SIZE FRACTIONATED ZOOPLANKTON C:N:P IN THE NORTH PACIFIC GYRE AND EQUATORIAL COUNTERCURRENT REGION

by

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Submitted in partial fulfilment of the requirements for
the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
July 2024

Dalhousie University is located in Mi'kma'ki,
the ancestral and unceded territory of the
Mi'kmaq. We are all Treaty people.

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ABSTRACT

Zooplankton play a critical role in carbon (C), nitrogen (N), and phosphorus (P) cycling in the ocean. There are fewer measurements of zooplankton C, N, and especially P, compared with phytoplankton and total particulate C:N:P. In this study, we measured the C:N:P in four size fractions (>2000 μm, 500-2000 μm, 250-500 μm, 64-250 μm) of zooplankton sampled using a WP-2 and ring-net from 18 stations in the North Pacific Subtropical Gyre (NPSG) and North Pacific Equatorial Countercurrent (NPEC) region. These regions cover a wide latitudinal range in temperature, nutrients, phytoplankton biomass and associated phytoplankton C:N:P. The overall median molar zooplankton C:N:P from the region is 116.4:25:1. Our data revealed significant differences in C:N:P across the four size fractions. The C:N was 2.1 (± 0.3) % higher in the 500-2000 μm size fraction compared to the overall median molar C:N, C:P was 18.5 (± 0.29) % higher in the 250-500 μm size fraction compared to the overall median molar C:P, and N:P was 15.2 (± 0.65) % higher in the 250-500 μm size fraction compared to the overall median molar N:P. The overall median zooplankton C, N, P content as a percent of dry weight from the region is 26 %, 8 %, 0.69 %. The N % dry weight was 24.1 (± 0.34) % lower in the >2000 μm size fraction compared to the overall median N % dry weight, and P % dry weight was 26.6 (± 0.37) % lower in the >2000 μm size fraction compared to the overall median P % dry weight, suggesting that gelatinous zooplankton may be a larger proportion of the mass in this size fraction. In addition, our low measurements of P as % dry weight in the >2000 μm are intermediate between P % dry weight measured from isolated gelatinous and euphausiid species from our samples. We did not observe strong changes in zooplankton C:N:P with latitude, temperature, nitrate concentration, or phytoplankton biomass (chlorophyll-*a* and carbon concentration) or C:N:P, however C:N in the >2000 μm and 500-2000 μm was correlated with C:N in phytoplankton. Overall, our results indicate that variability in zooplankton C:N:P is dominated by community composition, and not environmental conditions. If climate warming shifts the zooplankton community towards larger gelatinous zooplankton in the greater than 2000 μm fraction at the expense of smaller 250-2000 μm crustacean zooplankton, we may see shifts in the overall median molar C:N:P towards 125:26:1 in this region.

LIST OF ABBREVIATIONS AND SYMBOLS USED

ALOHA	A Long-term Oligotrophic Habitat Assessment
C	Carbon
Chl-*a*	Chlorophyll-*a*
ºC	Celsius
D	Day
DW	Dry weight
ENSO	El Niño-Southern Oscillation
EqPac	Equatorial Pacific Process Study
g	Grams
GZ	Gelatinous zooplankton
HCl	Hydrochloric acid
JGOFS	Joint Global Ocean Flux Study
L	Liters
m	Meters
m^{-2}	Meters squared
m^{-3}	Meters cubed
mg	Milligrams
mL	Milliliters
mmol	Millimoles
µM	Micromolar
µm	Microns
N	Nitrogen
Ni	Night
NO_2^-	Nitrite
NO_3^-	Nitrate
NPEC	North Pacific Equatorial Countercurrent
NPSG	North Pacific Subtropical Gyre
P	Phosphorus
POC	Particulate Organic Carbon
PON	Particulate Organic Nitrogen
PP	Primary Production
R/V	Research Vessel
S	Station
SCOPE	Simons Collaboration on Ocean Processes and Ecology
SD	Standard deviation
TPC	Total Particulate Carbon
TPN	Total Particulate Nitrogen
TPP	Total Particulate Phosphorus
WP-2 net	Wilkens and Plath double net

ACKNOWLEDGEMENTS

First, I would like to thank the Marine Microbial Macroecology Lab and all its members at Dalhousie University, especially Ying-Yu Hu, Niall McGinty, Laura Bretherton, Nuwanthi Samarasinghe, and Mohammad Amirianmatlob for their mentorship and advice.

My research achievements during my graduate studies are in large part due to the amazing support of my advisor. Thank you, Zoe, for your dedication and inspiration behind this project and all the amazing opportunities you presented me with along the way. I'd also like to extend my gratitude to the rest of my committee, Andrew, and Sarah, for their genuine support and feedback.

My favorite part about this experience was the multidisciplinary and collaborative approach that oceanography brings to solving problems, and in the process, I had the great privilege to work with some of the most inspirational minds and talents in this field. Everyone involved with the CBIOMES and SCOPE projects and in the Dalhousie oceanography department, including my other fellow graduate students, have left a mark on me during my time here and it's been a pleasure to be part of such a dedicated and gifted group of individuals.

I would like to thank my friends, partner, and family back home in the United States for their endless support and suffering through my rants.

Finally, I would like to end this section by thanking my aunt Patty Fagan, who gave me the courage to pursue this degree in the first place.

CHAPTER 1: INTRODUCTION

1.1 Importance Of The Zooplankton Community In The Ocean

The carbon, nitrogen, and phosphorus cycles are fundamental biogeochemical processes in the ocean, essential for ocean productivity and global climate regulation. The pelagic food web plays a critical role in the flux of CO_2 between the atmosphere and surface ocean and the transfer of organic carbon and nutrients (i.e., nitrogen and phosphorus) into the deep ocean. Zooplankton are a key link within these marine food webs, connecting phytoplankton, (responsible for approximately 50% of primary production, Field *et al.* 1998), to organisms in higher trophic levels such as fish and whales, and facilitating the transfer of particulate carbon and nutrients and recycling them into dissolved pools. For example, zooplankton contribute to the carbon cycle through multiple processes including consuming phytoplankton, respiration, and producing fecal pellets, which sink and transport carbon to the deep ocean, a process known as the biological pump (Steinberg & Landry 2017). This vertical transport of carbon is crucial for sequestering CO_2 from the atmosphere and storing it in the deep ocean over long time periods, influencing global climate regulation (Ducklow *et al.* 2001). In terms of the nitrogen and phosphorus cycle, zooplankton excrete excess nutrients back into the water column in the form of ammonia and phosphate as a waste product, which is a readily available form and contributing source of nitrogen and phosphorus for phytoplankton growth and primary production (Alcaraz *et al.* 2014; Moore *et al.* 2013; Ikeda & Mitchell 1985; Valdés *et al.* 2018). Despite their role in marine food webs and the cycling of carbon, nitrogen, and phosphorus in the ocean, zooplankton are still simplistically represented in biogeochemical models (Ratnarajah *et al.* 2023, Steinberg & Landry 2017).

Zooplankton are a diverse group of heterotrophic organisms and cover a wide range in body sizes. They are typically divided into two operationally defined size fractions: 20-200 μm in the microzooplankton, and 0.2-20 mm in the mesozooplankton (Steinberg & Landry 2017). Size is a key functional trait which affects the physiology and ecological roles of zooplankton, such as metabolic rates, growth rates, trophic structure and community composition of zooplankton in marine ecosystems (Andersen *et al.* 2016;

Hatton *et al.* 2022; Herbert *et al.* 2016; Ikeda 1985). For example, on a global scale microzooplankton grazing on phytoplankton constrains the fluxes of carbon in the ocean. Microzooplankton have been estimated to consume ~60 % of global phytoplankton biomass (Calbet & Landry 2004, Schmoker *et al.* 2013). The community composition of zooplankton is different across size fractions, with certain major groups being more abundant than others in certain size fractions. The microzooplankton size fraction (20-200 μm) is dominated by a mixture of protistan consumers (e.g. radiolaria and foraminifera, Stoecker *et al.* 1996) and juvenile stages of mesozooplankton like copepod nauplii (Steinberg & Landry 2017). The mesozooplankton size fraction (0.2-20 mm) consists mainly of crustacean zooplankton in the lower end of this size fraction (200-2000 μm), such as copepods which make up 70-90% of mesozooplankton abundance in marine ecosystems (Turner 2004). Larger crustaceans such as euphausiids, and a higher proportion of gelatinous and soft bodied zooplankton such as ctenophores and chaetognaths are more abundant in size fractions greater than 2000 μm. Together, the microzooplankton and mesozooplankton size fractions include functionally diverse groups of zooplankton, covering multiple trophic levels with complex feeding relationships (Hansen *et al.* 2014). For example, microzooplankton such as ciliates exhibit filter feeding like behavior, and raptorial feeding behavior. In the subtropical Pacific Ocean copepods dominate the total mesozooplankton abundance (~80 % of all organisms in 200-2000 μm, Landry *et al.* 2001). Copepods exhibit a variety of feeding modes, including suspension feeding in most herbivorous and omnivorous species, and raptorial feeding in carnivorous species. In the larger size fractions >2000 μm there are more chaetognaths (~40 % organisms) which are voracious carnivores that typically ambush their prey, and larger crustaceans like euphausiids which are more abundant at night (34 to 47 % organisms >2000 μm). Krill are primarily herbivorous, suspension feeders, however some species can be opportunistic carnivores. Gelatinous types of zooplankton such as siphonophores, medusae, and ctenophores also tend to be relatively more abundant in the >2000 μm size fraction (Harmelin-Vivien *et al.* 2015, Landry *et al.* 2001). Gelatinous zooplankton (GZ) are highly diverse and exhibit a variety of feeding modes. For example, GZ such as salps are typically herbivorous filter feeders, while ctenophores and siphonophores are typically carnivorous and can exhibit filter feeding

like behavior. We are beginning to understand how GZ are ecologically important, and their role in trophic structuring in marine food webs remains incompletely understood (Chi *et al.* 2020).

1.2 Zooplankton C:N:P Stoichiometry: Community Composition & Size

As we discussed in the previous section, zooplankton inhabit a wide range of sizes and diversity in community composition. In this section we will discuss how the elemental composition of zooplankton changes across different taxonomic groups and size fractions of zooplankton.

Carbon (C), nitrogen (N), and phosphorus (P) are essential elements for living organisms, forming essential biochemicals and macromolecules (Sterner & Elser, 2002). C, N, and P tend to make up a large percentage of an organism's dry weight because these elements are used to make structural macromolecules. For instance, carbon is prominent in lipids and carbohydrates, nitrogen in proteins, and phosphorus in nucleic acids like DNA and RNA. Zooplankton are no exception, and the evolutionary history and biochemical requirements of different taxonomic groups of zooplankton have shaped their C:N:P stoichiometry. The most widely used stoichiometric ratio used for plankton in the ocean (including phytoplankton and zooplankton) is the C:N:P ratio. Marine plankton communities in the surface ocean are expected to have molar C:N:P ratios of about 106:16:1, known as the Redfield ratio (Redfield 1934, 1958). Recent studies of phytoplankton C:N:P ratios have found systematic variability in the C:N, C:P, and N:P of phytoplankton through direct measurements of particulate organic matter (POM) and phytoplankton across different ocean regions and basins (Martiny *et al.* 2013; Martiny *et al*. 2014; Tanioka *et al.* 2022) demonstrating deviations from the Redfield ratio. The C:N, C:P and N:P of POM and phytoplankton are generally below or close to the Redfield ratio in high-latitude and equatorial upwelling regions with higher nutrient conditions, and above the Redfield ratio in the low nutrient subtropical gyres (Moreno *et al.* 2018). This variability in POM and phytoplankton C:N:P has been observed to be tied with community composition of phytoplankton and changes with environmental conditions.

Individual species and groups of phytoplankton usually do not display the Redfield ratio (La Geider & La Roche 2002, Quigg *et al.* 2003) and their internal C:N:P has been shown to widely vary and respond to nutrient concentration and supply ratio (Sterner & Elser 2002, La Geider & La Roche 2002) and temperature (Yvon-Durocher *et al*. 2015). All these studies focus on the C:N:P of phytoplankton and POM (which is derived largely from phytoplankton). In contrast, there are fewer studies specifically dedicated to examining the C:N:P ratios in zooplankton in the field and mechanisms influencing their variability in the ocean. Zooplankton tend to fall victim of being generalized under the blanket term "plankton" in studies that are focused specifically on C:N:P ratios in phytoplankton and POM. Measurements of POM largely exclude the majority of zooplankton size fractions and taxonomic groups. This is largely due to the different methods used for collecting phytoplankton and POM versus collecting zooplankton. Although Redfield's original measurements did include net tows with copepod species (Redfield 1934), today studies measuring C:N:P ratios in POM and phytoplankton typically collect samples on filters and will even intentionally remove larger organisms. For example, Tanioka *et al.* (2022) removed plankton and particles greater than 30 μm from their samples. Only a minor contribution of microzooplankton (20-200 μm) will contribute to these samples. Despite zooplankton communities accounting for ~40 % of the world's marine biomass (Hatton *et al.* 2022), existing measurements of carbon, nitrogen, and especially phosphorus is lacking for different sizes and taxonomic groups of zooplankton in the field compared with phytoplankton and POM, underscoring the need for further studies.

Before we begin to discuss different C:N:P ratios in zooplankton across different taxonomic groups and size fractions we need to first introduce the fundamental concept of homeostasis. C:N:P ratios have been observed to be different across taxonomic groups, but it is also relatively constant across a wide range of external conditions and responses to the C:N:P of their food resources (Andersen & Hessen 1991, Golz *et al*. 2015; Malzahan *et al.* 2010; Persson *et al.* 2010; Sterner & Elser 2002). This ability of zooplankton to regulate their internal C:N:P despite changes in their external environment is known as homeostasis or homeostatic regulation, which is defined by

Sterner & Elser (2002) as “physiological regulation of an organism’s internal nutrient content reducing changes within an organism, or a narrowing of variation in the chemical content in an organism compared to the resources it consumes”. The concept of homeostasis in zooplankton is not what we typically see in phytoplankton, which show more plasticity and variability in their C:N:P ratios with external conditions. This fundamental difference of zooplankton more as regulators, needing to excrete excess nutrients to maintain stable C:N:P, and phytoplankton as conformers in their environment in terms of their internal C:N:P means that they can have significantly different C:N:P from each other. For example, Elser and Hassett (1994) suggested that differences in the N:P ratio of zooplankton and phytoplankton can lead to amplifying nitrogen limitation in marine ecosystems. These elemental imbalances or mismatches between consumer and resource means that zooplankton may then indirectly influence both production and the community structure of marine food webs, by altering the quality of nutrients available for phytoplankton (Sterner 1990).

Starting with the smallest size fraction, microzooplankton (20-200 μm) tend to have C:N:P ratios closer to Redfield ratio values (C:N = 6.6, N:P = 16:1, C:P = 106:1) than larger size fractions of zooplankton. In a review study by Kiørboe (2013) C:N for protozoa was reported as 5.3 ± 0.7, which as discussed earlier make up a majority of the microzooplankton size fraction (protozoa include radiolaria, foraminifera, ciliates). In another review study Nugraha *et al.* (2010) reported an N:P of 21.50 ± 2.10 in microzooplankton. Similarly, Le Borgne *et al.* (1997) reported a C:N of 6, C:P of 178, and N:P of 21.7 in a 35-200 size fraction of zooplankton in the southwest Pacific Ocean. The microzooplankton size fraction also likely contains some phytoplankton and detritus. Gismervik (1997) attributed their higher C:N of 8.5 to 10, and C:P of 940 ± 478 and N:P of 40 to 200 and variability in samples containing mostly ciliates collected in the Oslofjord in Norway to containing some phytoplankton and detritus. Golz *et al.* (2015) reported ranges of C:N = 5 to 6, C:P = 40 to 120, and N:P = 7 to 25 for ciliates and rotifers, supporting the notion that Gismervik’s (1997) samples likely contained phytoplankton and detritus with higher C:N:P. Talmy *et al.* (2016) have also suggested that microzooplankton play a role in lowering the overall C:N in particulate organic

matter samples through grazing of phytoplankton. However, there are few marine studies on the C:N:P ratios in microzooplankton and the zooplankton community in this size range, and more data is needed for the microzooplankton size fraction.

The mesozooplankton size range (0.2–20 mm) typically contains the majority of crustacean zooplankton biomass. The organisms on the smaller end of this size range are primarily copepods (200-2000 μm), and on the larger end of the size range (>2000 μm), a higher proportion of organisms are more gelatinous and soft bodied zooplankton such as ctenophores and chaetognaths. However, the >2000 μm size fraction does contain some crustacean zooplankton such as euphausiids, with higher abundances at nighttime due to diel vertical migration behavior (Landry *et al.* 2001). The previous literature reviews of zooplankton C:N:P report copepods to have a C:N = 5.54 ± 1.60 , N:P = 23.32 ± 9.35, C:P = 131.80 ± 60.80 (Nugraha *et al.* 2010). Crustacean zooplankton like copepods in higher latitudes have higher C:N, and C:P than in lower latitudes (Plum *et al.* 2023, Steinberg *et al.* 2008) because of the accumulation of carbon-rich macromolecules like lipids and carbohydrates as high energy reserve to increase survival and reproductive success during unfavorable conditions (Kattner & Hagen 2009). Kiørboe (2013) reported that the C:N ratio is typically similar across all major zooplankton groups (C:N range = 4 to 5.9), with similarly low values in the larger sized carnivorous gelatinous zooplankton like ctenophores (C:N = 4.4 ± 0.5) and soft-bodied chaetognaths (C:N = 4.0 ± 0.2), and slightly higher but more variable values among crustacean zooplankton like copepods (C:N = 5.1 ± 0.4). Gelatinous zooplankton tend to have lower carbon and nitrogen % dry weight (C = 5.1 to 13.2 %, N = 1.1 to 3.7 %) compared with higher carbon and nitrogen % dry weight (DW) in crustacean zooplankton (C = 34.5 to 48 %, N = 6.8 to 10.4 %, Kiørboe 2013). It is difficult to fully dry gelatinous zooplankton for elemental analyses because of their high water-content in tissues (Lüskow *et al.* 2022), and consequently measurements of carbon, nitrogen, and phosphorus content % DW are typically low.

C:N is thought to be constrained across all these zooplankton groups (including microzooplankton) because all these groups have high protein content which is a nitrogen-rich molecule (Sterner & Elser 2002).

In the few studies that do examine C, N, and P in zooplankton, we tend to observe more variability in P than C and N across different taxonomic groups. The "Growth Rate Hypothesis" states that differences in organismal C:N:P ratios are caused by differential allocation to phosphorus-rich RNA molecules necessary to meet the protein synthesis demands of rapid rates of biomass growth and development (Sterner & Elser 2002). For example, faster growing organisms with high specific growth rates should then have lower N:P (and C:P) than slower growing organisms due to the increase in phosphorus-rich ribosomes containing RNA. This relationship has been observed across multiple studies and organisms including both zooplankton and phytoplankton (Elser & Sterner 2002; Main *et al.* 1997; Moreno *et al.* 2018). However, this hypothesis relies on the assumption that ribosomal content is an important component of the phosphorus pool in organisms. In a classic study by Beers (1966), carbon, nitrogen, and phosphorus were measured across major taxonomic groups of zooplankton in the Sargasso Sea as a % dry weight (DW), illustrating low values in gelatinous zooplankton (P = 0.05 to 0.12 %) and higher values in crustacean zooplankton like copepods (P = 0.69 %) and euphausiids (P = 1.39 %). The calculated N:P based off these measurements is N:P = 37 to 47 for gelatinous zooplankton, and N:P = 14 to 27 for crustacean zooplankton (i.e. copepods and euphausiids). The calculated C:P based on these measurements is C:P = 108 to 200 in gelatinous zooplankton, and C:P = 71 to 135 in crustacean zooplankton. The N:P and C:P is less constrained than C:N across zooplankton taxa because of these large differences in phosphorus content, with typically lower phosphorus values in gelatinous zooplankton and higher values in crustacean zooplankton. This is consistent with Nugraha *et al*. (2010) literature review values of lower C:P and N:P in marine crustacean zooplankton (C:P = 94.2 to 131.8, N:P = 17.3 to 26.1) and higher C:P and N:P in larger marine gelatinous zooplankton (C:P = 108.7 to 200, N:P = 30 to 46.7). In addition, more recent studies comparing the C:N:P between crustacean and gelatinous zooplankton (Plum *et al.* 2023) agree with these earlier measurements, and attribute lower C, N, and P % DW and

higher C:P, and N:P with lower nutrient demands in gelatinous zooplankton compared with crustacean zooplankton. Higher C:P and N:P in gelatinous zooplankton indicates that they have higher resilience towards phosphorus limitation (Lüskow *et al.* 2022), and lower nutrient requirements than crustacean zooplankton. Additionally, higher C:P and N:P in gelatinous zooplankton represents poor food quality for organisms in higher trophic levels. The ideal C:N:P of prey for higher trophic levels are near or below the Redfield ratio, which are observed more frequently in crustacean zooplankton that represent good food quality. Larger vertebrate organisms like fish and whales tend to have higher P requirements because of the presence of bones, which contain a higher proportion of P (Sterner & Elser 2002). The smaller sized portion of the mesozooplankton size fraction <2000 μm can then be expected to be more representative of crustacean zooplankton C:N:P ratios, while the >2000 μm sized portion of mesozooplankton will likely be more reflective of the gelatinous zooplankton.

Differences in the C:N:P of zooplankton have also been observed over the life history or ontogeny of single species across different developmental stages. This has been documented in marine copepods, which have complex life histories, starting with earlier nauplii stages having lower C:N, C:P and N:P ratios than adults (Villar-Argaiz *et al.* 2002). For example, Villar-Argaiz *et al.* (2002) observed a trend in the C:N:P in the freshwater copepod *Mixodiaptomus laciniatus* of 99:3:1 in nauplii, 165:13:1 for copepodites, and 234:25:1 for adults. According to the growth rate hypothesis, we may assume that N:P and C:P should increase with increasing size in zooplankton because larger organisms have slower metabolisms and growth rates than smaller ones (Andersen *et al*. 2016, Elser 1996). However, in the limited number of studies that do examine C:N:P in zooplankton size fractions there are conflicting observations that do not follow this assumption. Hannides *et al.* (2009) observed decreases in C:P and N:P with increasing size, and more constrained C:N ratios in five size fractions (i.e. 200-500 μm, 500-1000 μm, 1000-2000 μm, 2000-5000 μm, and >5000 μm) of marine zooplankton in the North Pacific Subtropical Gyre. C:N ranged from 4.54 to 5.0 across all 5 size fractions, while the C:P decreased from 108 to 72, and the N:P decreased from 21.4 to 15.3 with increasing size. This pattern of similar C:N and decreasing C:P and N:P with

increasing size has also been observed across other studies of size fractionated zooplankton (Baines *et al.* 2016, Elser 1994, Le Borgne *et al*. 1997), however increases in C:P and N:P has been observed with increasing size in the Indian Ocean (Scharler *et al.* 2016). There are only a limited number of studies that we could find including all three measurements of carbon, nitrogen, and phosphorus in different size fractions of marine zooplankton, including the five mentioned above, and each study uses different size fractions to describe the zooplankton in their study. This lack of consistency in size fractions across studies and lack of carbon, nitrogen, and phosphorus measurements in zooplankton size fractions makes comparisons and interpretations of patterns with size difficult across studies.

