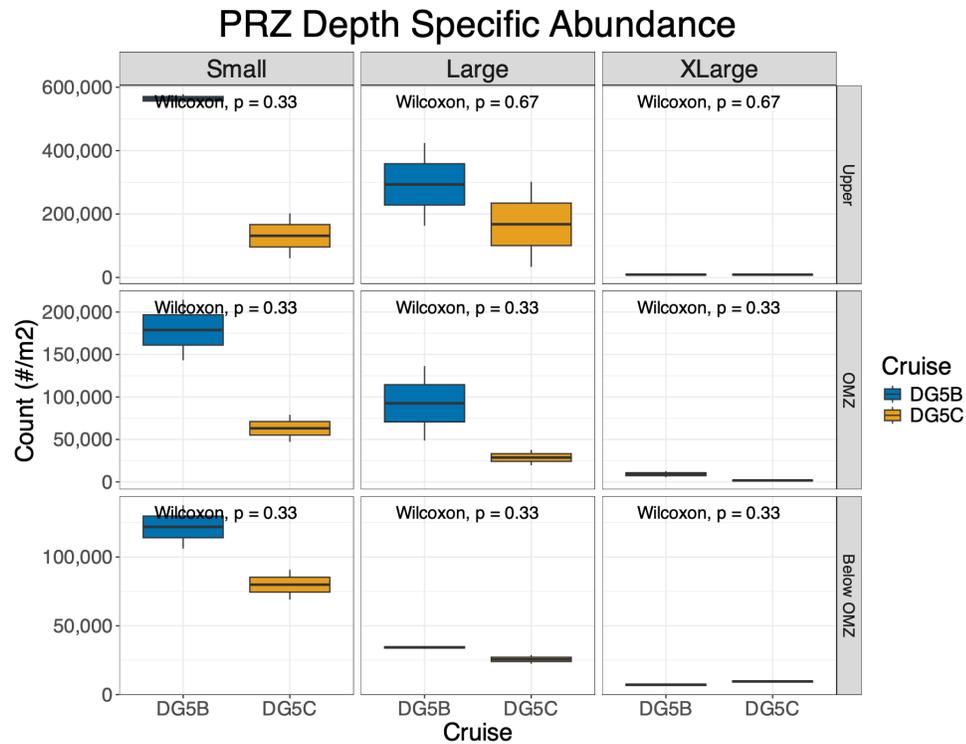


Figure 9. Boxplots of integrated zooplankton abundance ($\# \text{ m}^{-2}$) for three size fractions across three depth horizons (Upper, 0-100 m; OMZ, 100-700 m; Below OMZ, 700-1500 m) at the PRZ site in the spring (DG5B, blue) and fall (DG5C, yellow).

Seasonal trends in the CTA were a bit distinct from those observed in the PRZ (Fig. 10), with less seasonal change in abundance occurring above (0-100 m) and below (700-1500 m) the OMZ and greater seasonal differences in the small and large size fractions within the OMZ. Within the OMZ, there were 304,785 more small animals per m^2 (247% increase) and 406,628 more large animals per m^2 (752% increase) in the spring compared to the fall. However, these seasonal comparisons were also non-significant at this N of MOCNESS tows analyzed (Wilcoxon rank sum test, $p = 0.33$ small, $p = 0.33$ large)

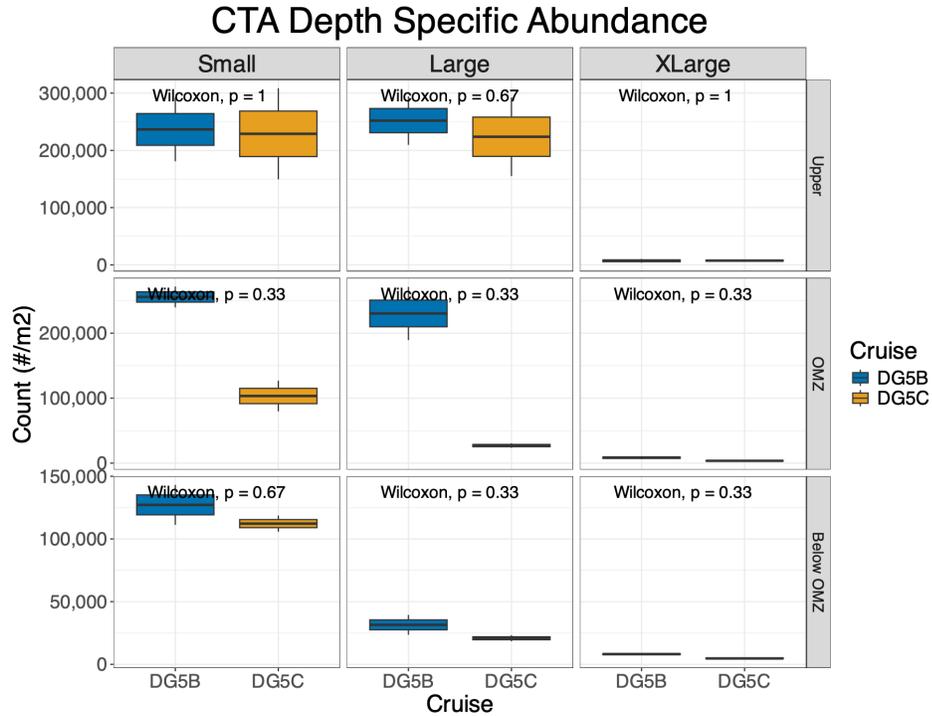


Figure 10. Boxplots of integrated zooplankton abundance ($\# \text{ m}^{-2}$) for three size fractions across three depth horizons (Upper, 0-100 m; OMZ, 100-700 m; Below OMZ, 700-1500 m) at the CTA site in the spring (DG5B, blue) and fall (DG5C, yellow).

Oxygen profiles at each site have a well oxygenated upper water column (0-50), an upper oxycline (UO) in the 50-100 m horizon, a strong oxygen minimum zone (OMZ) at 100-700 m, lower oxycline (LO) at ~ 700 -800 m, with gradual increases in oxygen at depths into the deep mesopelagic and bathypelagic (Figs. 11, 12). Some differences in water column oxygen occurred between seasons, with higher oxygen concentration in the upper OMZ in spring compared to fall (100-300m).

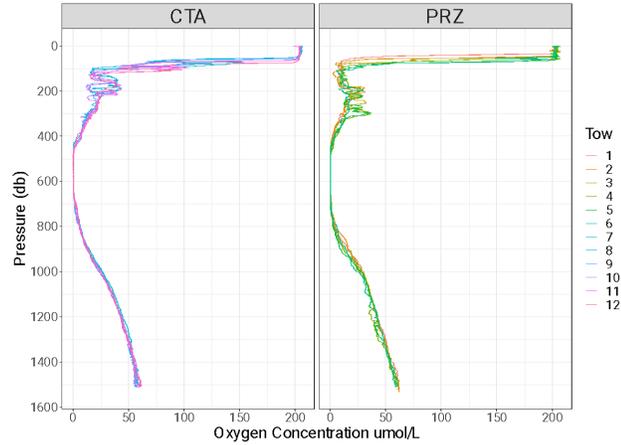


Figure 11. The oxygen concentration in umol/L with increasing depth of the DG5B cruise.

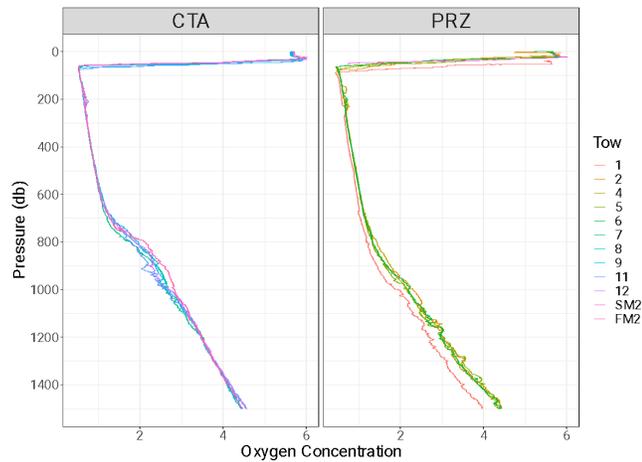


Figure 12. The oxygen concentration in mL O₂/L seawater with increasing depth of the DG5C cruise.

3.4 Taxon-specific patterns

Abundance of zooplanktonic taxa living within the OMZ varied by location, depth, and season. Total abundance at both locations was dominated by copepods. Overall, there was a greater abundance of animals in the spring than the fall in nearly all taxa (Figs. 13, 14, 15, 16). In the PRZ, copepod abundance from 100-300 m was greater in spring than fall in all taxonomic categories except *Eucalanus* (Fig. 13). *Eucalanus* was also the only copepod to show strong seasonality in the deeper, core depths of the OMZ

(300-700 m). Non-copepod zooplanktonic taxa in the PRZ were also more abundant in spring, especially in the upper portion of the OMZ (Fig. 14). Again, seasonality was limited or absent in the lower portion of the OMZ. Patterns in the CTA were similar to those in the PRZ, with greater copepod and non-copepod abundance in the spring (Figs. 15, 16). Abundance of nearly all taxa was higher in the spring, but exhibited very little seasonality at 500-700 m, except for *Eucalanus*, which showed relatively high abundance at 500-700 m compared to other taxa. In both regions, many taxa showed very low abundance from 300-700 m in the fall, with abundances increasing slightly in the spring in the 300-500 m depth range.

Across all 24 categories examined at the taxon-specific level, most seasonality was observed in the 100-300m depth range. Generally weaker seasonal trends were observed at depths of 300-500m, and 22 of 24 categories showed little or no seasonality at depths of 500-700m. Only *Eucalanus* and *Actinopterygii* showed strong seasonality in the core of the OMZ (500-700m).

Copepod Abundance By Taxa

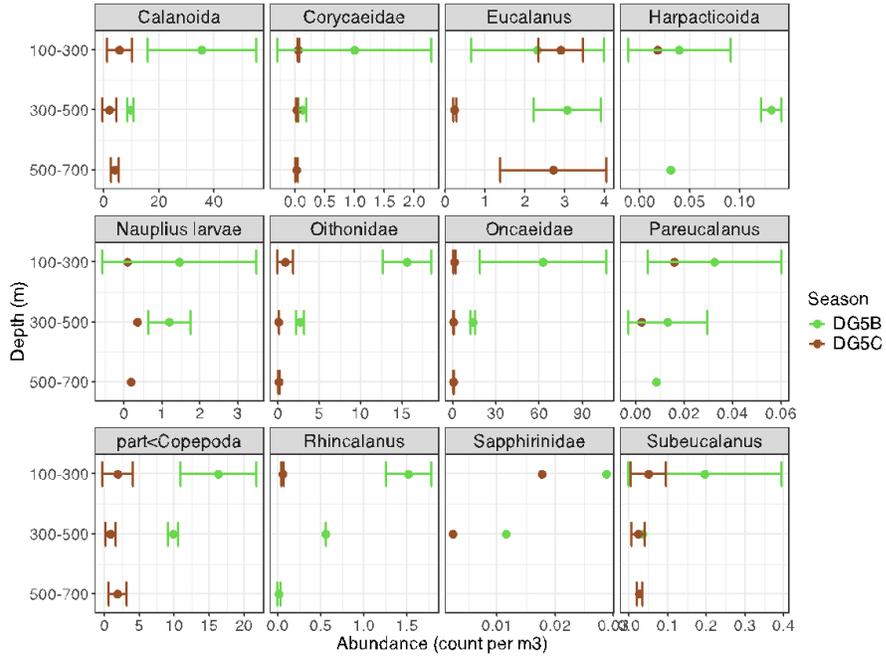


Figure 13. Boxplots of taxon-specific copepod abundance per m³ of seawater in the OMZ at the PRZ site in spring (green) and fall (brown).