1.3 Zooplankton C:N:P Stoichiometry: Potential Variability & External Conditions

In the previous section we introduced the concept of homeostasis in zooplankton, and most studies indicate homeostasis in internal C:N:P ratios of zooplankton regardless of external conditions, with differences being attributed to community composition in different size ranges and regions of the ocean. However, there are some cases in which we see variability in zooplankton C:N:P in response to temperature, nutrient concentrations, and resource quantity and C:N:P ratios. For example, experimental results from Matthews *et al*. (2018) observed increases in the C:P ratios of copepodite stages in the marine copepod species *Parvocalanus crassirostris* across three temperature treatments (i.e. 25, 28, and 32º C). C:P increased significantly with temperature in copepodites fed phosphorus-limited phytoplankton and the copepodites began to reflect the higher C:P of their resource C:P. In contrast C:P decreased with increasing temperature in copepodites fed phosphorus-replete phytoplankton. Increasing temperature should in theory increase metabolic rates of zooplankton (Ikeda 1985), and therefore growth rates and a higher demand for phosphorus-rich ribosomes, which should increase phosphorus content and consequently decrease the C:P (or N:P for that matter) of an organism with a high growth rate according to the growth rate hypothesis. Matthews *et al.* (2018) suggested the contradicting results to the growth rate hypothesis may be due to copepodites having the ability to relax their homeostatic regulation under temperature and food quality stress, with increased metabolic demands for carbon fueling higher C:P

with increasing temperature. Additionally, a study by Bellejos *et al.* (2014) observed a positive correlation in RNA content (higher P content) across freshwater zooplankton groups in 22 lakes with temperature in more phosphorus-rich lakes (> 9 µg P L^{-1}), and a negative trend in RNA and P content with temperature in less phosphorus-rich lakes (< 9 µg P L^{-1}). In another experimental study by Bi and Summer (2020) the C:N ratios in the copepod *Acartia tonsa* varied significantly with different food quantity and quality treatments, showing higher C:N while fed N-deficient phytoplankton, and at higher food quantity. However, the C:P did not significantly change in response to food quantity or quality changes. There's inconsistency in these limited studies both in terms of concepts and evidence. we have the concept that zooplankton should follow homeostasis or these ideas that it should be more responsive to environment or changes in taxonomy, and there is a lack of clarity which is why we should further investigate these ideas. In addition, there is a big difference between collecting bulk zooplankton in the field and culturing a single species in a very regulated system such as an incubation experiment in a lab. To our knowledge, these variables have not been investigated separately in one study for zooplankton in the field, and it may strengthen our understanding of zooplankton C:N:P to address the lack of clarity put forth by these limited number of studies.

1.3 Region Of Interest: North Pacific Subtropical Gyre & North Paciific Equatorial Countercurrent Region

The regions of interest for this study are the North Pacific Subtropical Gyre (NPSG) and North Pacific Equatorial Countercurrent (NPEC) region. Some of the more influential papers in zooplankton ecology were conducted in these regions known as Joint Global Ocean Flux Study (JGOFS) Equatorial Pacific Process Study (EqPac). The JGOFS EqPac cruise studies were conducted along 140° W latitude, in which four cruises took place. The overall scientific objectives of these studies were to determine the fluxes of carbon and related elements, and the processes controlling these fluxes between the Central Equatorial Pacific region euphotic zone and the atmosphere and deep ocean using measurements from phytoplankton, particulate organic matter in seawater, and zooplankton (Bidigare & Ondrusek 1996; Dam *et al.* 1995; Landry *et al.* 1995; Roman *et al.* 1995; White *et al.* 1995; Zhang *et al.* 1995).

These studies provided important findings on the spatial distribution of zooplankton biomass and associated environmental conditions in this region. White *et al.* (1995) collected zooplankton samples along 140º W from 12º N to 12º S during a set of cruises, the first being in the spring months (February-March) and the second during the late summer months (August-September). For both cruise surveys chlorophyll-*a* concentration and zooplankton biomass increased towards the equator from the higher latitudes 12º N and 12º S. This study also showed changes in zooplankton size structure, with proportionally more large sized zooplankton towards lower latitudes near the equator. The higher latitude ranges from these cruises associated with the NPSG region have historically been characterized by warmer, highly stratified, low nutrient waters with low phytoplankton and zooplankton biomass compared to the cooler, less stratified, more nutrient rich water masses in the NPEC region (Dai *et al.* 2023, Karl 1999). The NPSG region is also characterized by low seasonal variability in plankton biomass relative to high polar latitude ecosystems (Steinberg *et al.* 2008), and plankton biomass is usually stimulated by large scale disturbances such as eddies (Karl & Church 2017) or during El Niño conditions. The NPSG region ecosystem is dominated by organisms that are well adapted to nutrient limited conditions. The microbial community, including cyanobacteria (<2 μm) such as *Prochlorococcus* and *Synechococcus* as well as diazotrophs like *Trichodesmium* which can fix atmospheric nitrogen into ammonium are well adapted to efficient nutrient acquisition in oligotrophic conditions and act as the basis for the food web for small herbivorous microzooplankton (20-200 μm), which are then available to larger omnivorous crustacean mesozooplankton. The mesozooplankton community in the NPSG region is largely dominated by copepods (Landry *et al.* 2001), but also tends to include a higher proportion of larger sized gelatinous zooplankton such as salps, ctenophores, and siphonophores which can thrive in oligotrophic conditions because of their low nutrient demands (Lüskow et al. 2022).

The NPEC region is characterized by higher nutrient concentrations through upwelling processes and associated higher chlorophyll-*a* concentrations with larger sized phytoplankton like diatoms and zooplankton with increased biomass (Bidigare & Ondrusek 1996, Chavez 1990). The microbial community still dominate this region with

most phytoplankton and microzooplankton biomass occurring above ~90 m (Stoecker *et al.* 1996). Seasonal variability is also low in the NPEC region with significant intra and interannual variability in the intensity of upwelling caused by El Niño Southern Oscillation events (ENSO). Fall and winter months tend to have relaxed ENSO conditions, and thus surface water temperatures are lower on average or closer to the climatological mean compared to spring and summer months (White *et al.* 1995). During these periods larger sized phytoplankton such as diatoms have been observed to support larger sized omnivorous crustacean zooplankton biomass in the equatorial region compared with higher latitudes such as the NPSG region (Le Borgne *et al*. 2003).

Both regions cover a wide latitudinal range in temperature, nutrients, phytoplankton biomass, and associated phytoplankton C:N:P. Since the JGOFS EqPac cruises in 1992-1993, there has been little data coverage on zooplankton elemental composition in these regions of the Pacific Ocean (~32 years ago).These elemental measurements of zooplankton did not include reports of elemental ratio data (C:N ratio) or measurements of phosphorus, therefore to our knowledge there has been no reports of the C:N:P ratios or measurements of phosphorus in zooplankton along 140º W in the NPSG and NPEC region. Additionally, the changes in zooplankton size structure reported by White *et al.* (1995) would suggest a potential change in zooplankton C:N:P, however the JGOFS EqPac studies did not investigate this either. These data gaps in our knowledge of zooplankton C:N:P need to be addressed. We need more direct measurements of carbon, nitrogen and especially phosphorus and we need to report the C:N:P ratios in the zooplankton community in this region of the Pacific Ocean.

1.4 Research Objectives

We are lacking measurements of marine zooplankton carbon, nitrogen, and especially phosphorus on a global scale, and even more so on a regional scale, specifically in the NPSG and NPEC region. The lack of phosphorus measurements is a high priority that urgently needs to be addressed, which we know is an important element in assessing growth rates, nutrient requirements and the overall elemental composition in the zooplankton community. There is a disparity in C:N:P measured zooplankton taxonomic

groups and size fractions, especially in the microzooplankton size fraction, and we do not have a clear pattern in how the C:N:P in zooplankton changes with increasing size with the current limited evidence in the literature. There is also a lack of clarity from field and experimental studies in how environmental conditions like temperature, nutrient concentrations, and resource C:N:P influence the variability in the C:N:P in zooplankton. These data gaps need to be addressed if we are to have a more complete understanding of variability in C:N:P in the zooplankton community and their role in the biogeochemical cycles of carbon, nitrogen, and phosphorus in the ocean.

To address these data gaps, we went back and measured the C:N:P in different size fractions of zooplankton, and revisited and even larger transect in this same region along 140º W as part of the Simons Collaboration On Ocean Processes and Ecology (SCOPE) program. We collected phytoplankton and zooplankton during a research cruise in the NPSG and NPEC region to characterize the C:N:P, macromolecular composition, and community structure of zooplankton and phytoplankton samples. This research cruise known as the SCOPE-Gradients IV cruise provided an excellent opportunity to build off their previous investigation and address our research objectives in this regional context. We want to know about the variability in zooplankton C:N:P. Is zooplankton C:N:P constant, or is it variable? In this thesis we address the following two main questions:

1.) Are there differences in zooplankton molar C:N:P ratios across size fractions?

2.) Are there differences in zooplankton molar C:N:P ratios with environmental conditions and biological conditions?

Our first hypothesis is that we will observe significant differences in the molar C:N, C:P, and N:P ratios across size fractions based on our previous knowledge of differences in the molar C:N, C:P, and N:P in zooplankton taxonomic groups and the community composition in each size fraction. We expect to observe significant differences in molar C:N, C:P and N:P across size fractions because of past studies reporting varying C:N:P across different taxonomic groups of zooplankton. We expect higher C:P and N:P in size

fractions >2000 μm because of the higher proportion of gelatinous zooplankton typically found in this size fraction with characteristically higher C:P and N:P compared with lower C:P and N:P characteristic of crustacean zooplankton in the 200-2000 μm size fraction.

Our second hypothesis is that we should not expect to observe significant differences in the molar C:N, C:P, or N:P ratios of zooplankton with environmental conditions based on the majority of previous studies indicating that C:N:P in zooplankton is homeostatic and should not vary with external conditions, including environmental variables like latitude, temperature, nitrate concentration, phytoplankton biomass (chlorophyll-*a* concentration and carbon concentration) and associated phytoplankton molar C:N:P ratios. Overall, we suspect that variability in zooplankton C:N:P will be driven by differences in taxonomic composition, and not environmental conditions. In the next chapter we will describe our approach and methodology we used to address our research objectives and answer these two main research questions.

CHAPTER 2: MATERIALS AND METHODS

2.1 Study Area: SCOPE-Gradients iv Cruise Track

The Simons Collaboration On Ocean Processes and Ecology (SCOPE) program aimed to enhance understanding of marine microbial communities in the North Pacific Subtropical Gyre (NPSG) and its transition zones. The SCOPE-Gradients IV cruise (TN397) focused on the environmental gradients in the transition zone between the NPSG and the North Pacific Equatorial Countercurrent (NPEC) region. Zooplankton and phytoplankton samples were collected aboard the R/V *Thomas G. Thompson* from November 18th to December 15th, 2021, departing from San Diego, California, and ending in Honolulu, Hawai'i. The transect was performed along the 140º W longitude line from ~20º N to 2.5º S latitude, including 18 stations (S1-S18), with paired day and night zooplankton sampling at four stations (S4, S7, S9, S11) and nighttime sampling at S13 (Figure 1, Table I). The environmental variables latitude, temperature, nutrients (i.e. nitrate concentration), phytoplankton biomass (i.e. chlorophyll-*a* concentration and C, N, P concentration), and phytoplankton molar C:N:P ratios used for our analyses, and other important hydrographic properties (i.e. salinity, fluorescence) during this study are presented at the beginning of the results section.

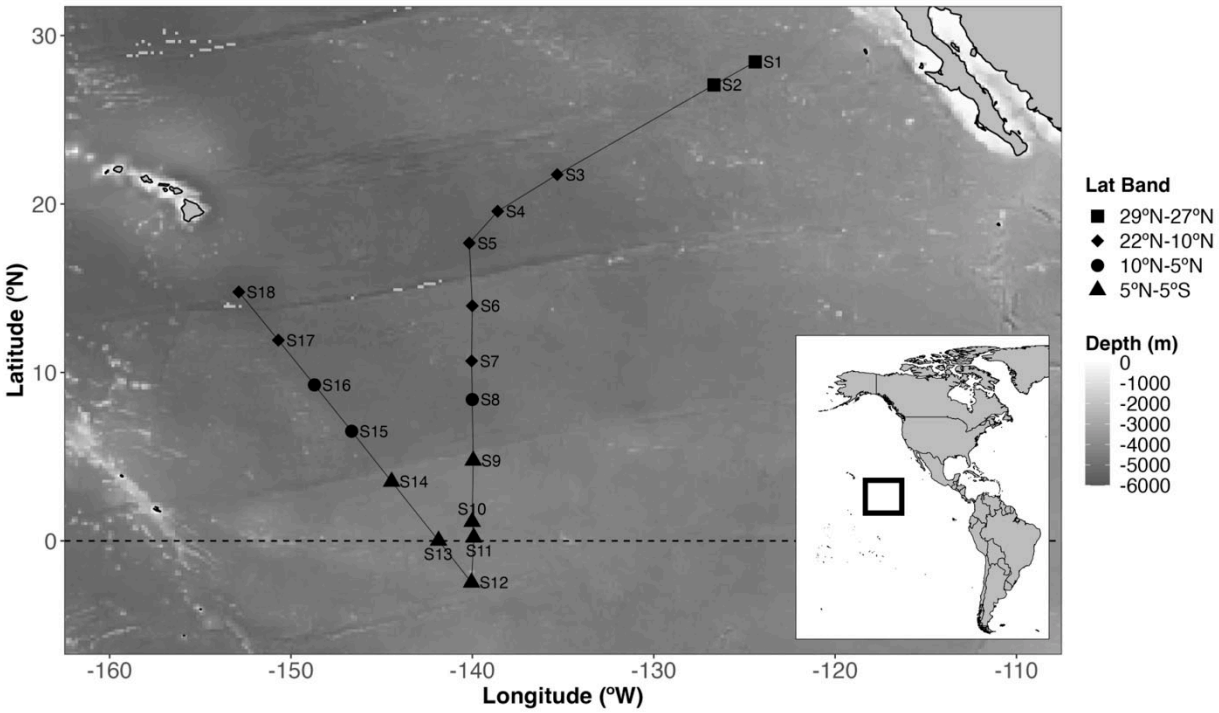

Figure 1. Map of the Gradients IV cruise (TN397) track, sampling stations, and bathymetry. Shapes represent stations within four latitudinal bands: Black squares represent stations in 29º N-27º N, black diamonds represent 22º N-10º N, black dots represent 10º N-5º N, black triangles represent 5º N-5º S. Thin black line represents cruise track path starting from S1 and ending at S18. The dotted line represents the equator.

The study area was divided into four latitudinal bands determined by latitudinal ranges used in previous regional studies: 29-27º N, 22-10º N, 10-5º N, and 5º N-5º S. The 29-27º N band included S1 and S2. The 22-10º N band included stations S3-S7, S17-S18, based on Dai *et al.'s* (2023) southern boundary of the NPSG at ~10º N. The 10-5º N band included stations S8, S15-S16, marking the transition between the NPSG and the equatorial upwelling zone. The 5º N-5º S band included stations S9-S14, following White *et al.*'s (1995) description of the upwelling zone at the equator. These latitudinal bands and their characteristics are described in the first section of the results chapter.

2.2 Meso-Zooplankton Collection: WP-2 Net

Mesozooplankton were collected using vertical net tows with a Wilkens and Plath double net (WP-2 net) with 0.57 m diameter openings equipped with 200 μm mesh nets. Each tow sampled from 0-250 m, where the majority of mesozooplankton biomass is found in the water column (La Borgne *et al.* 2003; Steinberg *et al.* 2008; White *et al.* 1995). A 35 lb weight was attached to the net to ensure negative buoyancy. A flow meter was positioned midway from the net mouth to rim to prevent flow overestimation. Volume filtered was calculated from the flowmeter measurements after each tow.

Each net's cod end was fitted with a plankton net bucket with 200 um mesh windows to collect the samples. The net was deployed at ~0.7 m/s to 250 m, then retrieved at ~0.5 m/s to minimize mesozooplankton escape (Sameoto *et al.* 2000). Upon retrieval, the nets were rinsed with unfiltered seawater from the underway of the ship using a hose to concentrate samples at the cod ends. The two net buckets were then carefully detached, and their contents transferred into separate containers for further processing.

2.3 Meso-Zooplankton Processing: WP-2 Net

Two containers were used (one for each net bucket described in the previous section): one for elemental and bulk macromolecule measurements (container A) and one for taxonomic identification (container B). Larger organisms and gelatinous zooplankton (>2000 μm) from container A were isolated in a sorting tray (with the exception of copepod species which were <2000 μm), transferred to 5 ml cryogenic vials (or, in cases where many individuals were collected, disposable plastic petri dishes), flash frozen and stored at -80º C for further analyses. Isolated individual zooplankton specimens were collected from a limited number of stations.

Container A's contents were sieved sequentially by mesh size (2000 μm, 500 μm, 250 μm) into three size fractions: >2000 μm, 500-2000 μm, and 250-500 μm. Sieves were gently submerged in seawater from container A to loosen clogged organisms. Each size fraction was then re-suspended, transferred into a plankton splitter, and homogenized with filtered seawater (~250 ml) using the stirring technique (Landry *et al.* 2001). Three-

quarters of each sample were collected for dry weight and elemental measurements, while the remaining quarter of sample was stored for other analyses. Samples were rinsed into 100 ml cylinder beakers with filtered seawater, vacuum filtered onto 52 μm nitex mesh, transferred to 5 ml cryogenic vials or plastic petri dishes, flash frozen, and stored at -80º C. Container B was not split, preserved in buffered formalin for microscopy and stored at room temperature (Postel *et al.* 2000).

2.4 Micro-Zooplankton Collection: Ring Net

Microzooplankton were collected using vertical net tows with a single-hoop ring net with a 0.25 m diameter opening equipped with a 52 μm mesh net. Each tow sampled from 0-150 m where the majority of microzooplankton biomass is found in the water column (Stoecker *et al.* 1996). A 25 lb weight, flow meter, and plankton net bucket with a 50 μm mesh window were attached and the net was deployed and retrieved as previously described for the WP2-double net in section 2.2.

2.5 Micro-Zooplankton Processing: Ring Net

Microzooplankton were processed from one container the same way for mesozooplankton samples described in section 2.3 except, they were separated into three size fractions: 250-2000 μm, 125-250 μm, and 64-125 μm (the 125-250 μm and 64-125μm size fraction samples were combined after the cruise into a single size fraction: 64-250 μm). Three-quarters of each sample were processed and stored the same way as mesozooplankton samples described in section 2.3, while the remaining quarter was used for future microscopy. Samples from this portion were further split in two (effectively 1/8 of total sample) using the plankton splitter. One sample was fixed using the Lugol's fixative described by Gifford & Caron (2000) while the other was flash frozen and stored at -80º C for other analyses.

2.6 Phytoplankton Collection And Processing

Samples for phytoplankton bulk elemental and macromolecule analyses were collected from 0.2-48 L whole seawater surface samples (~15 m) at each station using a trace metal-clean towfish surface water sampling system (Vink *et al.* 2000). Seawater was

filtered onto triplicate precombusted Whatman GF/F filters or 0.2 µm polycarbonate nucleopore filters, depending on the analyte being collected. All samples were stored in 2 ml cryovials, immediately flash frozen, and stored at -80º C until further analysis.

Table I. Actual depths of the zooplankton net sampling for Gradients IV cruise. Four stations collected zooplankton at nighttime (Ni). Phytoplankton were collected at stations indicated in bold text. Asterisks (*) indicate stations where sufficient biomass was collected by vertical nets for all four size-fraction elemental analyses. Notice that the ring net samples were not collected at 3 stations (S4(Ni) S7, S10). The ring net was deployed at S1 to test equipment, and no zooplankton samples were collected.

Station	Date	Time	Position	WP-2 net depth	Ring net depth
S1	20-Nov-2021	--	28º44'N, 124º41'W	--	0-161m
S2	21-Nov-2021	16:12 UTC	27º07'N, 126º68'W	0-287m	0-155m
S3	23-Nov-2021	17:00 UTC	21º74'N, 135º32'W	0-296m	0-162m
S4(Ni)*	24-Nov-2021	6:56 UTC	19º57'N, 138º60'W	0-265m	0-158m
S4	25-Nov-2021	17:30 UTC	19º57'N, 138º60'W	0-283m	--
S5*	26-Nov-2021	18:08 UTC	17º68'N, 140º17'W	0-294m	0-175m
S6*	27-Nov-2021	17:54 UTC	13º96'N, 140ºW	0-286m	0-170m
S7(Ni)*	28-Nov-2021	7:30 UTC	10º68'N, 140ºW	0-295m	0-115m
S7	29-Nov-2021	18:00 UTC	10º68'N, 140ºW	0-248m	--
S8*	30-Nov-2021	18:00 UTC	8º39'N, 140ºW	0-287m	0-165m
S9*	1-Dec-2021	18:00 UTC	4º77'N, 140ºW	0-287m	0-172m
S9(Ni)*	1-Dec-2021	6:30 UTC	4º77'N, 140ºW	0-298m	0-161m
S10	3-Dec-2021	17:50 UTC	1º13'N, 140ºW	0-281m	--
S11.1(Ni)*	3-Dec-2021	7:20 UTC	0º21'N, 139º92W	0-281m	0-171m
S11.2*	4-Dec-2021	2:15 UTC	0º21'N, 139º92W	0-295m	0-181m
S12	6-Dec-2021	10:50 UTC	2º47'S, 140ºW	0-297m	0-185m
S13(Ni)*	7-Dec-2021	8:20 UTC	0º02'N, 141º86'W	0-288m	0-176m
S14*	9-Dec-2021	18:20 UTC	3º52'N, 144º45'W	0-285m	0-175m
S15*	10-Dec-2021	18:25 UTC	6º51'N, 146º65'W	0-285m	0-178m
S16*	11-Dec-2021	19:30 UTC	9º26'N, 148º71'W	0-274m	0-168m
S17*	12-Dec-2021	19:30 UTC	11º92'N, 150º70'W	0-245m	0-165m
S18*	13-Dec-2021	19:00 UTC	14º78'N, 152º87'W	0-283m	0-170m

2.7 Dry Weight Measurements Of Zooplankton

Before elemental analysis of total particulate carbon (TPC), total particulate nitrogen (TPN), and total particulate phosphorus (TPP), zooplankton samples (including all four size fractions and individually selected organisms) were freeze-dried to a constant weight using a FreeZone Benchtop Freeze Dryer. Samples were removed and weighed every 24 hours, until weights remained constant. Freeze drying, which removes ~99.5% of water by vacuum and trapping water vapor in a cold condenser (Postel *et al.* 2000), provides higher recovery in elemental analyses than only oven drying at 60º C (Omori 1978). Dry weights were measured with an OAHUS semi-microbalance.

Samples were then ground into a homogeneous powder using 2 ml metal lysing tubes and an MP Fast Prep bead beater. In a biosafety cabinet, samples were transferred from their original containers to the lysing tubes to prevent contamination. For the >2000 μm size fraction, initial homogenization was done with an agate mortar and pestle before final homogenization in lysing tubes due to the larger size of organisms. Homogenized samples were transferred to glass scintillation or weighing vials, and residual biomass was stored in 2 ml or 5 ml RNase/DNase-free cryogenic vials at -80º C. For elemental analysis, samples in glass scintillation or weighing vials were weighed, oven-dried at 60º C for 2 days (Postel *et al.* 2000), transferred to a vacuum desiccator to cool to room temperature, and reweighed to ensure stable weight.

2.8 Elemental Analyses Of Zooplankton

Total particulate carbon (TPC) and total particulate nitrogen (TPN) content in zooplankton samples (including all four size fractions and some individually selected organisms) were measured using Carbon, Hydrogen, Nitrogen (CHN) analysis (Table II). The CHN analysis was conducted with an Elementar MicroCube elemental analyzer, standardized to acetanilide (C_8H_9NO), by Dr. Claire Normandeau in the Wallace Lab. Each sample was analyzed in triplicate using subsamples from homogenized dried powder, with dry weight (DW) measured on a microbalance before encapsulation in tin capsules. Each sample replicate had three technical replicates for CHN analysis. The minimum sampling zooplankton biomass used was 0.1 mg DW.

Total particulate phosphorus (TPP) content was measured using the eXtra High Temperature Dry Combustion (X-HTDC) ash-hydrolysis colorimetric method (Hu *et al.,* 2022). Potassium dihydrogen orthophosphate ($KH_2\ PO_4$) was the standard for phosphorus recovery. This method employs higher combustion temperatures (800° C vs. 450-500° C) to enhance phosphorus recovery in plankton field samples. As with CHN analysis, zooplankton samples (including all four size fractions and all individually selected organisms) were analyzed in triplicate using subsamples from homogenized dried powder, with dry weights measured on a microbalance before transfer to porcelain crucibles for the assay. Each subsample replicate had three technical replicates for the assay. The minimum sampling zooplankton biomass used was 0.4 mg DW.

2.9 Particulate Organic Carbon, Biogenic Silica, And Chlorophyll Concentration In Zooplankton

Particulate organic carbon (POC) content in zooplankton samples (including all four size fractions and all individually selected organisms) was analyzed and measured using CHN analysis by Dr. Claire Normandeau in the Wallace Lab. The preparation followed the same protocol in section 2.8, except with an initial acidification step to remove particulate inorganic carbonates (PIC). Triplicates were subsampled from homogenized dried powder, weighed, and fumigated with HCl acid for at least 24 hours. After air drying for 2-3 hours and drying at 60ºC for 48 hours, the samples were encapsulated in tin capsules for CHN analysis. However, due to an error in weighing samples post-fumigation (instead of *before*), POC content was not representative of the correct initial dry weight and required correction using the total particulate carbon (TPC) measured previously from the same samples. POC content for all four size fraction samples was corrected, while individually selected organism measurements were only corrected for euphausiids and ctenophores because TPC was also measured in these groups after learning about the incorrect POC measurements.