Non-Copepod Abundance By Taxa

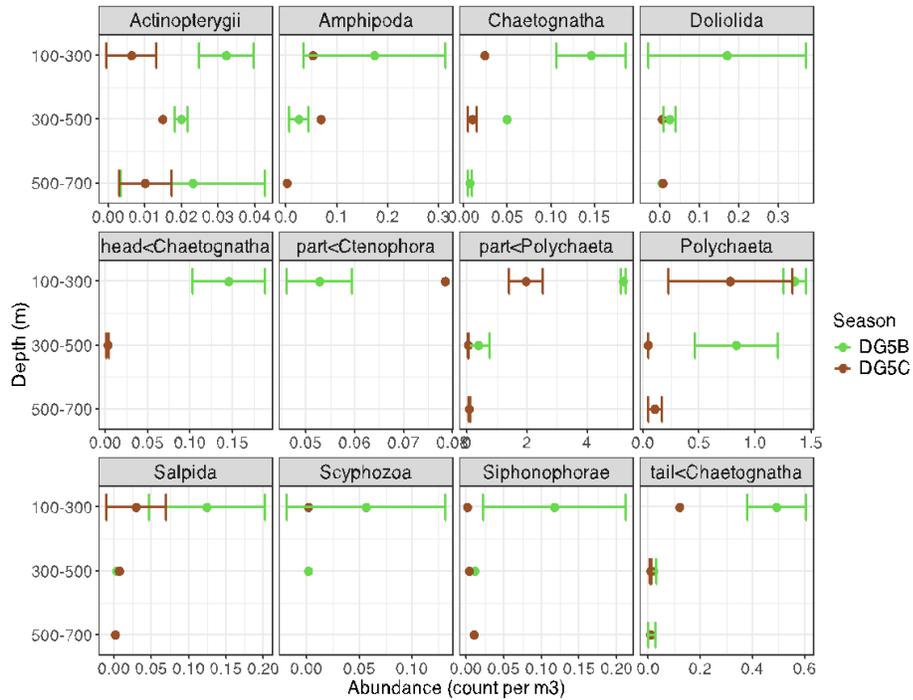


Figure 14. Boxplots of other (non-copepod) zooplankton abundances per m³ of seawater in the OMZ at the PRZ site in spring (green) and fall (brown).

Copepod Abundance By Taxa

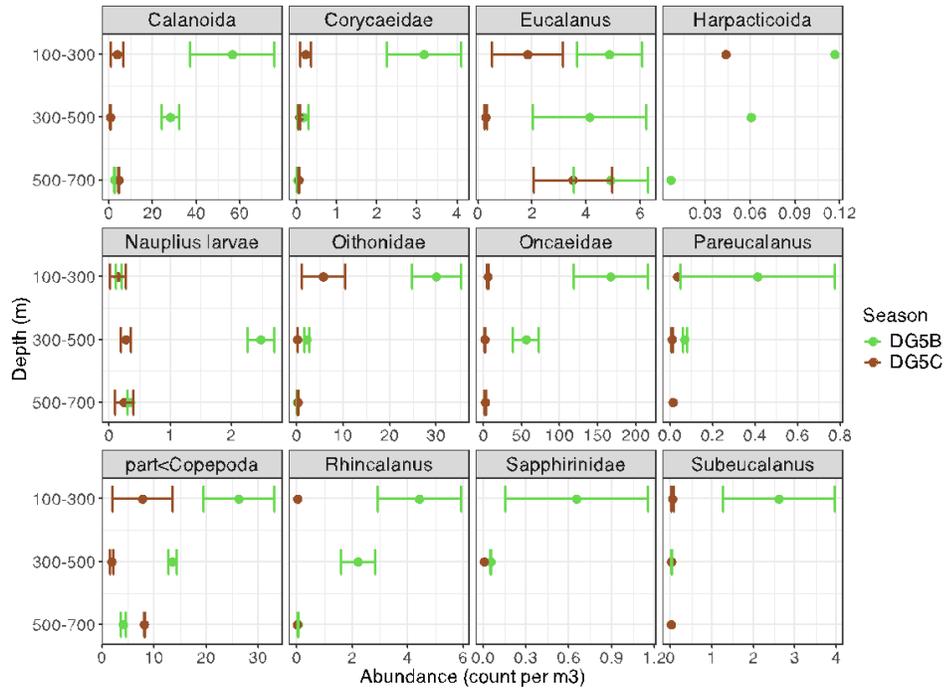


Figure 15. Boxplots of taxon-specific copepod abundance per m³ of seawater in the OMZ at the CTA site in the spring (green) and fall (brown).

Non-Copepod Abundance By Taxa

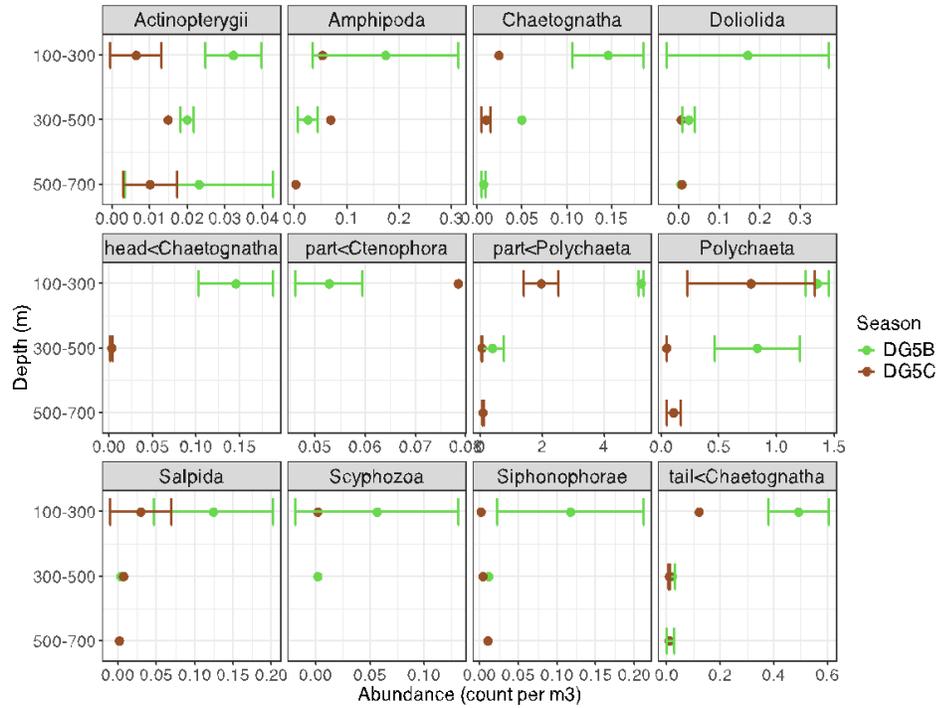


Figure 16. Boxplots of other (non-copepod) zooplankton abundances per m³ of seawater in the OMZ at the CTA site in spring (green) and fall (brown).

4.0 DISCUSSION

4.1 Seasonal variability

Results reported here are some of the first to examine temporal variability in zooplankton assemblages across the water column from the upper ocean through the mesopelagic and into the upper bathypelagic within the ETP. Prior studies have shown that zooplankton abundance in the ETP is typically highest in the spring, but it can be impacted by many factors (Fernández-Álamo & Färber-Lorda, 2006). Zooplankton abundance is high when upwelling is strong and surface ocean temperatures are cool, with peak abundance observed in March-May (Fernández-Álamo & Färber-Lorda, 2006). Solar forcing, winds, rainfall, surface temperature, and salinity all play a role in seasonality of the ETP, with wind driven upwelling the main driver of primary productivity (Fiedler, & Lavín, 2017).

Our results agree with these prior studies with higher abundance observed in spring compared to fall at both sites, but with the seasonal signal far more pronounced at the PRZ site than the CTA. The PRZ showed strong seasonality in all three depth strata in both the large and small size fractions (Fig. 10; Upper 0-100m, OMZ 100-700m, Below OMZ 700-1500m), suggesting that zooplankton show a numerical response to seasonal forcing that propagates down through the water column into the deep mesopelagic and bathypelagic. The seasonality shown in the CTA was primarily only seen in the small and large size fractions within the OMZ (Fig. 9), with a notable lack of seasonal differences both above and below the OMZ. One of the reasons for this may be due to oxygen concentrations in the upper OMZ (oxygen profiles shown in figs 11, 12). Compared to the fall, there were higher oxygen concentrations in the spring from about 100-200 m at

the CTA and about 200-300 m at the PRZ. These conditions could have allowed more zooplankton to sustain life in the upper portion of the OMZ in the spring. The increase in zooplankton seen throughout the whole OMZ could be heavily influenced by the upper portion of the OMZ, where oxygen levels were higher. It is also possible that there were different ocean currents or mesoscale eddies present at the two sites, which could cause zooplankton to be transported in/out of the sampling area (or cause alternate bottom-up forcing over weekly/monthly timescales). Additional field studies would help to determine if these seasonal patterns are recurrent or were driven by shorter-term oceanographic events. Interestingly, there was effectively no observed seasonality in zooplankton of the extra-large size fraction. This may be because the abundance of extra-large animals captured in these tows was much lower than that of the large and small animals, so changes in abundance in the extra-large size fraction may be less apparent.

Phytoplankton blooms and equatorial circulation are seasonally variable in this region (Feidler et al., 2013) and may influence seasonality in the zooplankton. Phytoplankton blooms and a weakening of the North Equatorial Countercurrent (NECC) often occur in spring, with greater wind-driven equatorial upwelling occurring later in the year (Feidler et al., 2013). These factors may contribute to seasonality in the zooplankton. One possible reason for greater seasonality being found above and below the OMZ in the PRZ is that the community may be more copepod/crustacean dominant in those depths at the PRZ. In our observations, we found that nearly all taxa reflect the overall OMZ trends in seasonality shown in Figures 10 and 11, with more animals present in spring than fall. More work needs to be done to classify images above and below the OMZ to see if all

taxa follow the same seasonal pattern as shown within the OMZ. Once such data are available, we may have a better understanding of what causes the lack of seasonality in the CTA at regions outside the OMZ. Changes in community composition, with lower dominance of copepods, may influence the strength of seasonality observed. Another possible reason for the difference in seasonality observed between the two sites is that oceanographic factors may change less between fall and spring at the CTA site in comparison to the PRZ. If oceanic conditions are more similar throughout the year at the CTA, there would likely be less seasonal variation in zooplankton abundance.

4.2 Zooplankton abundance

The observed abundance of zooplankton across both sites was mostly as expected. As shown in previous studies, small animals (0.2-1 mm) had highest abundance and extra-large animals (>5 mm) had lowest abundance in all samples analyzed (McCauley et al., 2015). Also as found in prior studies, there was a higher abundance of zooplankton near the surface, where there is more food and oxygen present (Saltzman and Wishner, 1997). It is easier to sustain life in regions where resources are plentiful, but avoiding predators can be difficult in the upper ocean. This is one reason why some animals reside within the OMZ; It can serve as a predation refuge (Seibel et al., 2016). The animals that live here have special metabolic adaptations that allow them to live under hypoxic conditions, some reducing their aerobic respiration by over 50% to conserve energy (Seibel et al., 2016). Vertical profiles of abundance across the water column were largely as expected, with higher abundance of zooplankton in the upper ocean, very low

abundance within the OMZ, and a (very) slightly higher abundance immediately below the OMZ (700-800m) than within it (hard to see on Figs 3, 4 due to the x-axis scale). There are often more animals below the OMZ, in the lower oxycline, than within the OMZ due to increased oxygen levels (Saltzman and Wishner, 1997). One surprising result was that there was very low abundance in tow 2 from the fall compared to the other analyzed tows. More tows will need to be analyzed to capture the variability in the region. Overall, there was far higher abundance of small animals compared to large or extra-large animals, as expected based on previous studies (McCauley et al., 2015).

4.3 Zooplankton DVM

Zooplankton typically migrate up in the water column to feed at night in the upper ocean, while also avoiding visual predators that could see and consume them during the day (Maas et al., 2014). This behavioral pattern was apparent in the fall as zooplankton abundance in the upper 100 m increased at night compared to the day. The expected trend was less apparent in the spring, as the typical migratory pattern was visible in only some size fractions and sites/locations. In a previous study conducted in the Eastern Tropical North Pacific (ETNP), myctophid fishes < 22 mm that resided between 200 and 600 m within the OMZ during the day migrated vertically to the top 100 m of the water column at night (Maas et al., 2014). Only animals with resting depths below 800 m did not migrate to the surface (Maas et al., 2014). Maas et al. (2014) also reported a similar pattern in euphausiids, with a significant number of organisms migrating from the upper OMZ and OMZ core to the upper 100 m. Fernández-Álamo & Färber-Lorda (2006) also

observed a similar trend with a 20-60% increase in animals in the upper 200 m of the ocean at night.