Biogenic silica (bSi) content in all four size fractions of zooplankton was determined using the wet-alkaline colorimetric method (Hu *et al.,* 2023), with Celite Silica diatomaceous earth as the standard. Triplicates were subsampled from homogenized dried powder, weighed, and transferred to 50 ml Falcon tubes for analysis. Each subsample replicate had three technical replicates for the assay. The minimum sampling zooplankton biomass used was 0.2 mg DW.

Chlorophyll-*a* concentration in zooplankton samples was measured fluorometrically with a Turner Designs fluorometer following the protocol of Arar and Collins (1997). Triplicates were subsampled from homogenized dried powder, weighed, and transferred to 15 ml glass tubes to extract chlorophyll-*a* in solvent for analysis. Each subsample replicate had three technical replicates. The minimum sampling zooplankton biomass used was 0.1 mg DW.

POC, bSi, and chlorophyll-*a* concentration was measured in zooplankton samples to investigate the presence of zooplankton with calcareous and silicious skeletons in size fractions, especially for microzooplankton (e.g. foraminifera and radiolaria) in the 64-250 μm size fraction (Stoecker *et al.* 1996). The bSi and chlorophyll-*a* concentration measurements are only provided in the Appendix (Table A1) because the focus of this study is on carbon, nitrogen, and phosphorus measurements in zooplankton samples.

2.10 Elemental Analyses Of Phytoplankton

CHN analysis of total particulate carbon (TPC) and total particulate nitrogen (TPN) in phytoplankton was measured using the same method described in section 2.8 except phytoplankton biomass was measured on precombusted GF/F filters. Each sample replicate had three technical replicates for CHN analysis. Phytoplankton samples were not fumigated in weak HCl acid to remove particulate inorganic carbonates (PIC). Total particulate phosphorus (TPP) analysis was conducted using the same method described in section 2.8 except phytoplankton biomass was measured on 0.2 μm polycarbonate nucleopore filters. Each sample replicate had three technical replicates for the TPP assay. The minimum sampling phytoplankton biomass is 0.19 μg P/ filter. Phytoplankton were collected, processed, and the data was analyzed and provided by Dr. Sing-How Tuo.

Table II. TPC, TPN and TPP analyses completed for all four zooplankton size fractions. Boxes with an X indicate that the elemental analyses were completed. Stations are described in Table I. Elemental analyses were completed for phytoplankton samples collected at stations indicated in bold text.

Station	**TPC, TPN, TPP >2000 µm**	**TPC, TPN, TPP 500-2000 µm**	**TPC, TPN, TPP 250-500 µm**	**TPC, TPN, TPP 64-250 µm**
S1	--	--	--	--
S2	X	X	X	--
S3	X	X	X	--
S4(Ni)*	X	X	X	X
S4	X	X	X	--
S5*	X	X	X	X
S6*	X	X	X	X
S7(Ni)*	X	X	X	X
S7	X	--	X	--
S8*	X	X	X	X
S9*	X	X	X	X
S9(Ni)*	X	X	X	X
S10	--	X	X	--
S11.1(Ni)*	X	X	X	X
S11.2*	X	X	X	X
S12	--	X	X	X
S13(Ni)*	X	X	X	X
S14*	X	X	X	X
S15*	X	X	X	X
S16*	X	X	X	X
S17*	X	X	X	X
S18*	X	X	X	X
Total	19	20	21	16

2.11 Data & Statistical Analyses: Environmental Variables

To assess which environmental variables best predict the molar TPC:TPN:TPP ratios of zooplankton we accessed the following 3 datasets on Simons CMAP (https://doi.org/10.5281/zenodo.7015515, https://doi.org/10.5281/zenodo.8400920, https://doi.org/10.5281/zenodo.7015528). Temperature (º C), nitrate and nitrite concentration (μM), chlorophyll-*a* concentration (μg/L), fluorescence (mg/m^3) and salinity (psu) were all measured from seawater collected from known depths using CTD-rosette sampling procedures during the Gradients IV cruise. Standard protocols for each measurement can be found online at the Hawaii Ocean Time-series (HOT) website (https://hahana.soest.hawaii.edu/hot/protocols/protocols.html?Chapter=12#).

2.12 Data & Statistical Analyses: C, N, P Measurements In Phytoplankton

All phytoplankton molar TPC:TPN:TPP ratios (mol:mol) and TPC, TPN, and TPP concentration in biomass measurements were provided in a dataset by Dr. Sing-How Tuo. Phytoplankton TPC, TPN, and TPP concentration measurements and elemental ratios were used to investigate the relationships between zooplankton molar TPC, TPN, and TPP concentrations in biomass and zooplankton molar TPC:TPN:TPP ratios in size fractions.

2.13 Data & Statistical Analyses: C, N, P Measurements In Zooplankton

All elemental ratio results in this study are presented as molar ratios. Molar TPC:TPN, TPC:TPP, and TPN:TPP ratios (mol:mol) in zooplankton size fractions and individually selected zooplankton were $\log_{10}$ transformed, averaged, anti-logged, and reported to meet the assumptions of normal distribution and variance homogeneity. To convert the standard deviation of the $\log_{10}$ of the ratios to a number to describe the error, we computed the standard deviation (±1 SD) as a percentage of error. Molar particulate organic carbon (POC) measurements were corrected by dividing the non-acidified dry weight from TPC measurements and acidified dry weight from POC measurements. This ratio was then multiplied with each incorrect POC measurement as a correction factor. This correction assumes nitrogen content (TPN) is not affected by fumigation in weak

HCl and its content will be the same for both acidified (i.e. POC measurements) and not acidified samples (i.e. TPC measurements). The corrected POC and TPC measurements were compared across all four size fractions to examine the POC:TPC ratio and potential patterns with latitude. POC:PON ratios (mol:mol) were calculated for individually selected zooplankton because both POC and PON measurements come from the same acidified sample during CHN analysis. From this point forward all TPC, TPN, and TPP measurements will be simply noted as C, N, and P. Only measurements of POC and PON will be fully abbreviated when referenced in the text. We calculated the mass of C, N and P content % dry weight (g:g) in zooplankton size fractions and individually selected zooplankton to provide additional context for our first question concerning significant differences in molar C:N:P across size fractions. The reason being to provide mass units representing absolute amounts to give us additional insight into our molar C:N:P results which represent relative amounts. C, N, and P % dry weight were calculated by converting molar measurements to mass measurements using the molecular weights of carbon (12.01 g per mol), nitrogen (14 g per mol), and phosphorus (30.97 g per mol). All % dry weight measurements are mass measurements (g:g) in this study.

Molar C, N and P concentration in zooplankton biomass (mmol m^{-3}) was calculated based on the dry weight (DW) biomass sampled for each size fraction from each net tow (mg m^{-3}). DW (mg) was converted to mmol of C, N, and P from the known percentage of each element measured per DW from each sample, then converted to mols of each element, and then divided by the volume of water each sample was filtered through during net tows. Molar C, N, and P concentrations in zooplankton size fractions were calculated to provide additional context for our second question concerning differences in zooplankton molar C:N:P ratios with environmental conditions. Molar C, N, P concentrations of zooplankton help us think about our molar C:N:P ratios in terms of the changes in zooplankton biomass along the cruise track. Sample size represents the number of individual samples that includes multiple organisms or bulk zooplankton samples, not the number of individual organisms.

2.14 Data & Statistical Analyses: Differences In Zooplankton C:N:P & C, N, P Content % Dry Weight In Size Fractions & Individual Species

To determine if there were significant differences in the molar C:N, C:P, and N:P, and C, N, and P % dry weight across the different size fractions a one-way (Analysis of variance) ANOVA was performed. The one-way ANOVA was chosen for its simplicity and specificity. We are only interested in examining the one independent variable of size for its effects on molar C:N:P ratios, and it allows for the comparison of means across at least three groups while controlling for the increased risk of Type I errors (rejecting the null hypothesis when it's true) that would result from conducting multiple pairwise t-tests. Following a significant ANOVA result, a Tukey (Honestly Significant Differences) HSD post hoc test was conducted to identify which specific size fraction means differed significantly. The Tukey HSD test was selected for its ability to control the Type I error rate when making multiple comparisons, providing a robust method for discerning specific size fraction differences. The same statistical protocol was conducted to identify differences in the molar POC:PON ratios and P % dry weight in selected zooplankton species. These statistical analyses were essential for addressing our first research question of determining if there are differences in the molar C:N:P ratios in the four size fractions. By identifying statistically significant differences between the size fractions, we can better understand the underlying patterns and relationships in our data. Statistical analyses and plots were run using data from both day and nighttime stations for molar C:N:P ratios in size fractions after finding no significant differences between the results using both methods.

Field measurements of molar C:N, C:P, and N:P ratios of size fractionated zooplankton were selected from eight published studies to provide a baseline for our molar C:N:P ratios in size fractions and compare measurements with this study. Published papers were compiled into an excel spreadsheet by Dr. Niall McGinty in the Finkel lab and selected upon the basis of containing measurements of C:N, C:P, and N:P ratios in size

fractionated marine zooplankton. Elemental ratios were given in the literature in either molar units or mass units. For comparability reasons, we converted all elemental ratios given by mass units to molar units. Average values were calculated for size fractions reported in each selected study.

2.15 Data & Statistical Analyses: Differences In Zooplankton C, N, P Measurements With Envrionmental Variables

To evaluate the differences in C, N, and P concentrations and molar C:N, C:P, and N:P ratios in zooplankton with environmental conditions we used the environmental variables latitude, temperature, nitrate concentration, chlorophyll-*a* concentration, phytoplankton C concentration, and molar C:N:P ratios. We chose latitude because zooplankton communities can vary spatially, and community changes may influence the molar C:N:P ratios. We chose temperature because of its unclear effect on increasing and decreasing in molar C:N:P ratios in zooplankton, warranting a second investigation as a potential mechanism. We chose nitrate concentration, chlorophyll-a concentration and phytoplankton C concentration because although nutrients aren't expected to directly influence the molar C:N:P ratios in zooplankton, they do influence and provide information on the quantity of phytoplankton as a food source for zooplankton. We examined phytoplankton biomass as both chlorophyll-a concentration and C concentration to account for potential misinterpretations associated with chlorophyll-*a* concentration. Chlorophyll-*a* concentration can vary significantly due to changes in light intensity, nutrient availability, and species composition, leading to variability in the chlorophyll-to-carbon ratio (Wang *et al.* 2009). These factors can cause chlorophyll measurements to overestimate or underestimate actual phytoplankton biomass. We examined both to be thorough in our analyses. Phytoplankton molar C:N:P ratios as resource quality have also been observed to influence the degree of homeostasis in zooplankton and warrants an investigation to examine this potential relationship in the field. Temperature, nitrate and nitrite concentration, chlorophyll-*a* concentration was depth integrated from CTD casts, and variation in measurements with depth across the wide latitudinal range of the study site. 100 m was chosen as the desired integration depth for temperature, nitrate and nitrite concentration, and chlorophyll-*a* concentration measurements because the majority of mesozooplankton, microzooplankton, and

phytoplankton biomass have been observed above this depth in the water column (Le Borgne *et al*. 2003; Steinberg *et al.* 2008; Stoecker *et al.* 1996; White *et al.* 1995).

Each environmental variable (latitude, temperature, nitrate and nitrite concentration, chlorophyll-*a* concentration, phytoplankton C, N, P concentration, phytoplankton molar C:N, C:P, and N:P) was tested separately as an independent predictor of the molar C:N, C:P, and N:P in each size fraction using linear regression models. These analyses were essential for addressing our second question of determining if zooplankton molar C:N:P ratios change with environmental conditions. Using individual linear regression models allows us to isolate effects by focusing on one environmental condition at a time. The simplicity of a linear regression model approach provides a straightforward interpretation of the relationship between each environmental condition and the molar C:N:P ratios in each size fraction. The insights gained from these individual analyses could inform subsequent multivariate analyses in the future, where the combined and interactive effects of multiple environmental conditions can be considered more in depth. With that being said, we acknowledge that the chosen environmental variables can often be interrelated and can have confounding effects on one another.

Statistical analyses and plots were run using data from both day and nighttime stations for molar C:N:P ratios after finding no significant differences between the results using both methods. However, we did find differences between results using data from both nighttime and daytime data and only daytime data for C, N, and P concentrations so we decided to only use daytime data for statistical analyses and plots of zooplankton elemental concentrations. All measurements used in plots and analyses are presented as averages of triplicates (i.e. one point = 3 replicates). Replicates are helpful for quantifying variability in measurements within each tow. C, N, and P measurements of zooplankton from S1 and S2 are excluded from all plots and analyses because 1.) No samples were collected at S1 (i.e. test station for equipment), and 2.) we are interested in pelagic ecosystems and associated water masses, not water masses with potential coastal

influences. All plots were created using the *ggplot2* package in R (Wickman, 2016) and statistical analyses were conducted in R version 4.4.1.

CHAPTER 3 RESULTS

3.1 Latitudinal Gradients In Environmental Variables & Hydrogroaphy Along The Cruise Track

Latitudinal gradients in environmental variables and corresponding phytoplankton biomass (chlorophyll-*a* concentrations and C, N, P concentrations) and molar C:N:P ratios were observed along the Gradients IV cruise track (Figure 2). Temperature decreased from 23º C at approximately 20º N latitude to 19º C at around 10º N latitude before sharply increasing to 26.4º C at roughly 6º N latitude. Temperature then steadily decreased to 22.8º C at the equator and increased again to 26º C at -2.5º N latitude. Nitrate and nitrite concentrations were below detectable limits until about 12.5º N latitude, where they reached 2.5 µmol m^{-2}, and then steadily increased to approximately 9 µmol m^{-2} at the equator, before dropping to 7 µmol m^{-2} at -2.5º N. Notably, Stations S8 and S7 (10.7-8.4ºN latitude) had the highest nitrate and nitrite concentrations, measuring 16.8 and 13.2 µmol m^{-2}, respectively. Chlorophyll-*a* concentration increased from 0.18 µg m^{-2} at about 19º N latitude to 0.34 µg m^{-2}at 14º N, plateaued between 0.2 to 2.5 µg m^{-2} until it sharply increased to 0.44 µg m^{-2} at the equator, and then decreased again at -2.5º N latitude. Phytoplankton C, N, P concentrations (mmol m^{-3}) were relatively low around 20º N latitude, sharply increased at 10º N, peaked at the equator, and then decreased at -2.5º N. The phytoplankton molar C:N:P ratios were more variable with no clear latitudinal patterns from 20º N to -2.5º N latitude, except for phytoplankton C:N, which showed some patterns as detailed in Figure A2 in the appendix (Please see appendix for environmental variables with linear regressions).

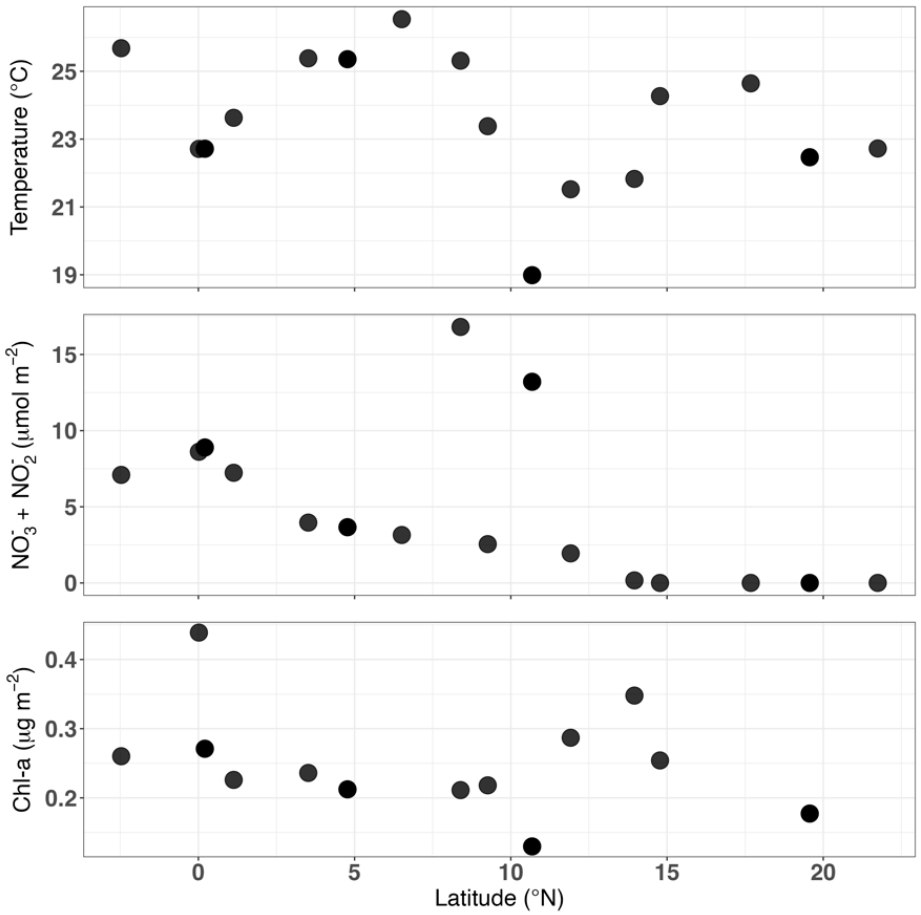

Figure 2. Average (0-100 m) temperature (ºC) (*top panel*), depth-integrated (0-100 m) nitrate and nitrite concentration (µmol m^{-2}) (*middle panel*), and depth-integrated (0-100 m) chlorophyll-*a* concentration (µg m^{-2}) (*bottom panel*) as a function of latitude (ºN). Protocols for these measurements were obtained from datasets in Simon's CMAP database for the TN397 cruise as described in section 2.11.

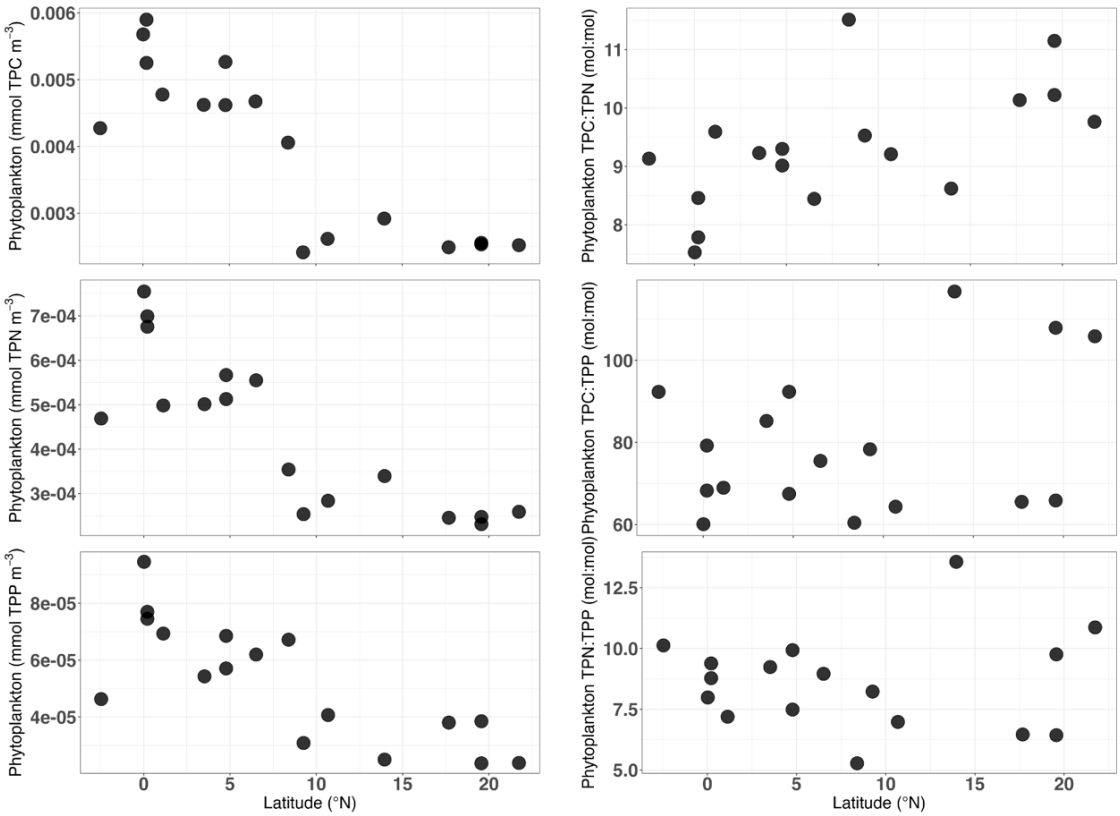

Figure 3. Phytoplankton C, N, and P concentration (mmol m^{-3}) (*left column panels*), and phytoplankton molar C:N, C:P, and N:P ratios (mol:mol) (*right column panels*) as a function of latitude (ºN).

After examination of the previous environmental variables and corresponding phytoplankton biomass (chlorophyll-*a* concentration and C, N, P concentrations) and C:N:P ratios as a function of latitude we were motivated to further examine how hydrographic properties such as temperature, nitrate and nitrite concentration, salinity and chlorophyll fluorescence change with depth along the cruise track. We examined fluorescence and nitrate and nitrite concentration with depth to provide a general indication of the location of the deep chlorophyll maximum and nutricline gradient with latitude and depth. We identified four latitudinal bands with the following characteristics (Figure 4).

29º N-27º N. Located ~500 km from the west coast of Baja California and Baja California Sur, Mexico. The temperature was the coldest at all depths (0-200 m) out of all 4 latitudinal bands, with surface temperatures at ~20º C from the surface to ~50 m before steadily decreasing with depth. The salinity profile was the least saline out of all 4 latitudinal bands, with a surface salinity of ~33.6 psu with some slight variability with increasing depth. The nutrient profile of nitrate and nitrite concentration was the lowest for all 4 latitudinal bands with undetectable values until 100 m and a steady increase to ~15 µM with depth. The fluorescence profile had a peak of ~0.8 mg m^{-3} at 100 m depth. This latitudinal band possibly contains different water masses associated with the California current indicated by the lower temperature and salinity profiles.

22º N-10º N. The surface temperature of ~25º C sharply decreased with depth, the salinity profile of 34.25 psu at the surface increased to ~34.75 psu at 50 m before steadily decreasing to 34 psu with depth, the nitrate and nitrite concentration was undetectable until ~50 m depth, and then increased to ~22 µM at 200 m depth, and the fluorescence profile had a peak of ~0.9 mg m^{-3} at 100 m depth.

10º N-5º N. The highest surface temperature of all 4 latitudinal bands with ~27º C at the surface before steadily decreasing with depth at 50. The salinity profile at the surface of ~34 psu until sharply increasing at 50 m depth to 34.75 psu with some variability with increasing depth, the nitrate and nitrite concentration was undetectable until a steep

increase at 50 m depth to 60 m depth and increasing to ~ 33 µM at 200 m depth. The fluorescence profile had a peak of ~1.6 mg m^{-3} at 75 m depth.

10º N-5º S. The temperature was ~25º C at the surface before steadily decreasing below 50 m depth, the salinity at the surface was the highest out of all 4 latitudinal bands with 34.75 psu, with slight variability with depth, the nitrate and nitrite concentration was highest at the surface out of all 4 latitudinal bands with ~10 µM until a steady increase at 50 m depth to ~27 µM. The fluorescence profile had a peak of ~1.9 mg m^{-3} at 40 m depth.