In our study, the same DVM trends were seen, but not in the spring (Fig. 6). In the fall, many zooplankton in the small and large size fractions migrated from 100-500 m to the upper 100 m of the ocean, with most of them migrating up to the top 50 m. In the PRZ, the extra-large size fraction mostly migrated from 50-100 m to the top 50 m of the ocean. In the CTA, most of the animals migrated from the mid to upper OMZ (100-500 m) to the top 50 m of the water column. The spring cruise showed signs of DVM, but the patterns were less clear (Fig. 5). In the small size fraction, they seemed to migrate from 50-100 m to 0-50 m in the PRZ, while in the CTA, there was only a significant increase in abundance in the 50-100 m depth range. In the large size fraction, there was a large decrease in abundance at night in the upper 100 m of the ocean, the opposite of expectations. Causes of the discrepancy are currently unclear. The extra-large size fraction showed an increase in animals in the 50-100 m depth at night. In the PRZ, it appeared that the animals migrated to that depth from the 0-50 m and the 300-500 m depths while in the CTA, the animals migrated from the 300-500 m depth. Differences between sites may have been due to factors that altered zooplankton DVM behaviors, in particular, a mesoscale eddy passing through the region in spring may have affected zooplankton behavior. Previous studies have suggested that increased DVM intensity can be associated with eddy features (Goldthwait and Steinberg, 2008; Landry et al., 2008). This may be the reason for the differences in DVM between the fall and spring cruises, but the results from the spring cruise may also be a result of stochastic variation that can

be better accounted for when a larger number of tows have been analyzed. Overall, the trends seen in our study were broadly similar to results of prior studies. The abnormalities in our data may be resolved when a larger number of tows are scanned.

4.4 Caveats and future work

Our study has scanned and analyzed a relatively small number of MOCNESS tows. Only one day and night pair from each site and each cruise have been processed to date (8 total tows), given that the sample handling and scanning process is fairly laborious, taking approximately 54 hours to process and scan each tow and many more hours to classify and validate each image in ecotaxa. In order to draw confident conclusions regarding the zooplankton community, more samples need to be analyzed to see if the results reported here are similar across all tows. This would help to eliminate uncertainty regarding more mesoscale oceanographic variability or other processes perturbing zooplankton communities in the ETP.

We also continue to further analyze the taxonomic composition of the ETP zooplankton community using image-based classification from our scans. We have classified much of the OMZ diversity into 51 separate categories, but much more work still remains to be done. Once enough taxonomic information is acquired, this data can be used to analyze the abundance, DVM, and seasonality of specific taxonomic groups within the ETP.

4.5 Future implications

Information gathered in this study may be used to inform deep-sea mining regulations that are needed to protect zooplankton communities. The impacts of mining could be felt throughout the water column through mining generated sediment plumes and noise pollution, which could have varying effects on different pelagic animals (Drazen et al., 2020). The ocean seafloor and regions in proximity to the depth of the sediment plume discharge (~1200 m) would likely be most affected. Our results suggest that zooplankton communities in some regions of the water column are living at the edges of suitable habitat (e.g., oxygen habitats), and they may be at risk if mining occurs. Below a depth of about 100 m, zooplankton abundance drops significantly, suggesting that life is difficult to sustain in these deeper strata. Environmental disturbance may cause additional declines in abundance of these communities. The region below the OMZ (700-1500 m) has higher zooplankton abundance than within the OMZ. While the abundance is still low relative to the upper ocean (100-0 m), zooplankton in deep midwater are still very important to ecosystem function. The midwater sediment discharge plume could smother animals living in proximity to the discharge pipe and it could also cause respiratory distress, toxicity, and mortality to the animals in this region. These animals are significant in forming the base of the food web, making higher trophic level animals in the area dependent on them (Drazen et al., 2020). If deep pelagic organisms are negatively impacted by mining, it may be years before they can fully recover, even if mining ceases.

This baseline survey also will be used to evaluate the damage caused by commercial mining if it does begin. Our study surveyed the zooplankton community prior to impact. Test mining operations occurred in the fall of 2022, and we anticipate that test mining and possibly exploitation-scale mining may occur in the next few years. If mining does occur, zooplankton samples could be taken from the same areas following mining in order to assess how zooplankton are being impacted by the mining process.

5.0 CONCLUSION

This study analyzed zooplankton communities across midwater in the eastern tropical Pacific and gathered abundance, diel vertical migration, and some of the first seasonality data in this region. Zooplankton had high abundance from 0-100 m, low abundance within the OMZ, and a slightly higher abundance below the OMZ within the lower oxycline. The most abundant organisms throughout all depth zones were small animals (0.2-1 mm), while extra-large animals (>5 mm) were the least abundant. DVM was seen in the fall cruise, with less clear patterns in the spring. In the fall, animals in the small and large size fractions migrated from the upper OMZ (100-300 m) to the surface (0-100 m) at night. In the spring, small and extra-large animals exhibited in some cases the expected pattern of animals migrating from the OMZ to the upper ocean at night, but large animals largely did not. Seasonality was present at both sites with higher animal abundance in spring, but it was much more pronounced at the PRZ site. The PRZ showed signs of seasonality with an increase in organisms in the spring in all three depth zones (upper, OMZ, below OMZ) in the large and small animal size fractions. The CTA

exhibited strong signs of seasonality only within the OMZ. One possible underlying driver of this seasonality was the increase in oxygen levels in the upper portion of the OMZ (100-300 m) in the spring compared to the fall. If commercial-scale deep-sea mining proceeds, it could have lasting impact on zooplankton communities and the ecosystem services that they provide. This study provides valuable baseline observations from which it would be possible to observe and measure how deep-sea mining impacts zooplankton communities in the ETP.

APPENDIX

APPENDIX 1

Table 1. Overview of the MOCNESS tows included in this study. Latitude and Longitude are recorded at the start of each tow, dates and times are local. CTA and TMA are the same sites.								
Event No.	Tow #	Net #	Site (PRZ/CTA)	Latitude	Longitude	Date	Start Time	D/N
DG5B_PRZ_1MOC2	2	1	PRZ	10 ° 55.5479 N	116 ° 19.268 W	3/21/2021	20:34	Night
DG5B_PRZ_1MOC2	2	2	PRZ	10 ° 55.5479 N	116 ° 19.268 W	3/21/2021	20:34	Night
DG5B_PRZ_1MOC2	2	3	PRZ	10 ° 55.5479 N	116 ° 19.268 W	3/21/2021	20:34	Night
DG5B_PRZ_1MOC2	2	4	PRZ	10 ° 55.5479 N	116 ° 19.268 W	3/21/2021	20:34	Night
DG5B_PRZ_1MOC2	2	5	PRZ	10 ° 55.5479 N	116 ° 19.268 W	3/21/2021	20:34	Night
DG5B_PRZ_1MOC2	2	6	PRZ	10 ° 55.5479 N	116 ° 19.268 W	3/21/2021	20:34	Night
DG5B_PRZ_1MOC2	2	7	PRZ	10 ° 55.5479 N	116 ° 19.268 W	3/21/2021	20:34	Night

DG5B_PRZ_1MOC2	2	8	PRZ	10 ° 55.5479 N	116 ° 19.268 W	3/21/2021	20:34	Night
DG5B_PRZ_1MOC2	2	9	PRZ	10 ° 55.5479 N	116 ° 19.268 W	3/21/2021	20:34	Night
DG5B_PRZ_1MOC5	5	1	PRZ	10 ° 48.479 N	116 ° 14.372 W	3/26/2021	6:40	Day
DG5B_PRZ_1MOC5	5	2	PRZ	10 ° 48.479 N	116 ° 14.372 W	3/26/2021	6:40	Day
DG5B_PRZ_1MOC5	5	3	PRZ	10 ° 48.479 N	116 ° 14.372 W	3/26/2021	6:40	Day
DG5B_PRZ_1MOC5	5	4	PRZ	10 ° 48.479 N	116 ° 14.372 W	3/26/2021	6:40	Day
DG5B_PRZ_1MOC5	5	5	PRZ	10 ° 48.479 N	116 ° 14.372 W	3/26/2021	6:40	Day
DG5B_PRZ_1MOC5	5	6	PRZ	10 ° 48.479 N	116 ° 14.372 W	3/26/2021	6:40	Day
DG5B_PRZ_1MOC5	5	7	PRZ	10 ° 48.479 N	116 ° 14.372 W	3/26/2021	6:40	Day
DG5B_PRZ_1MOC5	5	8	PRZ	10 ° 48.479 N	116 ° 14.372 W	3/26/2021	6:40	Day
DG5B_PRZ_1MOC5	5	9	PRZ	10 ° 48.479 N	116 ° 14.372 W	3/26/2021	6:40	Day
DG5B_TMA_1MOC8	8	1	CTA	10 ° 26.8541 N	117 ° 10.3205 W	4/3/2021	8:11	Day
DG5B_TMA_1MOC8	8	2	CTA	10 ° 26.8541 N	117 ° 10.3205 W	4/3/2021	8:11	Day
DG5B_TMA_1MOC8	8	3	CTA	10 ° 26.8541 N	117 ° 10.3205 W	4/3/2021	8:11	Day
DG5B_TMA_1MOC8	8	4	CTA	10 ° 26.8541 N	117 ° 10.3205 W	4/3/2021	8:11	Day
DG5B_TMA_1MOC8	8	5	CTA	10 ° 26.8541 N	117 ° 10.3205 W	4/3/2021	8:11	Day
DG5B_TMA_1MOC8	8	6	CTA	10 ° 26.8541 N	117 ° 10.3205 W	4/3/2021	8:11	Day
DG5B_TMA_1MOC8	8	7	CTA	10 ° 26.8541 N	117 ° 10.3205 W	4/3/2021	8:11	Day
DG5B_TMA_1MOC8	8	8	CTA	10 ° 26.8541 N	117 ° 10.3205 W	4/3/2021	8:11	Day

DG5B_TMA_1MOC8	8	9	CTA	10 ° 26.8541 N	117 ° 10.3205 W	4/3/2021	8:11	Day
DG5B_TMA_1MOC10	10	1	CTA	10 ° 25.937 N	117 ° 13.157 W	4/7/2021	20:55	Night
DG5B_TMA_1MOC10	10	2	CTA	10 ° 25.937 N	117 ° 13.157 W	4/7/2021	20:55	Night
DG5B_TMA_1MOC10	10	3	CTA	10 ° 25.937 N	117 ° 13.157 W	4/7/2021	20:55	Night
DG5B_TMA_1MOC10	10	4	CTA	10 ° 25.937 N	117 ° 13.157 W	4/7/2021	20:55	Night
DG5B_TMA_1MOC10	10	5	CTA	10 ° 25.937 N	117 ° 13.157 W	4/7/2021	20:55	Night
DG5B_TMA_1MOC10	10	6	CTA	10 ° 25.937 N	117 ° 13.157 W	4/7/2021	20:55	Night
DG5B_TMA_1MOC10	10	7	CTA	10 ° 25.937 N	117 ° 13.157 W	4/7/2021	20:55	Night
DG5B_TMA_1MOC10	10	8	CTA	10 ° 25.937 N	117 ° 13.157 W	4/7/2021	20:55	Night
DG5B_TMA_1MOC10	10	9	CTA	10 ° 25.937 N	117 ° 13.157 W	4/7/2021	20:55	Night
DG5C_PRZ_1MOC2	2	1	PRZ	10 ° 58.227 N	116 ° 20.9 W	10/6/2021	9:31	Day
DG5C_PRZ_1MOC2	2	2	PRZ	10 ° 58.227 N	116 ° 20.9 W	10/6/2021	9:31	Day
DG5C_PRZ_1MOC2	2	3	PRZ	10 ° 58.227 N	116 ° 20.9 W	10/6/2021	9:31	Day
DG5C_PRZ_1MOC2	2	4	PRZ	10 ° 58.227 N	116 ° 20.9 W	10/6/2021	9:31	Day
DG5C_PRZ_1MOC2	2	5	PRZ	10 ° 58.227 N	116 ° 20.9 W	10/6/2021	9:31	Day
DG5C_PRZ_1MOC2	2	6	PRZ	10 ° 58.227 N	116 ° 20.9 W	10/6/2021	9:31	Day
DG5C_PRZ_1MOC2	2	7	PRZ	10 ° 58.227 N	116 ° 20.9 W	10/6/2021	9:31	Day
DG5C_PRZ_1MOC2	2	8	PRZ	10 ° 58.227 N	116 ° 20.9 W	10/6/2021	9:31	Day
DG5C_PRZ_1MOC2	2	9	PRZ	10 ° 58.227 N	116 ° 20.9 W	10/6/2021	9:31	Day