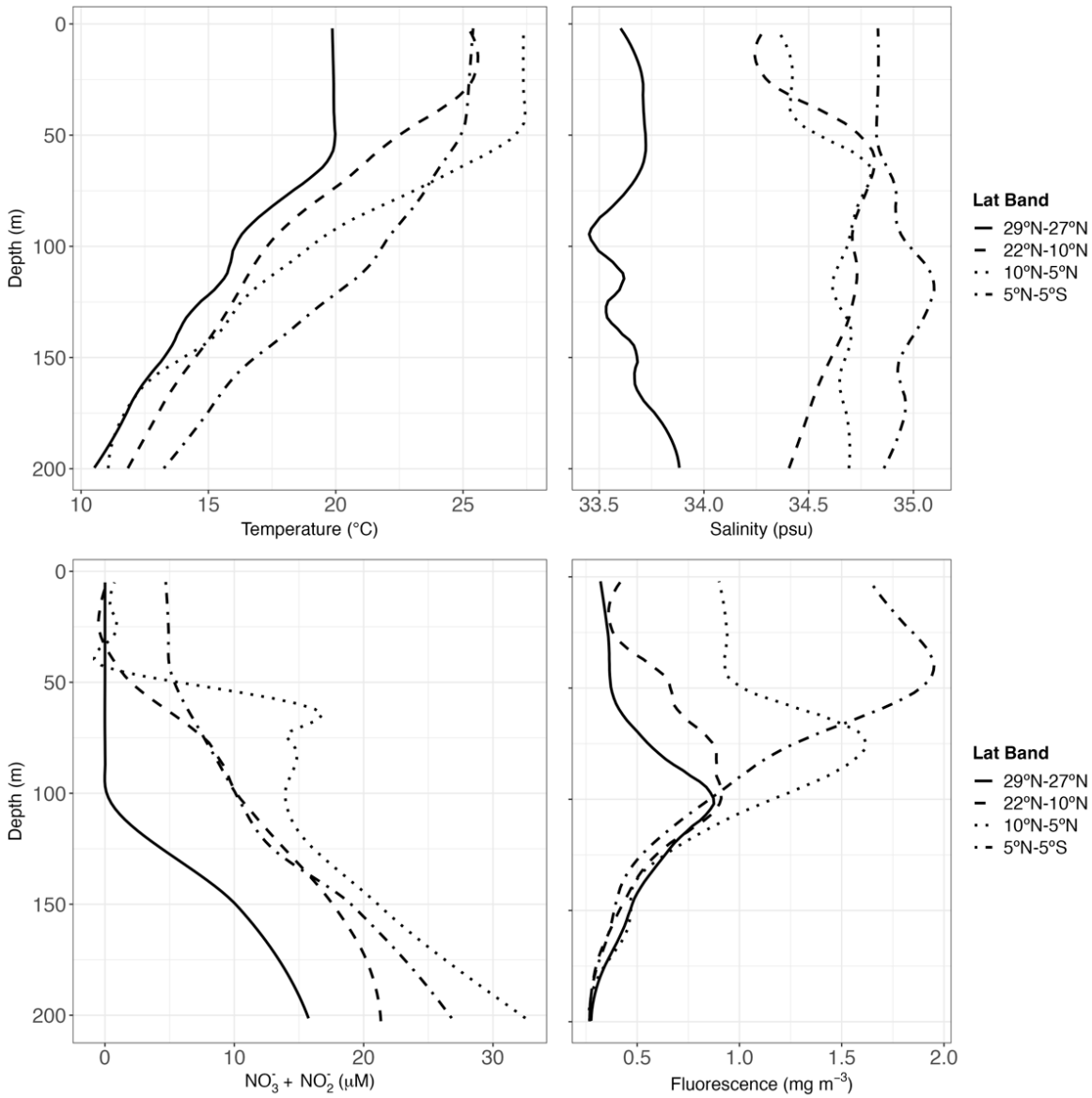

Figure 4. Average depth profiles 0-200 m of clustered stations within four latitudinal bands for temperature (ºC) (*top left*), salinity (psu) (*top right*), nitrate and nitrite concentration (μM) (*bottom left*), and CTD fluorescence (mg m^{-3}) (*bottom right*). Number of observations for nitrate and nitrite concentration were made every ~50 m, while the other three measurements were made every ~1 m along the CTD cast. Stations in each latitudinal band are described in Table I. Protocols for these measurements were obtained from datasets in Simon's CMAP database for the TN397 cruise as described in section 2.11.

3.2 Zooplankton C:N:P Stoichiometry & C, N, P % Dry Weight In Size Fractions & Species

The molar C:N:P ratios (mol:mol) were variable among the zooplankton size fractions however, there were no clear increases or decreases with size. C:N ratios did not differ much with size (Figure 5), although C:N for the 500-2000 μm fraction was significantly less than the 250-500 μm and 64-250 μm fraction (post-hoc Tukey HSD test, p-value<0.05, Tables III, IV). The average C:N was 4.88 ± 0.44 (mean ± 1SD, weighted by all four size fractions, n = 73). C:P ratios did not differ much with size, however the 250-500 μm fraction was significantly greater than the >2000 μm, 500-2000 μm, and 64-250 μm fractions (post-hoc Tukey HSD test, p-value<0.05). The average C:P was 130.65 ± 32.69 (mean ± 1SD, n = 73). N:P ratios were more variable with size, the 64-250 μm fraction was significantly less than the 500-2000 μm and 250-500 μm fraction, and the <2000 μm fraction was significantly less than the 250-500 μm fraction (post-hoc Tukey HSD test, p-value<0.05). The average N:P was 27.93 ± 6.20 (mean ± 1SD, n = 73). The overall median molar zooplankton C:N:P from the region was 116.4:25:1 (weighted using biomass contributions of stations including all four size fractions, n = 15). The median C:N was 2.1 ± 0.3 % higher in the 500-2000 μm size fraction compared to the overall median molar C:N (4.7 ± 0.42), median C:P was 18.5 ± 0.29 % higher in the 250-500 μm size fraction compared to the overall median molar C:P (116.4 ± 17), and the median N:P was 15.2 ± 0.65 % higher in the 250-500 μm size fraction compared to the overall median molar N:P (25 ± 2.9).

The corrected POC measurements and TPC measurements were compared across all four size fractions to examine the POC:TPC ratio (Figure 6) and potential pattern with latitude (Figure 7). The POC:TPC ratio across all four size fractions was 0.966 suggesting there was minimal inorganic carbonates across all zooplankton samples so we concluded that TPC and POC measurements in zooplankton could be comparable. The POC:PON ratio did not differ much among the individually collected zooplankton species (Figure 8). There were no significant differences in POC:PON detected by a one-way ANOVA test.

The overall average POC:PON in the copepods, siphonophores, euphausiids, ctenophores, fish larvae, and chaetognaths species was 4.81 ± 0.68 (mean ± 1SD, n = 24).

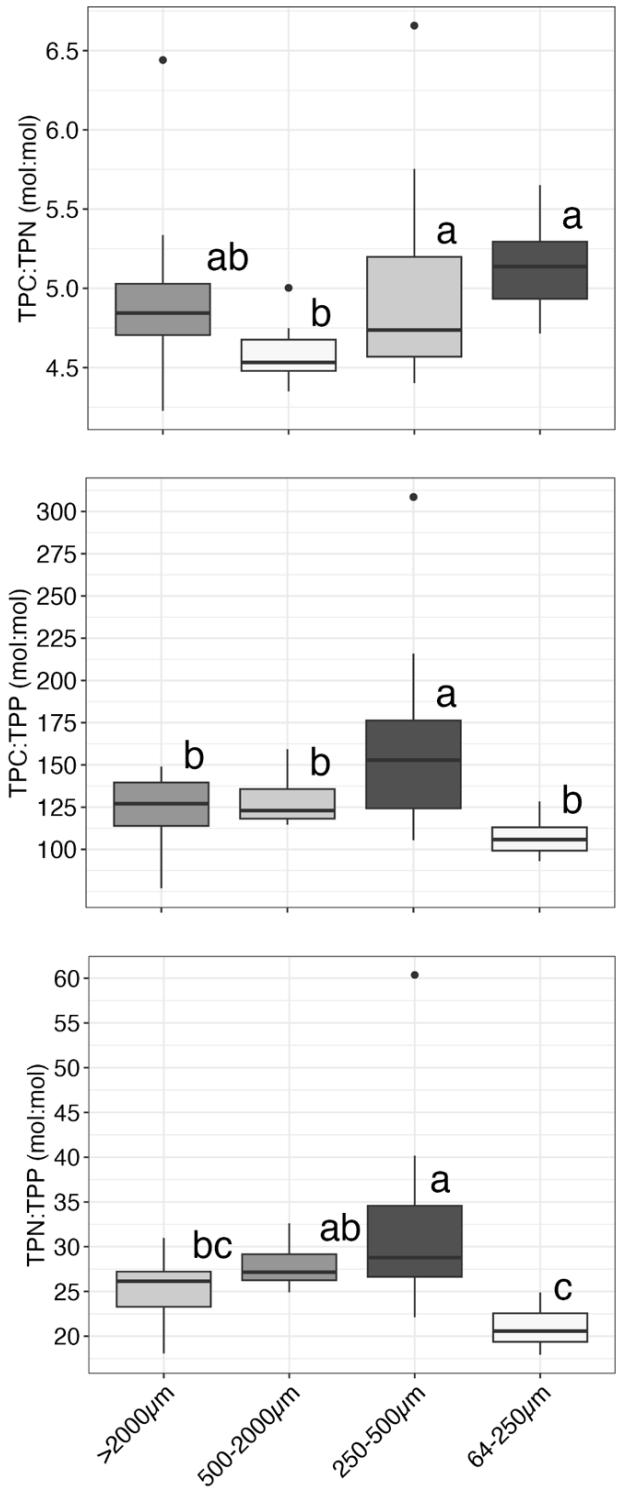

Figure 5. Zooplankton C:N:P ratios (mol:mol) in four size fractions. Boxes show data between 25th and 75th percentiles, with the median represented as a line. The whiskers extend as far as the minimum and maximum values not considered as outliers. An outlier is defined as a value beyond 1.5 x the interquartile range (75th and 25th percentile). Darker gray colors indicate higher median values. Letters indicate significant differences (p-value<0.05): Sample size (n) for >2000 µm = 18, 500-2000 µm = 19, 250-500 µm = 20, 64-250 µm = 16. One-way Analysis of variance (ANOVA) and Tukey HSD post hoc test results are given in Tables VI, and VII.

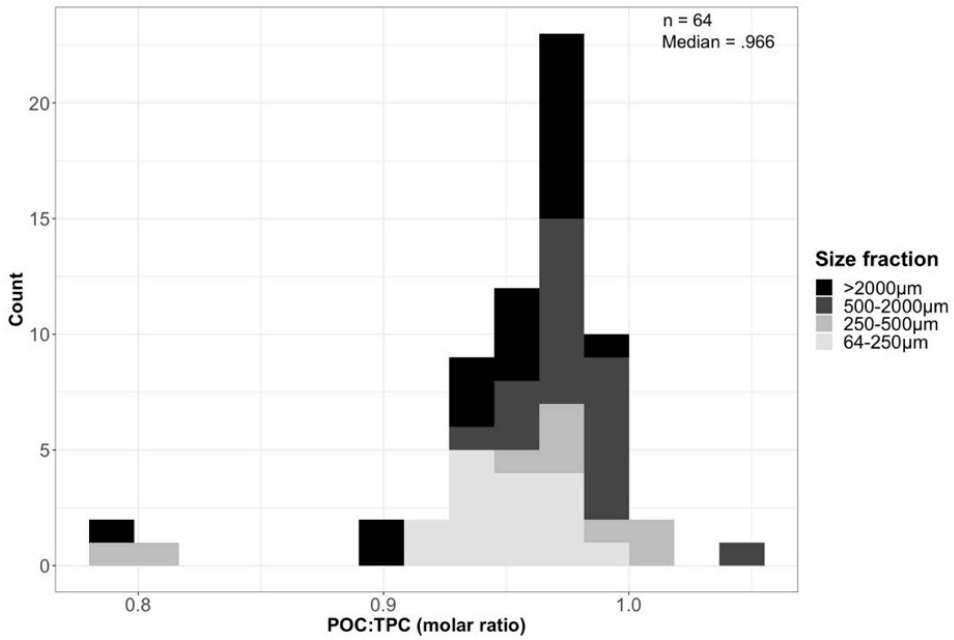

Figure 6. Molar POC:TPC (mol:mol) in four size fractions for 64 samples with a median POC:TPC value of 0.966).

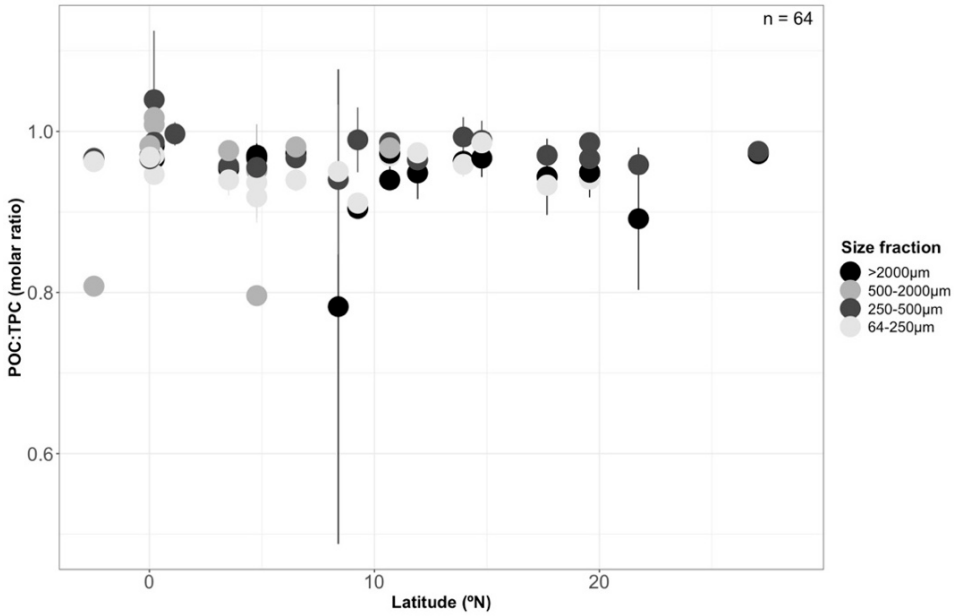

Figure 7. Molar POC:TPC ratio (mol:mol) as a function of latitude (ºN). Vertical lines represent ± 1SD. Sample size (n) = 64.

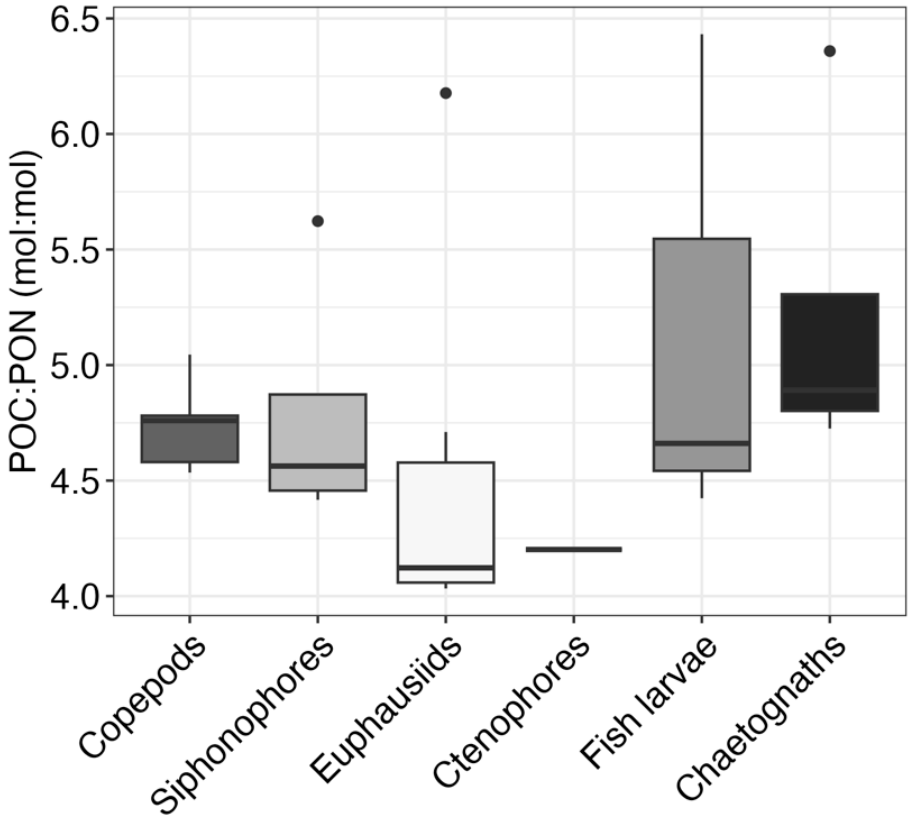

Figure 8. POC: PON ratio (mol:mol) of individually selected zooplankton groups. Boxes show data between 25th and 75th percentiles, with the median represented as a line. The whiskers extend as far as the minimum and maximum values not considered as outliers. An outlier is defined as a value beyond 1.5 x the interquartile range (75th and 25th percentile). Darker gray colors indicate higher median values. Sample size (n) for Copepods = 5, Siphonophores = 5, Euphausiids = 6, Ctenophores = 1, Fish larvae = 3, Chaetognaths = 4. No significant differences in POC:PON ratios following One-way Analysis of variance (ANOVA) test ($p<0.05$).

3.3 Zooplankton C:N:P Stoichiometry In Size Fractions - Literature Survey

The field data on zooplankton size fractions including all three measurements of molar C:N:P ratios were variable among size fractions in the eight selected published studies (Table V). The average C:N in all studies was 5.31 ± 1.11 (mean ± 1SD, n = 19), with a range of 4.4 to 8.5. There was no clear pattern of increase or decrease in C:N with size. The average C:P and N:P in all studies was 122.58 ± 35.12 and 23.56 ± 6.63, respectively (mean ± 1SD, n = 19). The C:P ranged from 75.52 to 174.4 and the N:P ranged from 16 to 34.2. There were patterns of decreasing C:P and N:P with increasing size for three regional studies: one in the North Pacific Ocean at station ALOHA, and one in the Costa Rica Dome in the Northeast Pacific Ocean, and one in New Caledonia in the Tropical Pacific. One study showed the opposite pattern, with increasing C:P and N:P with size in the Southeast Indian Ocean. The C:N:P ratios of zooplankton size fractions in our study fell within the ranges observed in these studies (Average C:N = 4.88 ± 0.44, C:P =130.65 ± 32.69, N:P = 27.93 ± 6.20) reported in the previous section (Table VI).

3.4 Zooplankton C, N, P Content % Dry Weight In Size Fractions & Species

The C, N, and P content as a percent (%) of dry weight (g:g) were variable among the size fractions (Figure 9). C % dry weight in the >2000 μm size fraction was significantly lower than the 500-2000 μm and 250-500 μm fraction (post-hoc Tukey HSD test, p-value<0.05, Table III, IV). The average C % dry weight was 32.49 ± 6.33 (mean ± 1SD, weighted by all four size fractions, n = 73). N % dry weight in the >2000 μm size fraction was significantly lower than the 500-2000 μm and 250-500 fraction, and the N % dry weight in the 64-250 μm fraction was significantly lower than the 500-2000 μm fraction. The average N % dry weight was 7.96 ± 2.04 (mean ± 1SD, weighted by all four size fractions, n = 73). P % dry weight in the >2000 μm size fraction was significantly lower than the 64-250 μm fraction. The average P % dry weight was 0.68 ± 0.18 (mean ± 1SD, weighted by all four size fractions, n = 73). The overall median zooplankton C, N, P content as % dry weight from the region was 26 ± 5.43 % for C, 8 ± 1.91 % for N, and 0.69 ± 0.14 % for P (weighted using biomass contributions of stations including all four

size fractions, n = 15). The median C % dry weight was 0.04 ± 0.29 % lower in the > 2000 µm size fraction compared to the overall median C % dry weight, N % dry weight was 24.1 ± 0.34 % lower in the >2000 µm size fraction compared to the overall median N % dry weight, and P % dry weight was 26.6 ± 0.37 % lower in the >2000 µm size fraction compared to the overall median P % dry weight.

The P % dry weight was variable across the individually selected zooplankton species (Figure 10, Table VII). The P % dry weight in the euphausiids and fish larvae species was significantly higher than in the ctenophores, siphonophores, chaetognaths, and copepods (post-hoc Tukey HSD test, p-value<0.05). Average P % dry weight in ctenophores was 0.20 % ± 0.03 (mean ± 1SD, n = 1), in siphonophores it was 0.35 ± % 0.12 (mean ± 1SD, n = 4), in chaetognaths it was 0.64 ± 0.12 % (mean ± 1SD, n = 5), in copepods it was 0.63 ± 0.13 (mean ± 1SD, n = 5), in euphausiids it was 1.24 ± 0.22 % (mean ± 1SD, n = 6), and in fish larvae it was 1.32 ± 0.08 % (mean ± 1SD, n = 3). The overall average P % dry weight from the region for the copepods, siphonophores, euphausiids, ctenophores, fish larvae, and chaetognaths species was 0.80 ± 0.40 (mean ± 1SD, n = 24).

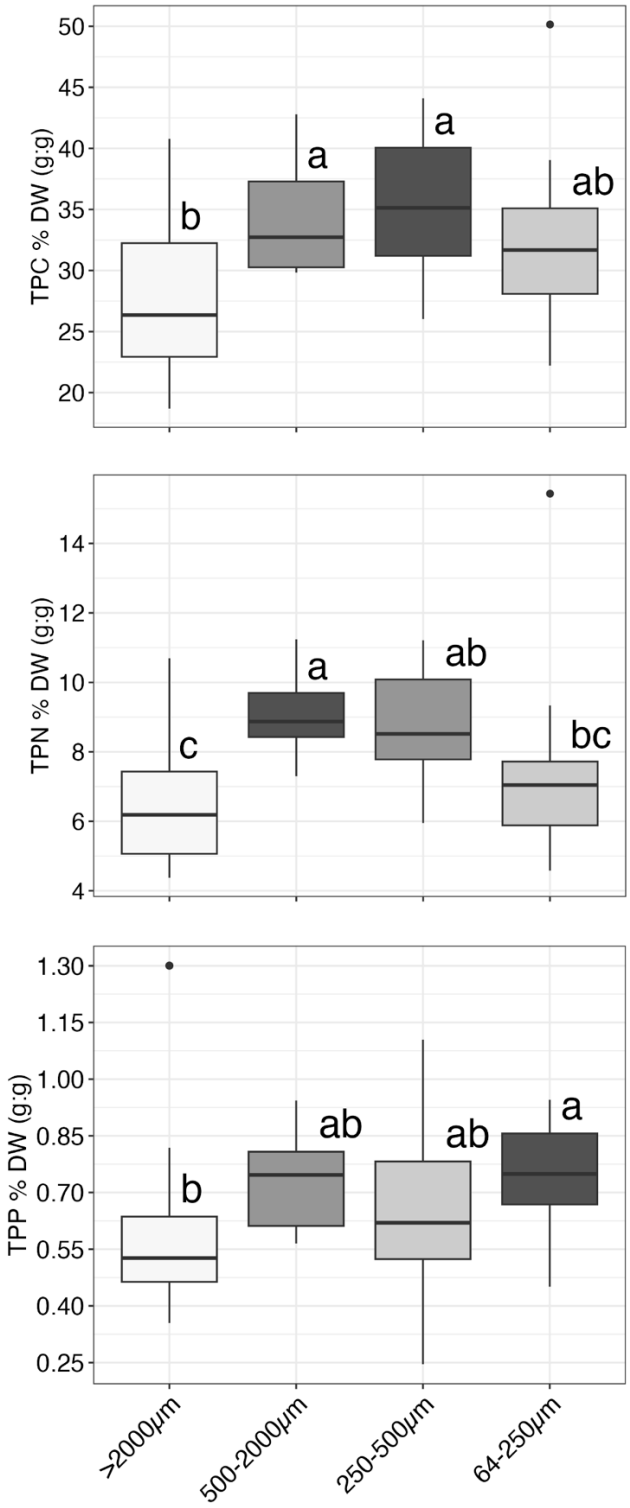

Figure 9. Zooplankton C as percent (%) dry weight (*top panel*), N % dry weight (*middle panel*), P % dry weight (*bottom panel*) in four size fractions (g:g). Boxes show data between 25th and 75th percentiles, with the median represented as a line. The whiskers extend as far as the minimum and maximum values not considered as outliers. An outlier is defined as a value beyond 1.5 x the interquartile range (75th and 25th percentile) Darker gray colors indicates higher median values. Letters indicate significant differences ($p<0.05$). Sample size (n) for >2000 μm = 18, 500-2000 μm = 19, 250-500 μm = 20, 64-250 μm = 16. Sample size means individual samples that included many organisms. One-way Analysis of variance (ANOVA) and Tukey HSD post hoc test results are given in Tables III, and IV.