DG5C_PRZ_1MOC5	5	1	PRZ	11 ° 00.143 N	116 ° 18.1189 W	10/10/2021	19:36	Night
DG5C_PRZ_1MOC5	5	2	PRZ	11 ° 00.143 N	116 ° 18.1189 W	10/10/2021	19:36	Night
DG5C_PRZ_1MOC5	5	3	PRZ	11 ° 00.143 N	116 ° 18.1189 W	10/10/2021	19:36	Night
DG5C_PRZ_1MOC5	5	4	PRZ	11 ° 00.143 N	116 ° 18.1189 W	10/10/2021	19:36	Night
DG5C_PRZ_1MOC5	5	5	PRZ	11 ° 00.143 N	116 ° 18.1189 W	10/10/2021	19:36	Night
DG5C_PRZ_1MOC5	5	6	PRZ	11 ° 00.143 N	116 ° 18.1189 W	10/10/2021	19:36	Night
DG5C_PRZ_1MOC5	5	7	PRZ	11 ° 00.143 N	116 ° 18.1189 W	10/10/2021	19:36	Night
DG5C_PRZ_1MOC5	5	8	PRZ	11 ° 00.143 N	116 ° 18.1189 W	10/10/2021	19:36	Night
DG5C_PRZ_1MOC5	5	9	PRZ	11 ° 00.143 N	116 ° 18.1189 W	10/10/2021	19:36	Night
DG5C_CTA_1MOC8	8	1	CTA	10 ° 28.1634 N	117 ° 08.6963 W	10/15/2021	19:05	Night
DG5C_CTA_1MOC8	8	2	CTA	10 ° 28.1634 N	117 ° 08.6963 W	10/15/2021	19:05	Night
DG5C_CTA_1MOC8	8	3	CTA	10 ° 28.1634 N	117 ° 08.6963 W	10/15/2021	19:05	Night
DG5C_CTA_1MOC8	8	4	CTA	10 ° 28.1634 N	117 ° 08.6963 W	10/15/2021	19:05	Night
DG5C_CTA_1MOC8	8	5	CTA	10 ° 28.1634 N	117 ° 08.6963 W	10/15/2021	19:05	Night
DG5C_CTA_1MOC8	8	6	CTA	10 ° 28.1634 N	117 ° 08.6963 W	10/15/2021	19:05	Night
DG5C_CTA_1MOC8	8	7	CTA	10 ° 28.1634 N	117 ° 08.6963 W	10/15/2021	19:05	Night
DG5C_CTA_1MOC8	8	8	CTA	10 ° 28.1634 N	117 ° 08.6963 W	10/15/2021	19:05	Night
DG5C_CTA_1MOC8	8	9	CTA	10 ° 28.1634 N	117 ° 08.6963 W	10/15/2021	19:05	Night
DG5C_CTA_1MOC12	12	1	CTA	10 ° 23.5291 N	117 ° 00.8386 W	10/21/2021	7:07	Day

DG5C_CTA_1MOC12	12	2	CTA	10 ° 23.5291 N	117 ° 00.8386 W	10/21/2021	7:07	Day
DG5C_CTA_1MOC12	12	3	CTA	10 ° 23.5291 N	117 ° 00.8386 W	10/21/2021	7:07	Day
DG5C_CTA_1MOC12	12	4	CTA	10 ° 23.5291 N	117 ° 00.8386 W	10/21/2021	7:07	Day
DG5C_CTA_1MOC12	12	5	CTA	10 ° 23.5291 N	117 ° 00.8386 W	10/21/2021	7:07	Day
DG5C_CTA_1MOC12	12	6	CTA	10 ° 23.5291 N	117 ° 00.8386 W	10/21/2021	7:07	Day
DG5C_CTA_1MOC12	12	7	CTA	10 ° 23.5291 N	117 ° 00.8386 W	10/21/2021	7:07	Day
DG5C_CTA_1MOC12	12	8	CTA	10 ° 23.5291 N	117 ° 00.8386 W	10/21/2021	7:07	Day
DG5C_CTA_1MOC12	12	9	CTA	10 ° 23.5291 N	117 ° 00.8386 W	10/21/2021	7:07	Day

LITERATURE CITED

- Amon, D. J., Levin, L. A., Metaxas, A., Mudd, G. M., & Smith, C. R. (2022). Heading to the deep end without knowing how to swim: Do we need deep-seabed mining? *One Earth*, 5(3), 220-223.
- Amon, D. J., Gollner, S., Morato, T., Smith, C. R., Chen, C., Christiansen, S., Bronwen, C., Drazen, J., Fukushima., T., Gianni, M., Gjerde, K. M., Gooday, A. J., Guillen Grillo, G., Haeckel, M., Joyini, T., Ju, S., Levin, L., Metaxas, A., Mianowicz, K., Molodtsova, T., & Pickens, C. (2022). Assessment of scientific gaps related to the effective environmental management of deep-seabed mining. *Marine Policy*, 138, 105006.
- Cuyvers, L., Berry, W., Gjerde, K., Thiele, T. and Wilhem, C. (2018). Deep seabed

mining: a rising environmental challenge. Gland, Switzerland: IUCN and Gallifrey Foundation. x + 74pp.

- Drazen, J. C., Smith, C. R., Gjerde, K. M., Haddock, S. H., Carter, G. S., Choy, C. A., Clark, M. R., Dutrieux, P., Goetze, E., Hauton, C., Hatta, M., Koslow, A. J., Leitner, A. B., Pacini, A., Perelman, J. N., Peacock, T., Sutton, T. T., Watling, L., & Yamamoto, H. (2020). Midwater ecosystems must be considered when evaluating environmental risks of deep-sea mining. *Proceedings of the National Academy of Sciences*, 117(30), 17455-17460.
- Fernández-Álamo, M. A., & Färber-Lorda, J. (2006). Zooplankton and the oceanography of the eastern tropical Pacific: A review. *Progress in Oceanography*, 69(2-4), 318-359.
- Fiedler, P. C., & Lavín, M. F. (2017). Oceanographic conditions of the eastern tropical Pacific. *Coral reefs of the Eastern Tropical Pacific: Persistence and loss in a dynamic environment*, 59-83.
- Fiedler, P. C., Mendelsohn, R., Palacios, D. M., & Bograd, S. J. (2013). Pycnocline variations in the eastern tropical and North Pacific, 1958–2008. *Journal of Climate*, 26(2), 583-599.
- Goldthwait, S.A., Steinberg, D.K. (2008). Elevated biomass of mesozooplankton and enhanced fecal pellet flux in cyclonic and mode-water eddies in the Sargasso Sea. *Deep Sea Research Part II: Topical Studies in Oceanography* 55 (10-13), 1360.
- Gorsky, G., Ohman, M. D., Picheral, M., Gasparini, S., Stemmann, L., Romagnan, J. B.,