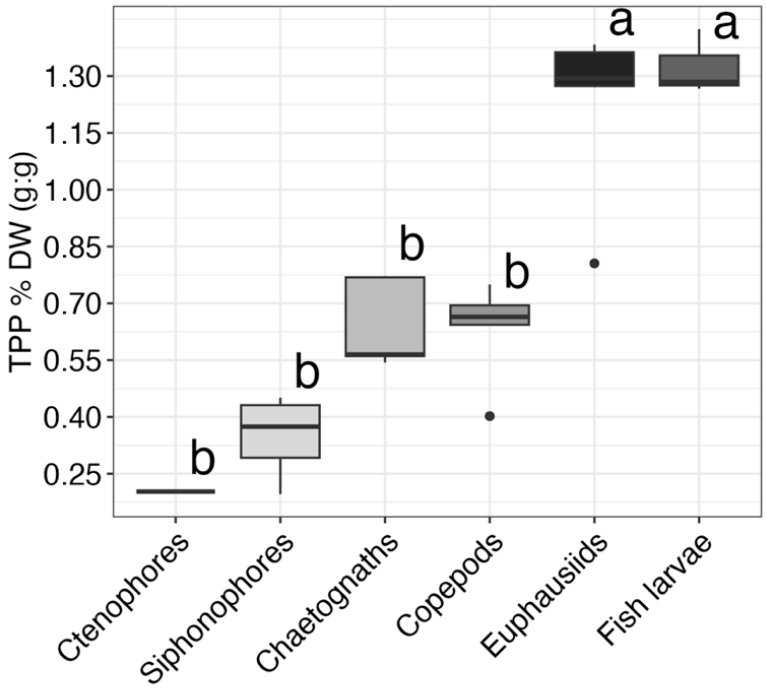

Figure 10. P as a percent (%) dry weight in individually selected zooplankton groups (g:g). Boxes show data between 25th and 75th percentiles, with the median represented as a line. The whiskers extend as far as the minimum and maximum values not considered as outliers. An outlier is defined as a value beyond 1.5 x the interquartile range (75th and 25th percentile). Darker gray colors indicate higher median values. Sample size (n) for Copepods = 5, Siphonophores = 5, Euphausiids = 6, Ctenophores = 1, Fish larvae = 3, Chaetognaths = 4. Letters indicate significant differences following one-way Analysis of variance (ANOVA) and Tukey HSD post hoc test (p-value = 0.05)

Table III. One-way Analysis of variance (ANOVA) results for significant differences in zooplankton molar C:N:P ratios (mol:mol) and C, N, and P % dry weight (g:g) in four size fractions. Sample size is indicated as (n) for each ANOVA model. Significant p-values are indicated with an asterisk (*). P-values less than 0.05 are flagged as *, 0.01 are flagged as **, less than 0.0001 are flagged as ***.

One-way ANOVA results

Source	**n**	**Df**	**Sum sq.**	**Mean sq.**	**f-Statistic**	**p-value**
TPC:TPN (mol:mol) 4 size fractions	73	3	2.65	0.89	5.32	**
Residuals	73	69	11.5	0.16		
TPC:TPP (mol:mol) 4 size fractions	73	3	23,662	7887	10.2	***
Residuals	73	69	53,323	773		
TPN:TPP (mol:mol) 4 size fractions	73	3	1057	352	14.2	***
Residuals	73	69	1712	25		
TPC % DW (g:g) 4 size fractions	73	3	607	202	6.11	***
Residuals	73	69	2284	33.1		
TPN % DW (g:g) 4 size fractions	73	3	75.2	25	7.65	***
Residuals	73	69	226.2	3.28		
TPP % DW (g:g) 4 size fractions	73	3	0.30	0.10	3.19	*
Residuals	73	69	2.15	0.03		

Table IV. Tukey-HSD post hoc test results for significant pairwise comparisons of zooplankton molar C:N:P ratios (mol:mol) and C, N, and P percent dry weight (g:g) in four size fractions. Sample size (n) for >2000 µm = 18, 500-2000 µm = 19, 250-500 µm = 20, 64-250 µm = 16. Significant p-values are indicated with an asterisk (*). P-values less than 0.05 are flagged as *, 0.01 are flagged as **, less than 0.0001 are flagged as ***.

Group Pair	Mean diff.	95% CI (lower, upper)	Adjusted p-value
TPC:TPN (mol:mol)			
>2000 µm vs. 500-2000 µm	0.30	(-0.05, 0.65)	0.12
250-500 µm vs. 500-2000 µm	**0.37**	**(0.03, 0.71)**	*
64-250 µm vs. 500-2000 µm	**0.52**	**(0.16, 0.89)**	**
250-500 µm vs. >2000 µm	0.07	(-0.27, 0.42)	0.95
250-64 µm vs. >2000 µm	0.22	(-0.14, 0.59)	0.38
250-64 µm vs. 250-500 µm	0.15	(-0.20, 0.51)	0.68
TPC:TPP (mol:mol)			
>2000 µm vs 64-250 µm	16.4	(-8.7 ,41.5)	0.32
500-2000 µm vs. 64-250 µm	21.3	(-3.6 46)	0.12
250-500 µm vs. 64-250 µm	**49.8**	**(25.2, 74.3)**	***
500-2000 µm vs. >2000 µm	4.89	(-19.2, 28.9)	0.95
250-500 µm vs. >2000 µm	**33.4**	**(9.6, 57.2)**	**
250-500 µm vs. 500-2000 µm	**28.5**	**(5.0, 51.9)**	*
TPN:TPP (mol:mol)			
>2000 µm vs 64-250 µm	4.3	(-0.19, 8.81)	0.07
500-2000 µm vs. 64-250 µm	**7**	**(2.56, 11.46)**	***
250-500 µm vs. 64-250 µm	**10.5**	**(6.15, 14.94)**	***
500-2000 µm vs. >2000 µm	2.7	(-1.61, 7.01)	0.36
250-500 µm vs. >2000 µm	**6.23**	**(1.97, 10.49)**	**
250-500 µm vs. 500-2000 µm	3.53	(-0.66, 7.73)	0.13
TPC % DW (g:g)			
64-250 µm vs. >2000 µm	4.77	(-0.43, 9.97)	0.08
500-2000 µm vs. >2000 µm	**6.30**	**(1.32, 11.28)**	**
250-500 µm vs. >2000 µm	**7.53**	**(2.61,12.45)**	***
500-2000 µm vs. 64-250 µm	1.53	(-3.60, 6.67)	0.86
250-500 µm vs. 64-250 µm	2.76	(-2.31, 7.84)	0.48
250-500 µm vs. 500-2000 µm	1.23	(-3.62, 6.0)	0.90
TPN % DW (g:g)			
64-250 µm vs. >2000 µm	0.79	(-0.71, 2.47)	0.58
250-500 µm vs. >2000 µm	**2.14**	**(0.59, 3.68)**	**
500-2000 µm vs. >2000 µm	**2.50**	**(0.93, 4.06)**	***
250-500 µm vs. 64-250 µm	1.34	(-0.25, 2.94)	0.12
500-2000 µm vs. 64-250 µm	**1.70**	**(1.05, 4.05)**	*
500-2000 µm vs. 250-500 µm	0.36	(-1.16, 1.88)	0.92
TPP % DW (g:g)			
250-500 µm vs. >2000 µm	0.07	(-0.08, 0.22)	0.59
500-2000 µm vs. >2000 µm	0.14	(-0.009,0.28)	0.07
64-250 µm vs. >2000 µm	**0.16**	**(0.006, 0.32)**	*
500-2000 µm vs. 250-500 µm	0.07	(-0.084, 0.22)	0.59
64-250 µm vs. 250-500 µm	0.09	(-0.06, 0.25)	0.38
64-250 µm vs. 500-2000 µm	0.02	(-0.13, 0.18)	0.97

Table V. Comparison of molar C:N:P (mol:mol) (Mean ±1 SD) in size fractionated zooplankton (µm) between this study and 8 selected published values.

Size fraction	C:N	n	C:P	n	N:P	n	Location	Climate	References
>2000	4.9(0.5)	19	123.3(19)	19	25.1(3.5)	19	Central Pacific (140°W)	Tropical	**This study**
500-2000	4.6(0.1)	20	130(14)	20	28.3(2.6)	20			
250-500	5.0(0.6)	21	155.5(46)	21	31.3(8.2)	21			
64-250	5.1(0.3)	16	107.4(11)	16	21(2.1)	16			
200-2000	4.4(1.3)	18	75.52(61)	18	17(9.4)	18	East Greenland	Polar Arctic	Alcaraz *et al*., (2010)
2000-5000	4.6	24	130.3	24	28.2	24	Costa Rica Dome	Tropical	Baines *et al*., (2016)
1000-2000	4.8	27	137.9	27	28.5	27	Northeast Pacific		
500-1000	4.8	26	153.9	26	31.9	26			
200-500	4.9	27	161.1	27	32.9	27			
> 5000	4.7	17	77.5	12	15.9	12	Station ALOHA	Subtropical	Hannides *et al*., (2009)
2000-5000	4.6	37	78	26	16.7	26	(NPSG)		
1000-2000	4.6	38	86	38	18.8	38			
500-1000	4.7	38	95.5	38	20.1	38			
200-500	5	38	106.5	37	21.4	37			
35-2000	5.7(0.1)	2	107.4(11)	2	19(2.4)	2	Tikehau, Atoll South Pacific	Tropical	Le Borgne *et al*., (1989)
200-2000	8(1.7)	10	113.3(16)	10	16.9(4)	10	New Caledonia, Atoll	Tropical	Le Borgne *et al,.* (1997)
35-200	8.5(2.3)	8	178(42)	8	21.7(4.4)	8	Coral Sea		
200-2000	6(0.1)	15	117.7(9)	15	19.8(1.6)	15	Equatorial Pacific	Tropical	Le Borgne *et al.,* (2003)
150-2000	5.3(0.8)	8	85.8(43)	8	16(6.4)	8	Southern Ocean	Polar	Plum *et al*., (2021)
750-1600	5.1(0.2)	3	174.4(78)	3	34.2(16.3)	3	KwaZulu - Natal Bight,	Temperate	Scharler *et al*., (2016)
500-750	5.2(0.2)	3	176(90)	3	33.8(17.8)	3	Southeast Indian Ocean		
200-500	5.2(0.3)	3	128.3(25)	3	24.9(6.3)	3			

Table VI. Mean molar C:N:P (mol:mol) and C, N, P % dry weight (g:g), in bulk zooplankton aggregated across all stations. C:N:P ratios were calculated using zooplankton molar concentrations for stations including measurements from all four size fractions as described in Table I. Error is described as (±1 SD). Number of samples used for each measurement is represented as (n).

Station	TPC:TPN:TPP	n	TPC:TPN	TPC:TPP	TPN:TPP	TPC % DW	TPN % DW	TPP % DW	n
SG4(Ni)	116:22.6:1	4	5.5(0.9)	102.9(35)	18.5(4.5)	23.4(4.9)	6.4(1.0)	0.63(0.1)	4
SG5	143.1:28.3:1	4	5.2(0.9)	122.9(35)	23.8(8.1)	26.6(5.3)	7.4 (1.5)	0.56(0.1)	4
SG6	132.9:27.2:1	4	5.1(0.4)	111.1(20)	21.8(3.4)	21.3(4.7)	6.3 (1.5)	0.51(0.1)	4
SG7(Ni)	116.7:24.5:1	4	4.7(0.2)	92.7(15)	19.8(3.9)	24.1(5.1)	7.4(1.5)	0.66(0.1)	4
SG8	144.6:24.3:1	4	5.3(0.8)	121.1(17)	23.1(6.0)	27.1(8.8)	7.5(2.8)	0.63(0.1)	4
SG9	150:33.2:1	4	4.8(0.3)	118.3(19)	24.6(4.7)	28.1(10)	9.4(4.0)	0.54(0.1)	4
SG9(Ni)	128.1:25.4:1	4	5(0.6)	103.7(37)	20.5(5.4)	29.4(3.8)	8.1(2.3)	0.76(0.1)	4
SG11.1(Ni)	86.7:19.7:1	4	4.7(0.4)	85.6(27)	18.4(6.2)	32.2(4.2)	10(1.6)	1.0(0.3)	4
SG11.2	114.6:25.7:1	4	4.6(0.2)	93.4(9)	20.5(2.3)	30.4(4.0)	9.9(1.6)	0.86(0.1)	4
SG13(Ni)	106.7:25:1	4	4.7(0.3)	100.4(29)	21.5(5.5)	26.3(1.1)	8.5(1.2)	0.72(0.1)	4
SG14	130.3:27.6:1	4	4.8(0.3)	107.3(37)	22.9(9.2)	25.7(2.1)	8.1(0.7)	0.69(0.2)	4
SG15	116.4:25.5:1	4	4.7(0.2)	92.3(15)	19.6(3.6)	26.8(4.9)	8.6(2.0)	0.78(0.1)	4
SG16	112.6:24.4:1	4	4.8(0.3)	91.8(19)	19(3.9)	25.4(9.4)	8(2.6)	0.77(0.3)	4
SG17	112:23.5:1	4	4.8(0.1)	90.8(12)	19.1(3.1)	23.3(7.3)	7.4(2.1)	0.69(0.2)	4
SG18	105:24.4:1	4	4.6 (0.1)	99.1(13)	21.4(2.9)	25.1(8.6)	8.2(2.2)	0.71(0.2)	4

Table VII. P % dry weight, molar POC:PON, and C:N ratio (mol:mol) measured in individually selected zooplankton groups. Individual groups were classified and sorted from net tows before processing size fractions as described in the methods chapter. Samples were grouped together by stations according to latitude (ºN) and nighttime or daytime sampling to have sufficient biomass for CHN analysis and the TPP assay. The euphausiids and ctenophores were the only groups with both TPC and POC measurements. Sample size is indicated by (n) and error in measurements is represented by (±1 SD).

Major Group	Size structure	Clustered stations	Latitude Range	TPP % DW	n	POC:PON	TPC:TPN	n
Copepods	250-2000 µm	S2, S3, S4, S6	27.07 - 10.68	0.74(0.06)	3	4.5(1.0)	--	3
		S7, S9, S15	10.68 - 6.51	0.69(0.07)	3	4.78(0.3)	--	3
		S4(Ni), S7(Ni), S9(Ni)	19.57 - 4.77	0.66(0.01)	2	5.04(0.5)	--	3
		S10, S11.2	1.13 - 0.21	0.40(0.01)	3	4.75(2.0)	--	3
		S13(Ni)	0.02	0.64(0.02)	3	4.58(1.0)	--	3
Siphonophores	>2000 µm	S5, S6	17.68 - 13.95	0.45(0.11)	3	4.87(1.0)	--	3
		S7	10.68	--		4.45(9.1)	--	3
		S8, S9	8.39 - 4.77	0.19(0.20)	2	4.56(6.4)	--	2
		S10	1.13	0.42(0.01)	3	5.62(1.0)	--	3
		S13(Ni)	0.02	0.32(0.02)	3	4.41(0.5)	--	3
Euphausiids	>2000 µm	S2, S3	27.07 - 21.74	0.80(0.11)	3	6.17(1.4)	--	3
		S4(Ni)	19.57	1.28(0.21)	3	4.71(2.4)	--	3
		S7(Ni)	10.68	1.38(0.13)	3	4.06(1.8)	4.18(0.54)	3
		S9(Ni)	4.77	1.27(0.08)	3	4.18(1.0)	4.34(0.75)	3
		S11.1(Ni)	0.21	1.38(0.17)	3	4.03(0.2)	4.00(0.8)	3
		S13(Ni)	0.02	1.30(0.09)	3	4.05(0.4)	4.20(0.12)	3
Ctenophores	>2000 µm	S6	10.68	0.20(0.03)	3	4.20(0.9)	4.35(1.6)	3
Fish Larvae	>2000 µm	S9(Ni)	4.77	1.26(0.06)	3	6.43(9.2)	--	3
		S11.1(Ni)	0.21	1.42(0.06)	3	4.66(16.9)	--	3
		S13(Ni)	0.02	1.28(0.04)	3	4.42(1.2)	--	3
Chaetognaths	>2000 µm	S3	21.74	0.77(0.44)	2	--	--	
		S4, S5	19.57 - 17.68	0.56(0.05)	3	4.95(3.1)	--	2
		S6	13.95	0.56(0.11)	3	4.82(1.3)	--	3
		S7(Ni)	10.70	0.76(0.14)	2	6.35	--	1
		S8, S9	8.39 - 4.77	0.54(0.01)	3	4.72(1.3)	--	3

3.5 Zooplankton C, N, P Concentrations & C:N:P Stoichiometry With Environmental Variables

The overall C concentration (mmol per m^{-3}) in zooplankton biomass increased from 20º N in the North Pacific Subtropical Gyre (NPSG) region towards ~0º in the North Pacific Countercurrent region (Figure 11.). C concentration increased until S7(N) (~10º N latitude), before dropping at S16 and increasing once again until S14 (~3.5º N latitude). The highest daytime total C concentrations was measured at station S9 and S14 around 5º N (C concentration = 0.48 and 0.59 mmol m^{-3}, respectively). The highest nighttime total C concentrations was observed at S13(Ni) and S9(Ni) (C = 0.85 and 0.79 mmol m^{-3}, respectively). Total C concentration was highest in nighttime stations when comparing stations with both nighttime and daytime sampling (i.e. S9 and S9(Ni), S11(Ni) and S11), with larger differences in daytime and nighttime biomass at stations near the equator. The lowest total C concentrations were at S5 and S4(Ni) above 10º N latitude (C = 0.09 and 0.07 mmol C m^{-3}, respectively). Among the zooplankton size fractions, on average the >2000 μm, 500-2000 μm, 250-500 μm, and 64-250 μm size fraction contributed 60.2 %, 27.7 %, 3.1 %, and 9 % of the C concentration measured in zooplankton biomass, respectively. The overall proportion of the larger >2000 μm size fraction increased from 20º N to ~0º latitude (i.e. except for S6 and S17 above 10º N) even with nighttime stations removed.

None of the C, N, P concentrations (mmol m^{-3}) in zooplankton had significant correlations as a function of temperature, nitrate concentration, chlorophyll-*a* concentration, except with latitude (ºN), and phytoplankton biomass (i.e. C, N, P concentrations mmol m^{-3}, Figures 12-16, Table VIII). The C and N concentration in the 500-2000 μm size fraction was significantly correlated with latitude (C concentration slope = -2.30×10^{-3}, $R^2 = 0.41$, p-value<0.05; and N concentration slope = -5.60×10^{-4}, $R^2 = 0.37$, p-value<0.05).

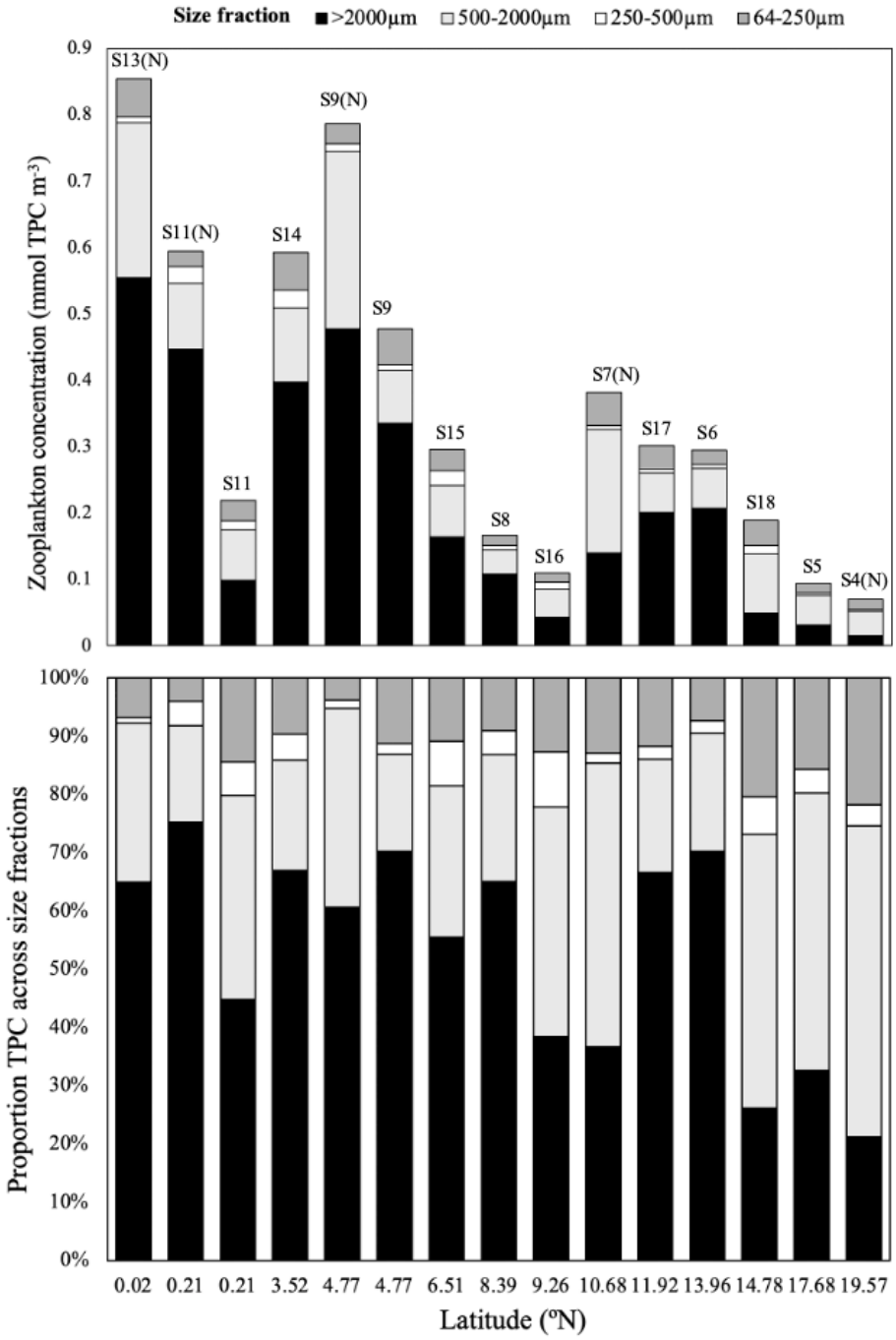

Figure 11. Zooplankton C concentration in four size fractions in each station as a function of latitude for daytime and nighttime stations (*top*), and the proportion of each size fraction in zooplankton C concentration for each respective station (*bottom*). Samples collected at night are noted with (Ni). The x-axis is categorical and does not represent actual latitude distances between stations, only the latitude where the station sampling occurred. Stations are only included that contained C concentration for all four size fractions.

C, N, and P concentration in the 500-2000 μm size fraction was significantly correlated with phytoplankton C, N, and P concentration (p-value<0.05, Figure 16, Table VII). The C concentration in the 64-250 μm size fraction was significantly correlated with phytoplankton C concentration (Slope = 10.6, R^2 = 0.47, p-value=<0.05).

We did not observe significant correlations in zooplankton molar C:N:P ratios with the environmental variables latitude, temperature, nitrate concentration, or phytoplankton biomass (chlorophyll-*a* and C concentration), except for the C:N in the >2000 μm size fraction (Slope = 0.35, R^2 = 0.59, p<0.05) and 500-2000 μm size fraction (Slope = 0.11, R^2 = 0.58, p = 0.05) with the molar C:N in phytoplankton (Figure 17-22, Table IX).