- Cawood, A., Pesant, S., García-Comas, C., & Prejger, F. (2010). Digital zooplankton image analysis using the ZooScan integrated system. *Journal of plankton research*, 32(3), 285-303.
- Hauton, C., Brown, A., Thatje, S., Mestre, N. C., Bebianno, M. J., Martins, I., Bettencourt, R., Canals, M., Sanchez-Vidal, A., Shillito, B., Ravaux, J., Zbinden, M., Duperron, S., Mevenkamp, L., Vanreusel, A., Gambi, C., Dell'Anno, A., Danovaro, R., Gunn, V., & Weaver, P. (2017). Identifying toxic impacts of metals potentially released during deep-sea mining-a synthesis of the challenges to quantifying risk. *Frontiers*.
- Kessler, W.S. (2006). The circulation of the eastern tropical Pacific: a review. *Progress in Oceanography* 69 (2-4), 181-217.
- Landry, M.R., Decima, M., Simmons, M.P., Hannides, C.C.S., Daniels, E. (2008). Mesozooplankton biomass and grazing responses to Cyclone Opal, a subtropical mesoscale eddy. *Deep Sea Research Part II: Topical Studies in Oceanography* 55 (10-13), 1378.
- Lavaniegos, B. E., & Ohman, M. D. (2007). Coherence of long-term variations of zooplankton in two sectors of the California Current System. *Progress in Oceanography*, 75(1), 42-69.
- Loescher, C. R., Bange, H. W., Schmitz, R. A., Callbeck, C. M., Engel, A., Hauss, H., Kanzow, T., Kiko, R., Lavik, G., Loginova, A., Meizner, F., Meyer, J., Neulinger, S. C., Pahlow, M., Riebesell, U., Schunck, H., Thomsen, S., & Wagner, H. (2016). Water column biogeochemistry of oxygen minimum zones in the eastern

- tropical North Atlantic and eastern tropical South Pacific oceans. *Biogeosciences*, 13(12), 3585-3606.
- Longhurst, A.R., Pauly, D. (1987). *Ecology of Tropical Oceans*. Academic Press, London, 407 pp.
- Maas, A. E., Frazar, S. L., Outram, D. M., Seibel, B. A., & Wishner, K. F. (2014). Fine scale vertical distribution of macroplankton and micronekton in the Eastern Tropical North Pacific in association with an oxygen minimum zone. *Journal of plankton research*, 36(6), 1557-1575.
- McCauley, D. J., Pinsky, M. L., Palumbi, S. R., Estes, J. A., Joyce, F. H., & Warner, R. R. (2015). Marine defaunation: animal loss in the global ocean. *Science*, 347(6219), 1255641.
- Ramirez-Illodra, E. Z., Levin, L. A., Metaxas, A., Gjerde, K. M., Copley, A., & Baker, M. (2019). Strategic environmental goals and objectives for seabed mining. *Deep-Ocean Stewardship Initiative*, 3 pp.
- Saltzman, J., & Wishner, K. F. (1997). Zooplankton ecology in the eastern tropical Pacific oxygen minimum zone above a seamount: 2. Vertical distribution of copepods. *Deep Sea Research Part I: Oceanographic Research Papers*, 44(6), 931-954.
- Seibel, B. A., Schneider, J. L., Kaartvedt, S., Wishner, K. F., & Daly, K. L. (2016). Hypoxia tolerance and metabolic suppression in oxygen minimum zone euphausiids: implications for ocean deoxygenation and biogeochemical

- cycles. *Integrative and comparative biology*, 56(4), 510-523.
- Sekerci, Yadigar, and Sergei Petrovskii. "Mathematical modeling of plankton–oxygen dynamics under the climate change." *Bulletin of mathematical biology* 77.12 (2015): 2325-2353.
- Sharma, R. (2015). Environmental issues of deep-sea mining. *Procedia Earth and Planetary Science*, 11, 204-211.
- Spearman, J., Taylor, J., Crossouard, N., Cooper, A., Turnbull, M., Manning, A., Lee, M., & Murton, B. (2020). Measurement and modeling of deep sea sediment plumes and implications for deep sea mining. *Scientific reports*, 10(1), 5075.
- Washburn, T., Turner, P., Durden, J., Jones, D., Weaver, P., and Van Dover, C. (2019) "Ecological risk assessment for deep-sea mining." *Ocean & coastal management* 176: 24-39.
- Washburn, T. W., Jones, D. O., Wei, C. L., & Smith, C. R. (2021). Environmental heterogeneity throughout the Clarion-Clipperton Zone and the potential representativity of the APEI network. *Frontiers in Marine Science*, 8, 661685.
- Wedding, L. M., Friedlander, A. M., Kittinger, J. N., Watling, L., Gaines, S. D., Bennett, M., Hardy, S. M., & Smith, C. R. (2013). From principles to practice: a spatial approach to systematic conservation planning in the deep sea. *Proceedings of the Royal Society B: Biological Sciences*, 280(1773), 20131684.
- Wedding, L. M., Reiter, S. M., Smith, C. R., Gjerde, K. M., Kittinger, J. N., Friedlander, A. M., Gaines, S. D., Clark, M. R., Thurnherr, A. M., Hardy, S. M., & Crowder, L. B. (2015). Managing mining of the deep seabed. *Science*, 349(6244), 144-145.

Wishner, K. F., Outram, D. M., Seibel, B. A., Daly, K. L., & Williams, R. L. (2013).

Zooplankton in the eastern tropical north Pacific: Boundary effects of oxygen minimum zone expansion. *Deep Sea Research Part I: Oceanographic Research Papers*, 79, 122-140.

Wishner, K. F., Seibel, B., & Outram, D. (2020). Ocean deoxygenation and copepods:

coping with oxygen minimum zone variability. *Biogeosciences*, 17(8), 2315-2339.