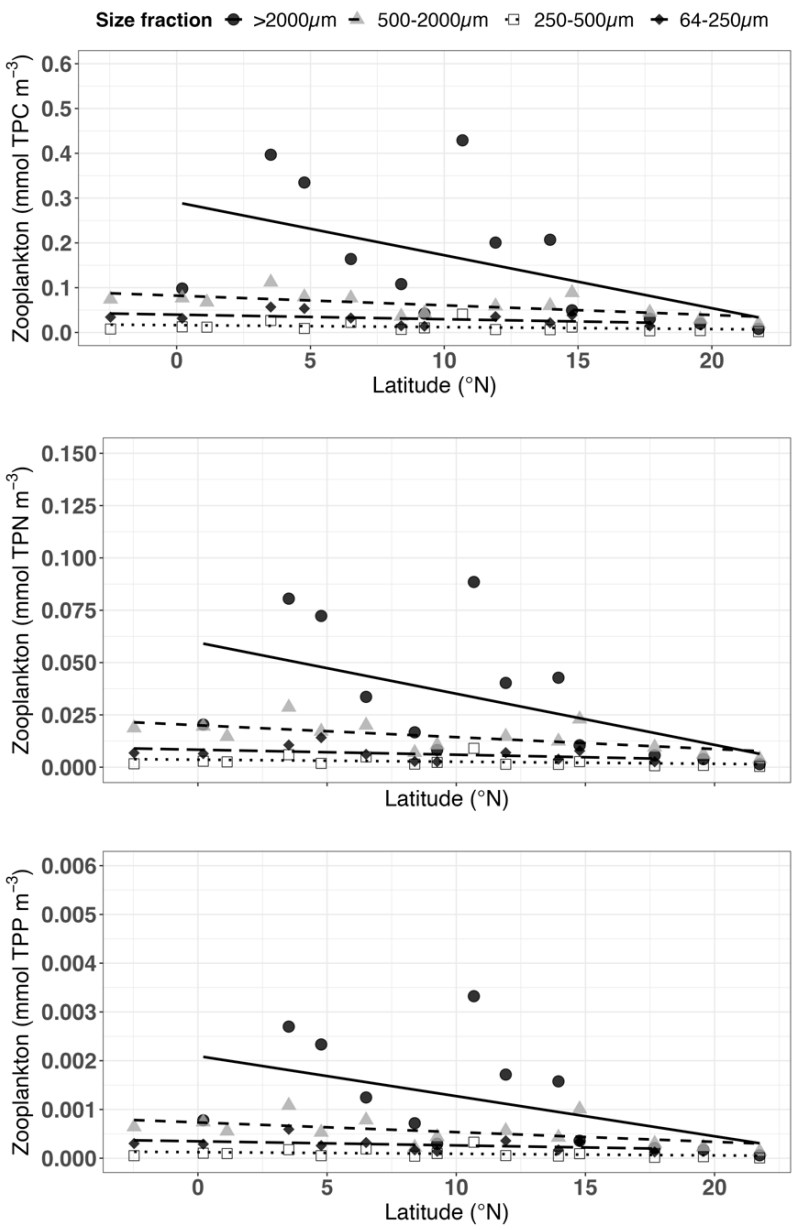

Figure 12. Zooplankton C, N, and P concentration (mmol m^{-3}) as a function of latitude (ºN). Shapes represent size fractions: black dot = >2000 μm, gray triangle = 500-2000 μm, white square = 250-500 μm, black diamond = 64-250 μm. Each size fraction was fitted with a linear regression model: solid line = >2000 μm, dashed line = 500-2000 μm, dotted line = 250-500 μm, long dashed line = 64-250 μm. Linear regression model coefficients are given in Table VIII.

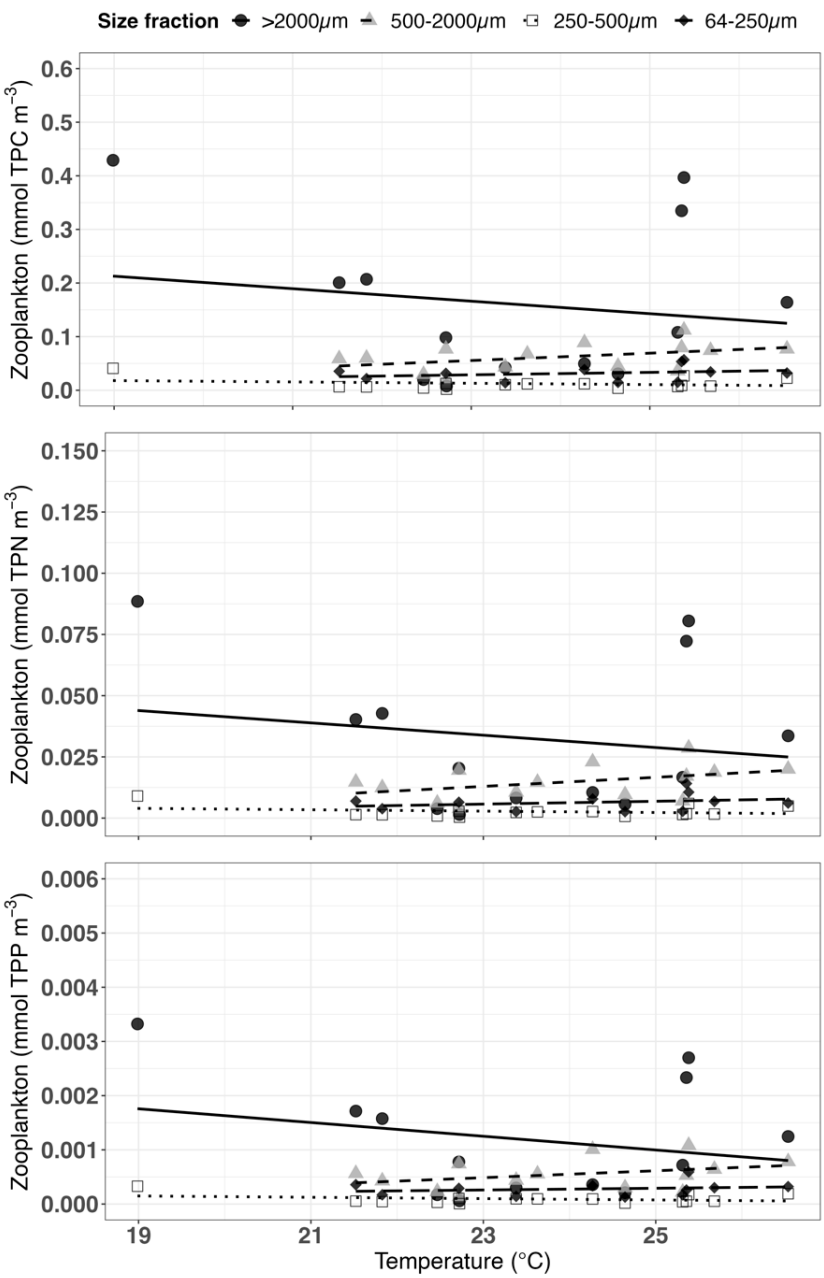

Figure 13. Zooplankton C, N, and P concentration (mmol m^{-3}) as a function of 100 m depth integrated temperature (ºC). Size fractions and linear regression models are described as in Figure 13. Linear regression model coefficients are given in Table VIII.

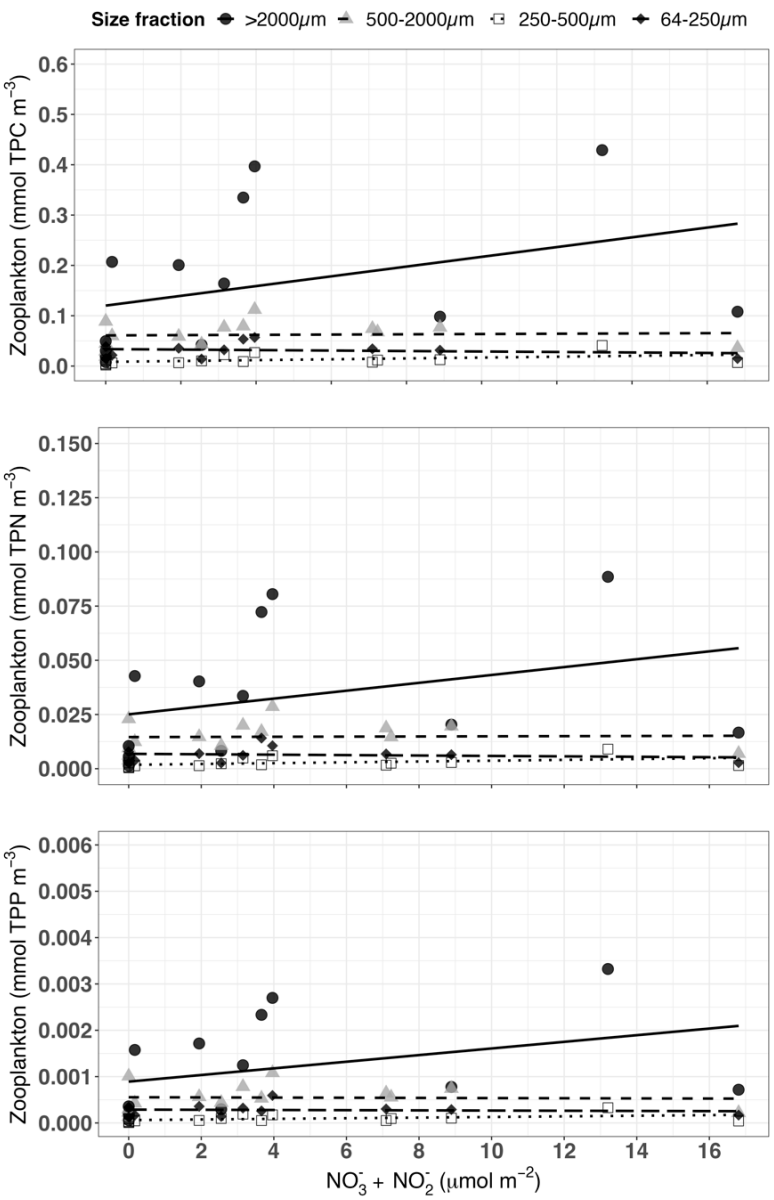

Figure 14. Zooplankton C, N, and P concentration (mmol m^{-3}) as a function of 100 m depth integrated nitrate and nitrite concentration (μmol m^{-2}). Size fractions and linear regression models are described as in Figure 13. Linear regression model coefficients are given in Table VIII.

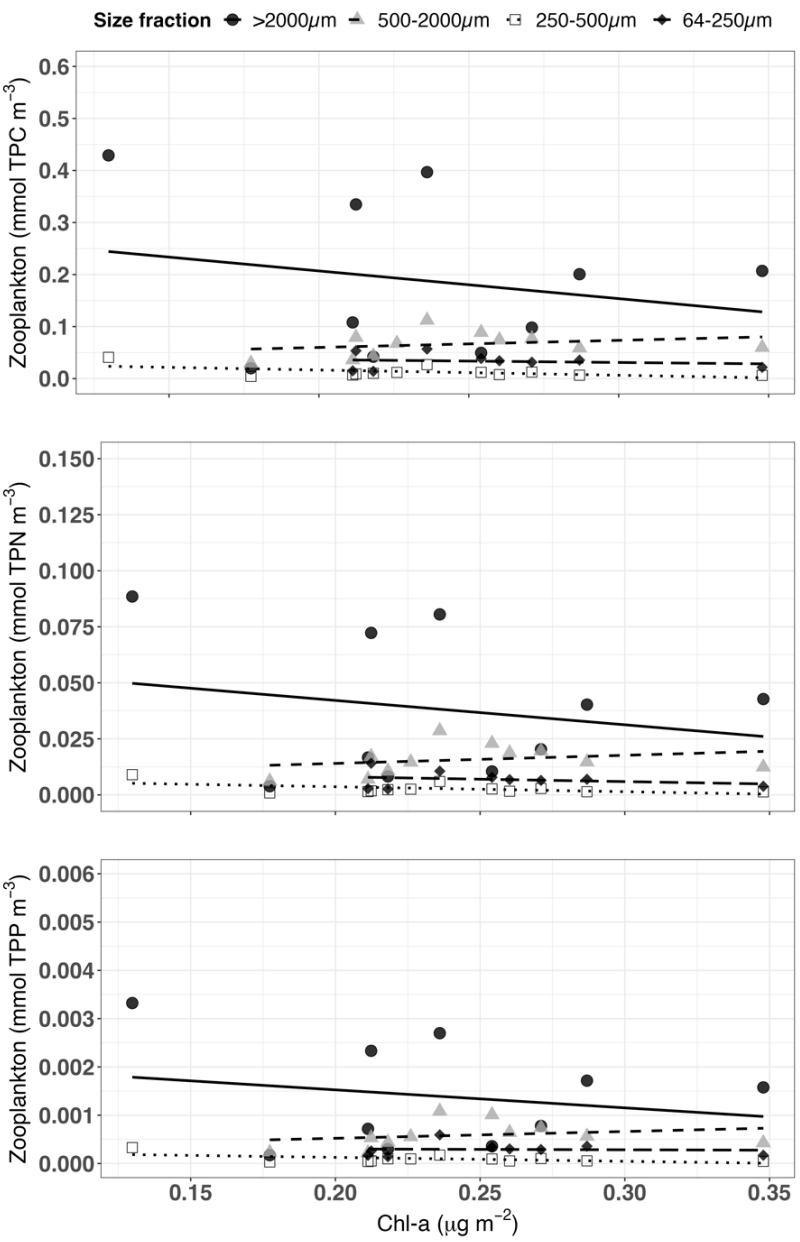

Figure 15. Zooplankton C, N, and P concentration (mmol m^{-3}) as a function of 100 m depth integrated Chlorophyll-*a* concentration (μg m^{-2}). Size fractions and linear regression models are described as in Figure 13. Linear regression model coefficients are given in Table VIII.

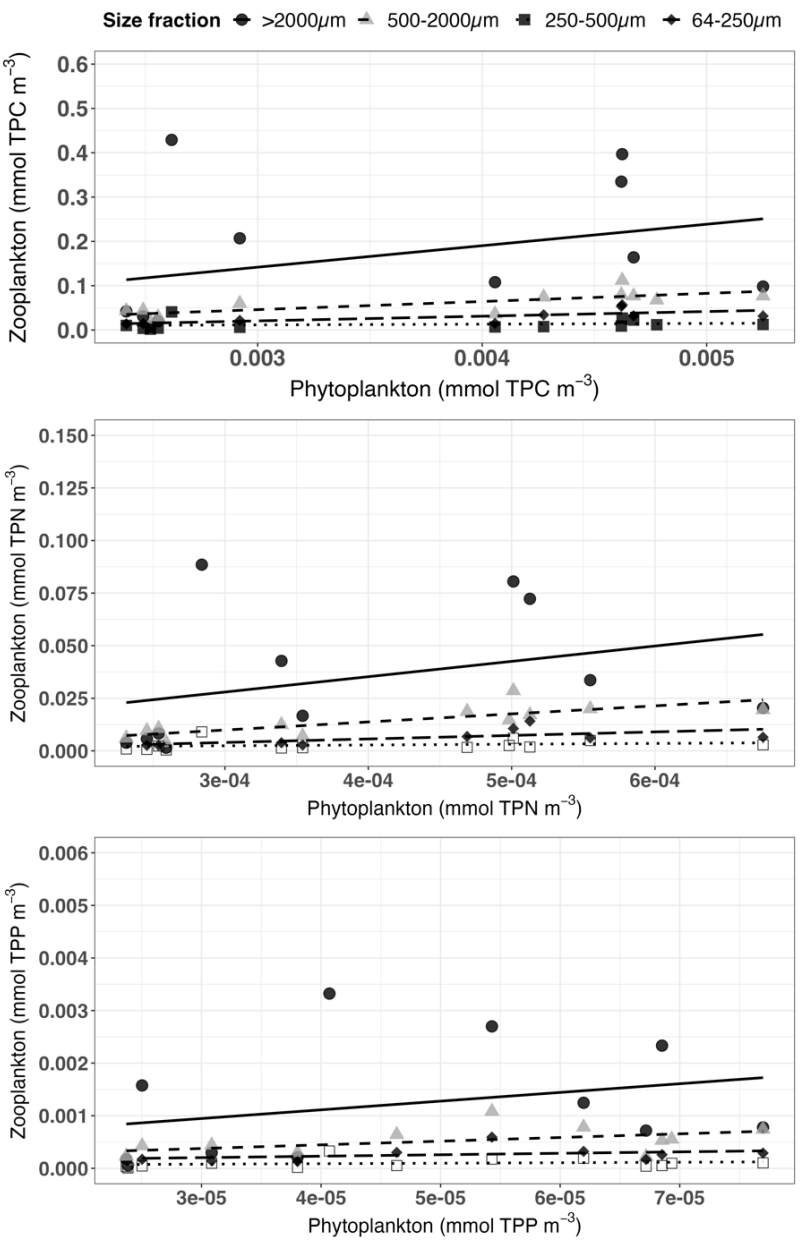

Figure 16. Zooplankton C, N, and P concentration (mmol m^{-3}) as a function of phytoplankton C, N, and P concentration (mmol m^{-3}). Size fractions and linear regression models are described as in Figure 13. Linear regression model coefficients are given in Table VIII.

Responses	Predictor variables	n	Slope	Std. error	t-statistic	p-value	R^2
	Latitude (ºN)	18	-0.01	<0.01	<0.01	0.06	0.27
Zoo TPC	Temperature (ºC)	18	-0.01	0.02	<0.01	0.59	0.02
(mmol m^{-3})	NO_3- + NO_2- (µmol m^{-2})	18	0.01	<0.01	1.30	0.22	0.13
>2000 µm	Chl-*a* (µg m^{-2})	15	-0.53	0.86	<0.01	0.55	0.04
	Phyto TPC (mmol m^{-3})	15	48.4	43.5	1.11	0.30	0.12
	Latitude (ºN)	**19**	**-2.3e-3**	**<0.01**	**<0.01**	*	**0.41**
Zoo TPC	Temperature (ºC)	19	0.006	<0.01	1.62	0.13	0.18
(mmol m^{-3})	NO_3- + NO_2- (µmol m^{-2})	19	2.7e-4	<0.01	0.18	0.86	<0.01
500-2000 µm	Chl-*a* (µg m^{-2})	16	0.14	0.16	0.80	0.44	0.06
	Phyto TPC (mmol m^{-3})	**16**	**18.4**	**4.97**	**3.69**	**	**0.58**
	Latitude (ºN)	20	-4.3e-4	<0.01	<0.01	0.27	0.09
Zoo TPC	Temperature (ºC)	20	-1.2e-3	<0.01	<0.01	0.39	0.05
(mmol m^{-3})	NO_3- + NO_2- (µmol m^{-2})	20	8.5e-4	<0.01	1.68	0.11	0.18
250-500 µm	Chl-*a* (µg m^{-2})	17	-0.10	0.05	<0.01	0.08	0.28
	Phyto TPC (mmol m^{-3})	17	1.66	3.02	0.55	0.59	0.03
	Latitude (ºN)	16	-1.0e-3	<0.01	<0.01	0.18	0.18
Zoo TPC	Temperature (ºC)	16	2.2e-3	<0.01	0.80	0.44	0.06
(mmol m^{-3})	NO_3- + NO_2- (µmol m^{-2})	16	4.7e-4	<0.01	<0.01	0.63	0.03
64-250 µm	Chl-*a* (µg m^{-2})	14	-0.05	0.13	<0.01	0.68	0.02
	Phyto TPC (mmol m^{-3})	**13**	**10.6**	**4.24**	**2.49**	*	**0.47**
	Latitude (ºN)	18	-2.4e-3	<0.01	<0.01	0.07	0.26
Zoo TPN	Temperature (ºC)	18	-2.5e-3	<0.01	<0.01	0.58	0.03
(mmol m^{-3})	NO_3- + NO_2- (µmol m^{-2})	18	1.8e-3	<0.01	1.14	0.27	0.10
>2000 µm	Chl-*a* (µg m^{-2})	15	-0.10	0.18	<0.01	0.57	0.04
	Phyto TPN (mmol m^{-3})	15	72.9	66.9	1.09	0.30	0.11
	Latitude (ºN)	**19**	**-5.6e-4**	**<0.01**	**<0.01**	*	**0.37**
Zoo TPN	Temperature (ºC)	19	1.8e-3	<0.01	1.59	0.13	0.17
(mmol m^{-3})	NO_3- + NO_2- (µmol m^{-2})	19	3.6e-4	<0.01	0.08	0.93	<0.01
500-2000 µm	Chl-*a* (µg m^{-2})	16	0.03	0.04	0.77	0.46	0.06
	Phyto TPN (mmol m^{-3})	**16**	**38.2**	**9.5**	**4.0**	**	**0.61**
	Latitude (ºN)	20	-9.8e-4	<0.01	<0.01	0.26	0.09
Zoo TPN	Temperature (ºC)	20	-2.8e-4	<0.01	<0.01	0.38	0.05
(mmol m^{-3})	NO_3- + NO_2- (µmol m^{-2})	20	1.8e-4	<0.01	1.68	0.11	0.18
250-500 µm	Chl-*a* (µg m^{-2})	17	-0.02	0.01	<0.01	0.08	0.27
	Phyto TPN (mmol m^{-3})	17	3.74	5.04	0.74	0.47	0.05
	Latitude (ºN)	16	-2.4e-4	<0.01	<0.01	0.19	0.17
Zoo TPN	Temperature (ºC)	16	-5.7e-4	<0.01	<0.83	0.42	0.07
(mmol m^{-3})	NO_3- + NO_2- (µmol m^{-2})	16	-9.6e-5	<0.01	<0.01	0.69	0.02
64-250 µm	Chl-*a* (µg m^{-2})	14	-0.02	0.03	<0.01	0.51	0.06
	Phyto TPN (mmol m^{-3})	13	16.8	8.2	2.05	0.08	0.37
	Latitude (ºN)	18	-8.2e-4	<0.01	<0.01	0.08	0.24
Zoo TPP	Temperature (ºC)	18	-1.2e-4	<0.01	<0.01	0.42	0.06
(mmol m^{-3})	NO_3- + NO_2- (µmol m^{-2})	18	7.1e-4	<0.01	1.30	0.21	0.13
>2000 µm	Chl-*a* (µg m^{-2})	15	-3.7e-3	<0.01	<0.01	0.57	0.04
	Phyto TPP (mmol m^{-3})	15	16.5	18.0	0.91	0.38	0.08
	Latitude (ºN)	19	-1.9e-5	<0.01	<0.01	0.053	0.27
Zoo TPP	Temperature (ºC)	19	6.3e-5	<0.01	1.30	0.22	0.12
(mmol m^{-3})	NO_3- + NO_2- (µmol m^{-2})	19	-1.7e-5	<0.01	<0.01	0.92	<0.01
500-2000 µm	Chl-*a* (µg m^{-2})	16	1.4e-3	<0.01	0.72	0.48	0.05
	Phyto TPP (mmol m^{-3})	**16**	**16.6**	**6.54**	**2.53**	*	**0.31**
	Latitude (ºN)	20	-3.3e-6	<0.01	<0.01	0.29	0.08
Zoo TPP	Temperature (ºC)	20	-1.2e-5	<0.01	<0.01	0.29	0.08
(mmol m^{-3})	NO_3- + NO_2- (µmol m^{-2})	20	6.7e-6	<0.01	1.65	0.12	0.17
250-500 µm	Chl-*a* (µg m^{-2})	17	-8.1e-4	<0.01	<0.01	0.07	0.29
	Phyto TPP (mmol m^{-3})	17	6.97	3.74	1.86	0.09	0.25

Responses	Predictor variables	n	Slope	Std. error	t-statistic	p-value	R^2
	Latitude (ºN)	16	-8.4e-6	<0.01	<0.01	0.22	0.16
Zoo TPP	Temperature (ºC)	16	1.6e-6	<0.01	0.61	0.56	0.04
(mmol m^{-3})	NO_3- + NO_2- (µmol m^{-2})	16	-2.0e-6	<0.01	<0.01	0.81	<0.01
64-250 µm	Chl-*a* (µg m^{-2})	14	-1.6e-4	<0.01	<0.01	0.89	<0.01
	Phyto TPP (mmol m^{-3})	13	2.74	2.80	0.98	0.36	0.12

Table VIII. Linear regression model coefficients for zooplankton (zoo) C, N, and P concentration (mmol m^{-3}) in size fractions as a function of latitude (ºN), temperature (ºC), nitrate and nitrite concentration (µmol m^{-2}), chlorophyll-*a* concentration (µg m^{-2}), and phytoplankton (phyto) C, N, and P concentration (mmol m^{-3}). Temperature, nitrate and nitrite concentration, and chlorophyll-*a* concentration is depth integrated to 100 m. Sample size is indicated as (n) for each linear regression model. Significant p-values are indicated with an asterisk (*). P-values less than 0.05 are flagged as *, 0.01 are flagged as **, less than 0.0001 are flagged as ***.

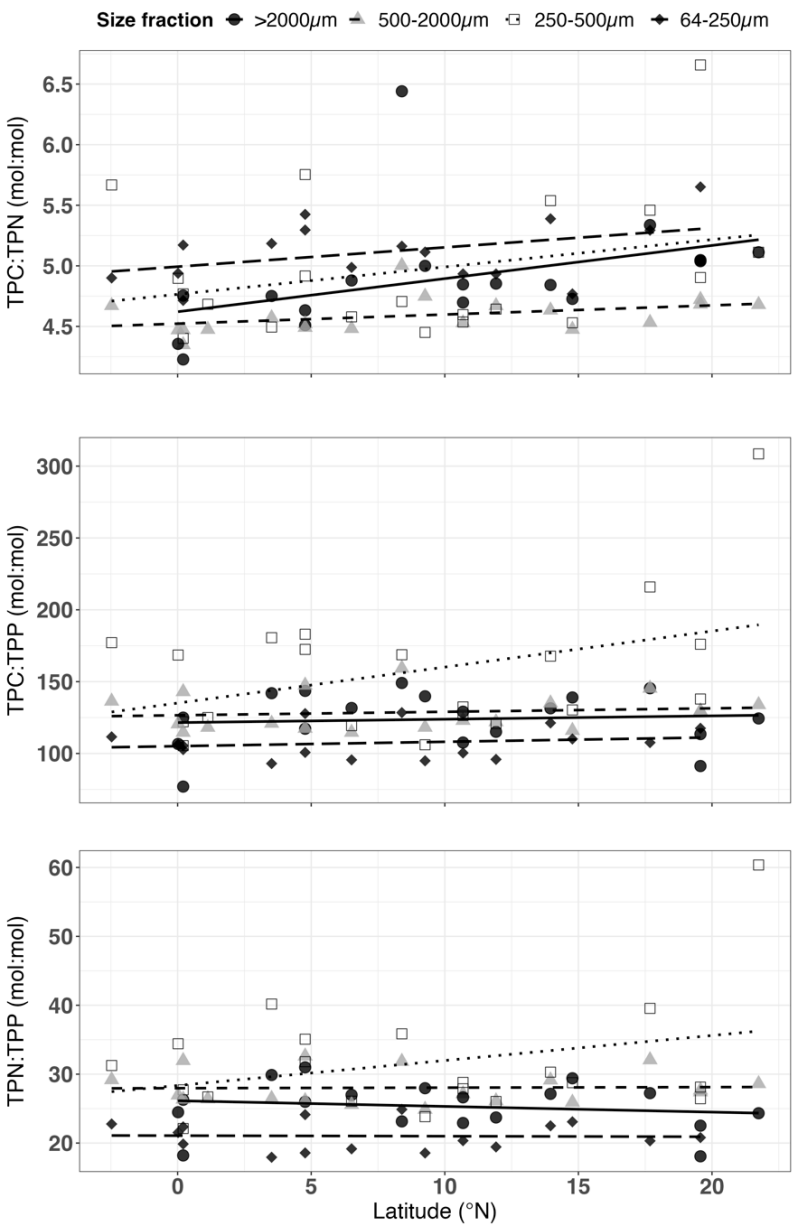

Figure 17. Zooplankton molar C:N, C:P, and N:P ratios (mol:mol) as a function of latitude (ºN). Shapes represent size fractions: black dot = >2000 µm, gray triangle = 500-2000 µm, white square = 250-500 µm, black diamond = 64-250 µm. Each size fraction was fitted with a linear regression model: solid line = >2000 µm, dashed line = 500-2000 µm, dotted line = 250-500 µm, long dashed line = 64-250 µm. Linear regression model coefficients are given in Table IX.

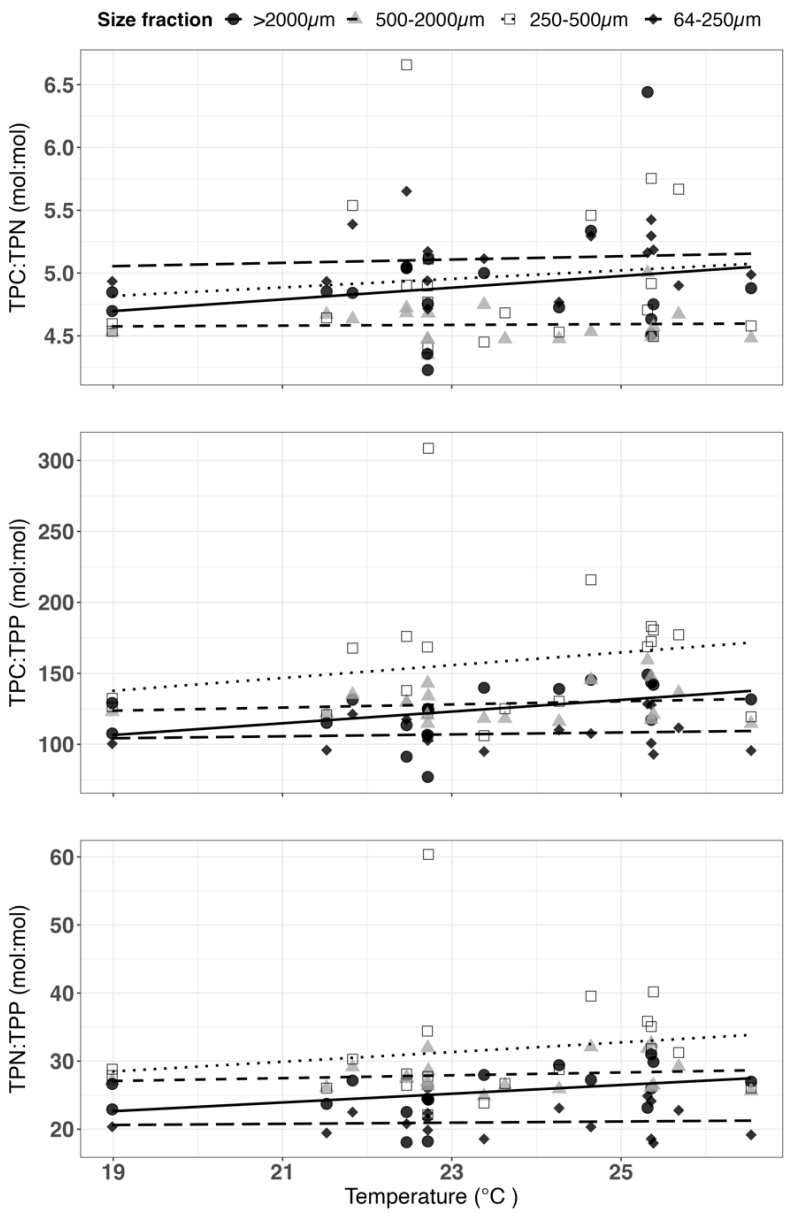

Figure 18. Zooplankton molar C:N, C:P, and N:P ratios (mol:mol) as a function of 100 m depth integrated temperature (ºC). Size fractions and linear regression models are described in Figure 18. Linear regression model coefficients are given in Table IX.

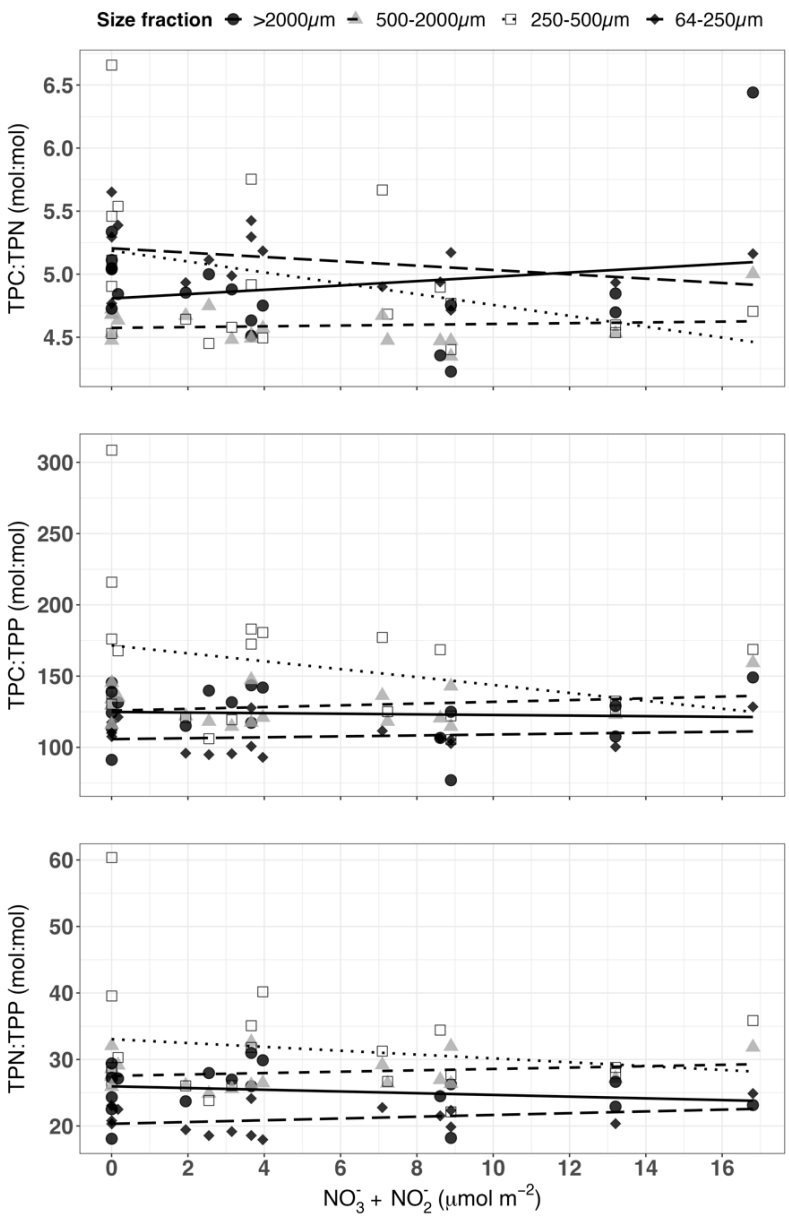

Figure 19. Zooplankton molar C:N, C:P, and N:P ratios (mol:mol) as a function of 100 m depth integrated nitrate and nitrite concentration (μmol m^{-2}). Size fractions and linear regression models are described in Figure 18. Linear regression model coefficients are given in Table IX.

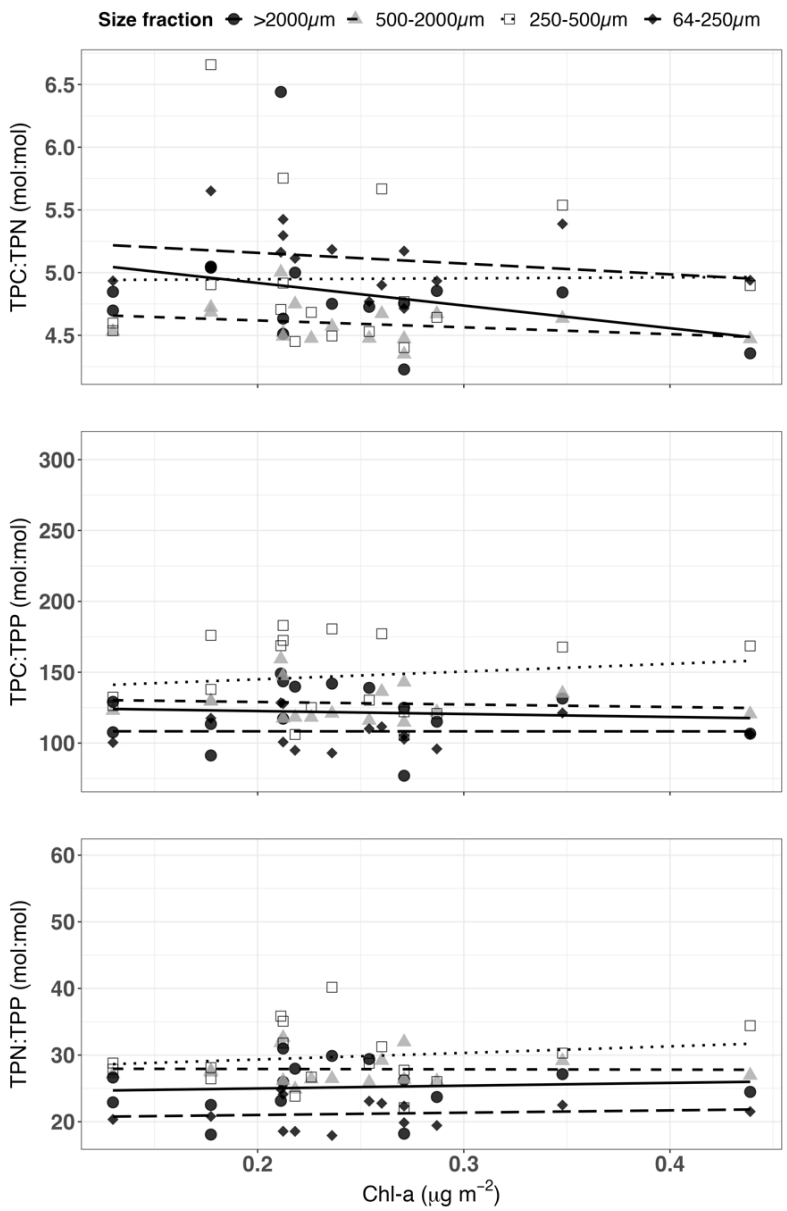

Figure 20. Zooplankton molar C:N, C:P, and N:P ratios (mol:mol) as a function of 100 m depth integrated chlorophyll-*a* concentration (μg m^{-2}). Size fractions and linear regression models are described in Figure 18. Linear regression model coefficients are given in Table IX.

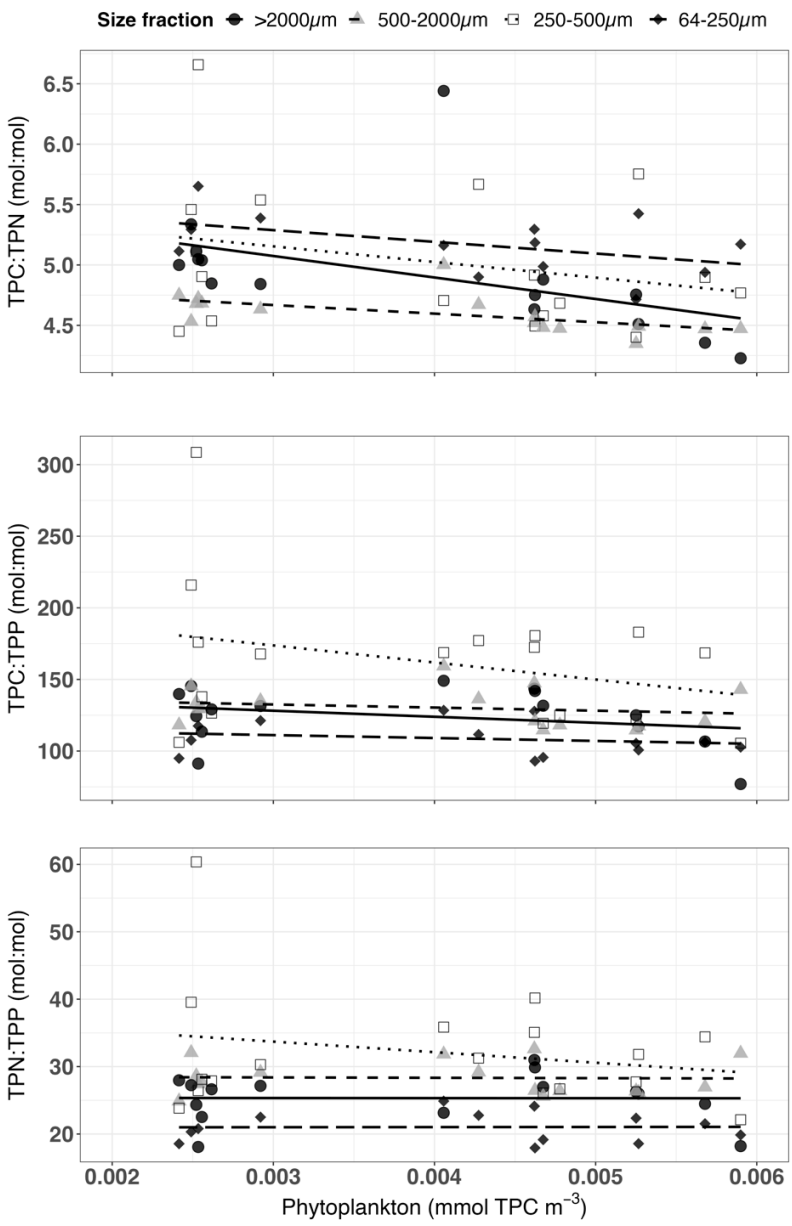

Figure 21. Zooplankton molar C:N, C:P, and N:P ratios (mol:mol) as a function of phytoplankton C concentration (mmol m^{-3}). Size fractions and linear regression models are described in Figure 18. Linear regression model coefficients are given in Table IX.

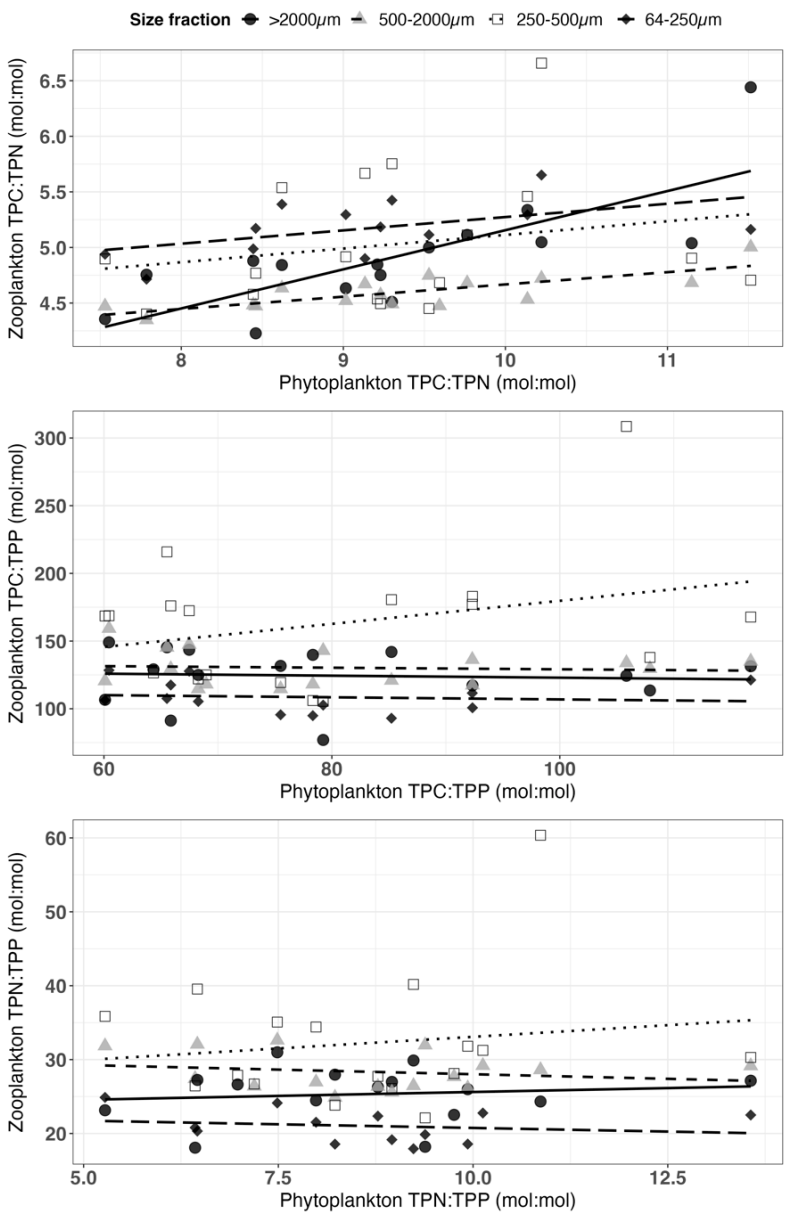

Figure 22. Zooplankton molar C:N, C:P, and N:P ratios (mol:mol) as a function of phytoplankton C:N, C:P, and N:P ratios (mol:mol). Size fractions and linear regression models are described in Figure 18. Linear regression model coefficients are given in Table IX.

Responses	Predictor variables	n	Slope	Std. error	t-statistic	p-value	R^2
	Latitude (ºN)	18	0.03	0.02	1.77	0.09	0.16
Zoo TPC:TPN	Temperature (ºC)	18	0.05	0.05	0.85	0.40	0.04
(mol:mol)	NO_3- + NO_2- (µmol m^{-2})	18	0.02	0.02	0.79	0.44	0.04
>2000 µm	Chl-*a* (µg m^{-2})	15	-1.8	1.64	<0.01	0.29	0.08
	Phyto TPC (mmol m^{-3})	15	-177	94.5	<0.01	0.08	0.21
	Phyto TPC:TPN (mol:mol)	**15**	**0.35**	**0.08**	**4.34**	***	**0.59**
	Latitude (ºN)	19	7.5e-3	<0.01	1.73	0.10	0.15
Zoo TPC:TPN	Temperature (ºC)	19	2.9e-3	0.02	0.15	0.88	<0.01
(mol:mol)	NO_3- + NO_2- (µmol m^{-2})	19	3.1e-3	<0.01	0.43	0.67	0.01
500-2000 µm	Chl-*a* (µg m^{-2})	16	-0.54	0.56	<0.01	0.35	0.06
	Phyto TPC (mmol m^{-3})	16	-68.1	43.4	<0.01	0.14	0.19
	Phyto TPC:TPN (mol:mol)	**16**	**0.11**	**0.02**	**4.43**	***	**0.58**
	Latitude (ºN)	20	0.02	0.02	1.28	0.22	0.08
Zoo TPC:TPN	Temperature (ºC)	20	0.03	0.06	0.52	0.61	0.01
(mol:mol)	NO_3- + NO_2- (µmol m^{-2})	20	-0.04	0.02	<0.01	0.09	0.14
250-500 µm	Chl-*a* (µg m^{-2})	17	0.07	2.08	0.04	0.97	<0.01
	Phyto TPC (mmol m^{-3})	17	-129	118	<0.01	0.29	0.07
	Phyto TPC:TPN (mol:mol)	17	0.12	0.14	0.85	0.41	0.05
	Latitude (ºN)	16	0.02	<0.01	1.72	0.11	0.17
Zoo TPC:TPN	Temperature (ºC)	16	0.01	0.03	0.39	0.70	0.01
(mol:mol)	NO_3- + NO_2- (µmol m^{-2})	16	-0.02	0.01	<0.01	0.20	0.11
64-250 µm	Chl-*a* (µg m^{-2})	14	-0.86	0.99	<0.01	0.40	0.06
	Phyto TPC (mmol m^{-3})	13	-96.8	53.1	<0.01	0.09	0.23
	Phyto TPC:TPN (mol:mol)	13	0.12	0.06	1.98	0.07	0.26
	Latitude (ºN)	18	0.23	0.70	0.33	0.74	<0.01
Zoo TPC:TPP	Temperature (ºC)	18	4.12	2.08	1.97	0.06	0.19
(mol:mol)	NO_3- + NO_2- (µmol m^{-2})	18	-0.21	0.90	<0.01	0.82	<0.01
>2000 µm	Chl-*a* (µg m^{-2})	15	-20.8	70.6	<0.01	0.77	<0.01
	Phyto TPC (mmol m^{-3})	15	-4218	4115	<0.01	0.32	0.07
	Phyto TPC:TPP (mol:mol)	15	-0.07	0.31	<0.01	0.82	<0.01
	Latitude (ºN)	19	0.24	0.41	0.59	0.56	0.02
Zoo TPC:TPP	Temperature (ºC)	19	1.09	1.68	0.65	0.52	0.02
(mol:mol)	NO_3- + NO_2- (µmol m^{-2})	19	0.61	0.62	0.98	0.34	0.05
500-2000 µm	Chl-*a* (µg m^{-2})	16	-17.7	47.5	<0.01	0.71	<0.01
	Phyto TPC (mmol m^{-3})	16	-2174	2797	<0.01	0.45	0.04
	Phyto TPC:TPP (mol:mol)	16	-0.06	0.20	<0.01	0.78	<0.01
	Latitude (ºN)	20	2.50	1.38	1.82	0.09	0.15
Zoo TPC:TPP	Temperature (ºC)	20	4.50	5.25	0.85	0.40	0.04
(mol:mol)	NO_3- + NO_2- (µmol m^{-2})	20	-2.8	2.06	<0.01	0.19	0.09
250-500 µm	Chl-*a* (µg m^{-2})	17	54.6	94.1	0.58	0.57	0.02
	Phyto TPC (mmol m^{-3})	17	-1.2e4	9533	<0.01	0.23	0.09
	Phyto TPC:TPP (mol:mol)	17	0.85	0.68	1.25	0.23	0.09
	Latitude (ºN)	16	0.30	0.45	0.68	0.50	0.03
Zoo TPC:TPP	Temperature (ºC)	16	0.68	1.51	0.45	0.66	0.01
(mol:mol)	NO_3- + NO_2- (µmol m^{-2})	16	0.32	0.60	0.54	0.59	0.02
64-250 µm	Chl-*a* (µg m^{-2})	14	-0.21	45.2	<0.01	0.99	<0.01
	Phyto TPC (mmol m^{-3})	13	-2029	2849	<0.01	0.49	0.04
	Phyto TPC:TPP (mol:mol)	13	-0.08	0.22	<0.01	0.73	0.01
	Latitude (ºN)	18	-0.08	0.13	<0.01	0.52	0.03
Zoo TPN:TPP	Temperature (ºC)	18	0.64	0.39	1.64	0.12	0.14
(mol:mol)	NO_3- + NO_2- (µmol m^{-2})	18	-0.13	0.35	<0.01	0.96	<0.01
>2000 µm	Chl-*a* (µg m^{-2})	15	4.17	13.2	0.31	0.76	<0.01
	Phyto TPC (mmol m^{-3})	15	-11.7	766	<0.01	0.98	<0.01
	Phyto TPN:TPP (mol:mol)	15	0.21	0.50	0.43	0.68	0.01

Responses	Predictor variables	n	Slope	Std. error	t-statistic	p-value	R^2
	Latitude (ºN)	19	7.7e-3	0.08	0.10	0.92	<0.01
Zoo TPN:TPP	Temperature (ºC)	19	0.20	0.32	0.65	0.52	0.02
(mol:mol)	NO_3- + NO_2- (µmol m^{-2})	19	0.10	0.11	0.87	0.39	0.04
500-2000 µm	Chl-*a* (µg m^{-2})	16	-0.48	8.80	<0.01	0.96	<0.01
	Phyto TPC (mmol m^{-3})	16	-53.2	540	<0.01	0.92	<0.01
	Phyto TPN:TPP (mol:mol)	16	-0.25	0.33	<0.01	0.47	0.04
	Latitude (ºN)	20	0.36	0.25	1.44	0.17	0.10
Zoo TPN:TPP	Temperature (ºC)	20	0.71	0.93	0.76	0.46	0.03
(mol:mol)	NO_3- + NO_2- (µmol m^{-2})	20	-0.29	0.37	<0.01	0.45	0.03
250-500 µm	Chl-*a* (µg m^{-2})	17	9.87	15.6	0.63	0.54	0.03
	Phyto TPC (mmol m^{-3})	17	-1564	1767	<0.01	0.39	0.05
	Phyto TPN:TPP (mol:mol)	17	0.63	1.15	0.55	0.59	0.02
	Latitude (ºN)	16	-7.9e-3	0.08	<0.01	0.93	<0.01
Zoo TPN:TPP	Temperature (ºC)	16	0.09	0.28	0.30	0.77	<0.01
(mol:mol)	NO_3- + NO_2- (µmol m^{-2})	16	0.13	0.10	1.25	0.23	0.10
64-250 µm	Chl-*a* (µg m^{-2})	14	3.44	8.34	0.41	0.69	0.01
	Phyto TPC (mmol m^{-3})	13	17.1	538	0.03	0.97	<0.01
	Phyto TPN:TPP (mol:mol)	13	-0.20	0.31	<0.01	0.55	0.03

Table IX. Linear regression model coefficients for zooplankton (zoo) molar C:N, C:P, and N:P ratios (mol:mol) in size fractions as a function of latitude (ºN), temperature (ºC), nitrate and nitrite concentration (µmol m^{-2}), chlorophyll-*a* concentration (µg m^{-2}), phytoplankton (phyto) C concentration (mmol m^{-3}) , and molar C:N, C:P, and N:P ratios (mol:mol). Temperature, nitrate and nitrite concentration, and Chlorophyll-*a* concentration are depth integrated to 100 m. Sample size is indicated as (n) for each linear regression model. Significant p-values are indicated with an asterisk (*). P-values less than 0.05 are flagged as *, 0.01 are flagged as **, less than 0.0001 are flagged as ***.

CHAPTER 4: DISCUSSION

4.1 Zooplankton C:N:P Stoichiometry & C, N, P % Dry Weight Across Size Fractions & Selected Organisms

The overall median molar C:N:P in our zooplankton samples was 116 : 25 : 1. The C:N, C:P, and N:P ratios in this study (Average C:N = 4.88 ± 0.44, C:P =130.65 ± 32.69, N:P = 27.93 ± 6.20) were very consistent and within the range of past literature values of zooplankton C:N:P in different size fractions (Average C:N = 5.31 ± 1.11 , C:P = 122.58 ± 35.12, N:P = 23.56 ± 6.63, Table V). These eight selected studies were the only ones that we could find that measured C, N, and P in size fractions of zooplankton and they included studies in both polar and tropical regions, covering a wide range in environmental conditions. After performing statistical analyses, we observed significant differences in molar C:N, C:P, and N:P ratios across the four size fractions as expected. The C:N ranged from 4 to 6.6 across the four size fractions in this study, which is consistent with the C:N reported for most major zooplankton groups (C:N range = 4 to 5.9, Kiørboe 2013). The C:N in the 500-2000 μm size fraction was significantly lower than our two smallest size fractions (Figure 5), and this is consistent with past literature in the NPSG region (Hannides *et al.* 2009). Additionally, based on our previous literature values of C:N from Kiørbe (2013) for chaetognaths (C:N = 4.2) and euphausiids (C:N = 4.6), perhaps the presence of smaller sized nitrogen-rich chaetognaths and euphausiids could be playing some role in the lower C:N in the 500-2000 μm size fraction, while the more carbon-rich smaller sized copepods (C:N = 5.5) could be playing some role in the higher C:N we observed in our 250-500 μm size fraction.

We also observed significant differences in C:P and N:P across size fractions, however we expected to observe higher C:P and N:P in the >2000 μm size fraction compared with our smaller ones. We hypothesized that the >2000 μm size fraction would contain a higher proportion of gelatinous zooplankton, which characteristically have higher C:P and N:P (Lüskow *et al.* 2022, Nugaraha *et al.* 2010), but instead we observed lower C:P and N:P (123.3 ± 19 and 25.1 ± 3.5, respectively, Figure 5) which suggests there may be

more hard bodied crustacean zooplankton in the largest size fraction. we also observed higher than expected C:P and N:P in the 250-500 μm size fraction, which suggests that there may be more gelatinous zooplankton in this size fraction. The smallest 64-250 μm size fraction representing the microzooplankton had the highest C:N (5.1 ± 0.3) lowest C:P (107.4 ± 11) and N:P (21 ± 2.1). The observed C:P and N:P in this size fraction are the closest to "Redfield ratio" values (C:P = 106, N:P = 16), which is consistent with a certain proportion of this size fraction containing phytoplankton. This size fraction contained the highest chlorophyll-*a* concentrations (Table A1), further supporting this suggestion. Additionally, in the previous literature on zooplankton C:N:P in size fractions Le Borgne *et al.* (1997) notes that their 35-200 μm mostly contained phytoplankton (54.4 % of total), and a smaller proportion of protists and copepod larvae and eggs (remaining 45.6 % of total). Therefore, predicting the C:N:P ratios in size fractions of bulk zooplankton samples based on the taxonomic composition reported from past literature may sometimes not be what we expected, and it is unclear with our current data. We will need taxonomic composition data and more direct measurements of the C:N:P in different major taxonomic groups of zooplankton to verify our observed variability in C:N:P in size fractions and address these questions.

We also calculated the C, N, and P % dry weight in our zooplankton samples, and we observed significant differences in the C, N, and P % dry weight across size fractions. The largest size fraction had the lowest C, N, and P % dry weight, and this is generally considered indicative of gelatinous zooplankton because their tissues hold onto water content even after drying, and so most reported values for gelatinous zooplankton are much lower than those reported for hard bodied crustacean organisms (Hubot *et al.* 2022, Plum *et al.* 2023). So, there are likely gelatinous zooplankton in this size fraction as we suspected, but it is still unclear why we don't see this signal in the C:N:P ratios of the largest size fraction.

In addition, we observed low measurements of P as % dry weight in our individually selected gelatinous and euphausiid zooplankton groups and compared their values with what we measured in the >2000 μm size fraction (average P = 0.58 ± 0.18 %). We see an intermediate value between the P % dry weight measured from our gelatinous zooplankton species (average P = 0.32 ± 0.12 % for ctenophores and siphonophores) and euphausiid species (Average P = 1.24 ± 0.22 %), which we would typically expect to find in this largest size fraction. The intermediate value of the gelatinous zooplankton and euphausiid species indicates that the >2000 μm size fraction likely represents a mixture of the bulk P % dry weight of these zooplankton groups in the community composition of this size fraction. Although we don't have C and N % dry weight for our individual zooplankton species, we speculate that these values would also be lower in the gelatinous zooplankton and higher in the euphausiid species and represent an intermediate value that we would expect to see in the >2000 μm size fraction. Similarly, we know that copepods in this region dominate the 200-2000 μm size fraction, and the average P % dry weight in our individual copepods (P = 0.63 ± 0.13 %) is strikingly like the combined average P % dry weight in the 250-500 and 500-2000 size fractions (P = 0.69 ± 0.17 %). These connections we observe between our individually selected zooplankton groups and their predicted associated size fractions is supporting evidence of our hypothesis addressing our first research question: that the variability in zooplankton C:N:P is dominated by differences in taxonomic composition in each of these size fractions.

4.2 Zooplankton C:N:P stoichiometry & C, N, P Concentrations Across Environmental Conditions

The Gradients IV cruise covered a wide latitudinal range and associated environmental gradients. This cruise track was useful because we had quite a bit of variability in temperature, nutrients, and the biomass of phytoplankton, and our second research question wanted to investigate their influence on the variability in zooplankton C:N:P. Does zooplankton C:N:P vary because of the environmental conditions, or the food, including the amount of food (i.e. phytoplankton C concentration) and the quality of the food (phytoplankton C:N:P)? After running our statistical analyses, we did not observe any significant correlations with zooplankton C:N:P and latitude, temperature, nitrate and nitrite concentration, and phytoplankton C concentration. Regarding changes in C:N:P

with temperature, a potential reason we did not observe any changes in C:N:P ratios could be that two processes that increase and decrease C:P in zooplankton may cancel each other out. zooplankton need more carbon to fuel their increasing metabolic demands with increasing temperature, which in turn increases their C:P, while simultaneously higher growth rates would increase P demand and decreasing their C:P. This hypothesis of the contrasting concepts of the metabolic theory and growth rate hypothesis were suggested by Mathews *et al.* (2018) for copepod C:P during their incubation experiments. There was only a significant correlation between the C:N in the two larger size fractions (>2000 μm and 500-2000 μm) of zooplankton and phytoplankton C:N (Figure 22). However, the slopes of both linear regressions were shallow (Slope = 0.35 and 0.11 for >2000 μm and 500-2000 μm size fractions, respectively) and not a 1:1 relationship which suggests that phytoplankton C:N is not a very strong effect. However, these slopes are significant, so perhaps the C:N of the food (i.e. phytoplankton) does have an impact on the C:N in these larger size fractions of zooplankton. We're not exactly sure why we observed these results, and we're not prepared to say that this correlation necessarily means causation, but it's a possibility that's worth investigating in future studies. Overall, these results further indicate that variability in zooplankton C:N:P is dominated by differences in taxonomic composition in each of these size fractions, and not environmental conditions. This is consistent with many zooplankton studies that observe strong homeostasis in the C:N:P of zooplankton across a wide variety of conditions in the field and in laboratory settings (Andersen & Hessen 1991; Golz *et al.* 2015; Malzahan *et al.* 2010; Persson *et al.* 2010; Sterner & Elser 2002).

As expected, we observed a general increase in zooplankton biomass with lower latitudes. This latitudinal gradient in zooplankton biomass was also previously observed during the JGOFS EqPac studies. For example, our measurements of C concentration in zooplankton biomass along the Gradients IV cruise ranged from 0.07 to 0.85 mmol C m^{-3} with a median value of 0.29 mmol C m^{-3}, with peaks of carbon concentration at 3.5 and 6.5º N. This is within the range reported by White *et al.* (1995) of 0.1 to 0.7 mmol C m^{-3} with similar peaks generally higher near the equator at 1 and 5º N. In a global context, zooplankton biomass in the Western Pacific Ocean reported by Ikeda (1985) is generally

highest at high latitudes >50º N (30 mg m^{-3}), lowest at mid-latitudes 40-10º N (~2.5 mg m^{-3}), and slightly higher ± 10º N from the equator (10 mg m^{-3}). If we convert Ikeda's measurements to mmol C m^{-3} for comparison, we see results within the range of our regional measurements with ~0.2 mmol C m^{-3} within the 40-10º N range, and ~0.8 mmol C m^{-3} ± 10º N from the equator. Therefore, our measurements of C concentration in zooplankton biomass are comparable regionally and globally. Other studies do not report zooplankton biomass in terms of nitrogen and lack of phosphorus measurements also prevent comparisons across other regional studies. Zooplankton biomass is typically represented as carbon biomass by plankton ecologists because it makes up the largest proportion of biomass, and nitrogen and phosphorus may be orders of magnitude lower, which we observe in our C, N, and P concentration measurements. Diel vertical migration behavior of certain zooplankton groups can also change the community structure of larger sized zooplankton in this study site. Chaetognaths and gelatinous predators make up a larger proportion of the >2000 μm zooplankton size fraction in the euphotic zone during the day, while crustaceans like euphausiids and shrimps are more abundant at night because of their nocturnal migration behavior to the euphotic zone (Hannides *et al.* 2009). We observed higher concentrations of zooplankton at night stations, particularly near the equator, and this needs to be taken into consideration for future studies in this region.

We observed a significant correlation between the C, N, and P concentration in the 500-2000 μm size fraction with the C, N and P concentration in phytoplankton. White *et al.* (1995) observed general increases in phytoplankton biomass (measured in mmol C m^{-3} d^{-1}) and chlorophyll-a concentration (mg m^{-3}) with peaks at 0º on the equator. We also observed a peak of phytoplankton carbon concentration (0.006 mmol C m^{-3}) and chlorophyll-a concentration (~0.45 μg m^{-2}) at 0º on the equator. Although the patterns in our measurements are similar, our measurements of phytoplankton C concentration and chlorophyll-*a* concentration are lower than values reported by White *et al.* (1995). Comparisons are difficult between measurements due to differences in units and lack of explanation for depth integration calculations for phytoplankton. White *et al.* (1995) did

not find any strong associations between zooplankton and phytoplankton biomass (C concentration or chlorophyll-*a* concentration). They speculated that phytoplankton develop rapidly in newly upwelled water compared with zooplankton populations, which respond to enhanced food abundance "downstream" from the upwelling center. We need more evidence of growth rates in zooplankton and phytoplankton in this region to confirm this speculation.

We also observed a significant correlation between the C concentration in the 64-250 μm size fraction with C concentration in phytoplankton. Landry *et al.* (1995) estimated that microzooplankton accounted for ~55% of grazing losses in phytoplankton during the Fall season JGOFS cruise series in this region. Since microzooplankton are important grazers on phytoplankton in this region it is not unexpected to observe this increase in their concentration with phytoplankton concentration. As expected, temperature and nitrate and nitrite concentration were not significant variables in changing zooplankton C, N, P concentrations. In this region, we observed a significant increase in nitrate and nitrite concentration and phytoplankton C, N, and P concentration with lower latitudes near the equator (Figure 2 and Figure 3). Previous studies in this region have observed an increase in phytoplankton concentration, and nutrient concentrations at the equator, increases in nutrients and thus the amount of food available to zooplankton may likely be the reason for changes in community structure and sizes of phytoplankton and consequently zooplankton towards the equator (Bidigare & Ondrusek 1996; Roman *et al.* 1995; White *et al*. 1995). Therefore, our zooplankton biomass measurements were consistent with the previous JGOFS EqPac studies.

4.3 Implications: Potential Future Shifts In The Zooplankton Community

Scientists are beginning to be concerned and modeling what might happen to marine ecosystems with climate change, and some recent studies predict that climate warming in future oceans, especially in tropical regions, with increasing stratification there might be less phytoplankton biomass, and this would tend to favor shifts in zooplankton communities towards larger sized filter feeding gelatinous zooplankton (i.e. which are

typically in the >2000 μm size fraction) at the expense of smaller omnivorous crustaceans like copepods (200-2000 μm size fraction) (Heneghan *et al.* 2023). These predictions are in part based on recent evidence from climate change projections and models of possible "tropicalization" of marine ecosystems, or an increase of stratification and oligotrophic conditions near this study region in the North Pacific Ocean (Polovina *et al.* 2008, Irwin & Oliver 2009). Enhanced stratification will likely enrich phytoplankton with carbon, increasing their C:nutrient ratios, which also favors the shift of zooplankton community composition towards less nutrient-demanding species (Van de Waal *et al.* 2010), like gelatinous zooplankton which we know have higher C:P, N:P, and lower C, N, and P % dry weight compared with crustacean zooplankton. Gelatinous zooplankton have also recently been observed to be responsible for top-heavy trophic structures observed in planktonic ecosystems worldwide, across both oligotrophic and eutrophic conditions (Lombard *et al.* 2024). Top-heavy trophic structures refer to an ecosystem where the biomass or abundance of predators (the higher trophic levels) is relatively high compared to the lower trophic levels, such as phytoplankton and herbivores like copepods and krill in this case, and the larger gelatinous zooplankton are the more abundant predators. Top-heavy or inverted trophic pyramids may be more characteristic of marine ecosystems than previously predicted (Woodson *et al.* 2020) and increasing contribution from larger sized gelatinous zooplankton may have potential consequences for marine ecosystems in terms of biogeochemical fluxes and food web structure. For example, gelatinous zooplankton can capture a wide size range of prey (i.e. high clearance rates) and increasing trophic efficiency, but gelatinous zooplankton represent lower food quality than hard bodied crustaceans like copepods for organisms in higher trophic levels like whales and fish, which typically prey on copepods and krill (Heneghan *et al.* 2023, Fabien *et al.* 2024). Shifts in zooplankton communities towards gelatinous zooplankton have also been observed in polar latitudes in the ocean. Shifts from Crustaceans like euphausiids to gelatinous zooplankton like salps has been observed in the Southern Ocean, with potential consequences for nutrient recycling and the whole Southern Ocean ecosystem (Alcaraz *et al.* 2014, Plum *et al.* 2023).

So, in the future, we may expect an increase in gelatinous zooplankton in our largest size fraction (>2000 μm) , and a decrease in omnivorous crustaceans in our smaller size fractions (250-500 μm and 500-2000 μm). Initially, we thought that we would see large differences in C:N:P in our zooplankton size fractions because the larger size fraction would contain gelatinous zooplankton. That's not what we observed in our direct measurements of zooplankton C:N:P in this region, and in fact our largest size fraction was quite similar in terms of C:N, C:P, and N:P compared to the smaller size fractions, suggesting that there were more hard bodied organisms in this size fraction than we expected. However, that may not be the case in the future, and more work needs to be done to monitor the C:N:P of this large size fraction and observe these potential future changes in the zooplankton community. If climate warming shifts the zooplankton community towards larger gelatinous zooplankton in the greater than 2000 μm size fraction, at the expense of smaller crustacean zooplankton in the 200-2000 μm size fraction, we may expect to see shifts in the overall median molar C:N:P towards 125:26:1 (the median C:N:P we observed in the >2000 μm) from our overall median molar C:N:P of 116:25:1 in this region of the Pacific Ocean.

4.4 Concluding Remarks & Future Directions

We've addressed our two research questions and extend our understanding of zooplankton C, N and P. And we now have this new overall median C:N:P of 116:25:1 in this region, and it's in agreement with past literature. And we also found that there are some differences in C:N:P across size fractions, which we think is because of taxonomic composition, and there's very little effect on variability in C:N:P from environmental conditions. If future climate change and ocean warming shifts the zooplankton community towards larger sized gelatinous zooplankton in the >2000 μm fraction at the expense of smaller 200-2000 μm crustacean zooplankton like copepods, we may expect to see shifts in the overall median molar C:N:P towards 125:26:1 in this region. Patterns in zooplankton C: N: P are not always what we may expect, and it shows that we still have much to learn and understand about the C:N:P of the bulk zooplankton community. We still require a better understanding of C:N:P differences across gelatinous and crustacean zooplankton groups to better understand gelatinous zooplankton's role in biogeochemistry, and how to better interpret overall signals in bulk zooplankton measurements of C:N:P in zooplankton size fractions. This cannot be ignored, and we need to increase our spatial, and temporal coverage (This was only one cruise with 18 stations for one season) to better capture variability of zooplankton carbon, nitrogen and especially measurements of phosphorus on a regional and global scale. Diel vertical migration behavior should also be considered more in future studies, as we can see higher zooplankton biomass associated with nighttime sampling closer to the equator than in the higher latitudes. The change in community composition from diel vertical migration behavior may also alter the C:N:P of bulk zooplankton measurements. More measurements of bulk macromolecules would also help to determine the allocation of carbon, nitrogen, and phosphorus into proteins, carbohydrates, lipids, RNA, and DNA and provide us with better insights into the metabolic and physiological status of organisms. Understanding the distribution of these elements into macromolecules is crucial because it reveals how organisms prioritize resource allocation under varying environmental conditions. These measurements of carbon, nitrogen, and phosphorus in zooplankton size fractions in this study will act as a valuable baseline for future studies

on the C:N:P of zooplankton in this region, especially with potential future shifts in the zooplankton community with future changes in our climate.

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APPENDIX

Table AI. biogenic silica (bSi) (µmol:mg) and chlorophyll-*a* concentration (µg:mg) measurements in 4 size fractions of zooplankton. Stations are described the same as Table I in the methods section. Sample size (n) = 2. Some stations are missing data because not enough zooplankton biomass was available for analysis.

Station	bSi >2000 µm	bSi 500-2000µm	bSi 250-500µm	bSi 64-250µm	Chl-*a* >2000µm	Chl-*a* 500-2000µm	Chl-*a* 250-500µm	Chl-*a* 64-250µm	n
SG1	--	--	--	--	--	--	--	--	
SG2	0.22	0.11	0.06	--	0.01	0.02	0.01	--	2
SG3	0.29	0.11	--	--	0.02	0.04	--	--	2
SG4(N)*	0.23	0.17	--	--	0.02	0.03	--	--	2
SG4	0.41	0.14	0.13	--	0.04	0.02	0.02	--	2
SG5*	0.62	0.15	--	1.15	0.07	0.05	--	--	2
SG6*	0.30	0.21	0.14	1.20	0.03	0.03	--	--	2
SG7(N)*	0.39	0.19	0.10	1.01	0.03	0.03	--	0.08	2
SG7	0.58	--	0.17	--	0.05	--	0.03	--	2
SG8*	0.29	0.18	0.11	--	0.02	0.03	--	0.10	2
SG9*	0.46	0.15	0.19	1.98	0.06	0.03	0.06	--	2
SG9(N)*	0.44	0.21	0.22	2.12	0.05	0.05	0.04	0.22	2
SG10	--	0.27	0.15	--	--	0.03	0.03	--	2
SG11.1(N)*	0.13	0.18	0.19	1.86	<0.001	0.01	0.02	--	2
SG11.2*	0.47	0.09	0.10	1.49	0.05	0.02	0.01	0.07	2
SG12	--	0.15	0.15	0.77	--	0.03	0.02	--	2
SG13*	0.24	0.10	0.09	0.77	0.03	0.02	0.01	0.14	2
SG14*	0.42	0.20	0.10	1.60	0.04	0.03	0.02	0.22	2
SG15*	0.54	0.15	0.11	0.54	0.04	0.03	0.02	0.06	2
SG16*	0.13	0.12	0.09	--	0.02	0.09	0.02	--	2
SG17*	0.20	0.17	0.20	0.61	0.01	0.04	0.02	0.10	2
SG18*	0.11	0.14	0.10	0.28	0.02	0.04	0.03	--	2

COMPLIMENTARY MEASUREMENTS

This section includes a table of biogenic silica (bSi) and chlorophyll-*a* (Chl-*a*) concentration measured in the four size fractions of zooplankton from this study (Table AI). Methods for bSi and Chl-*a* measurements are described in Chapter 2: Methods. We also have two figures of the 100 m depth integrated predictor variables temperature, nitrate concentration, chlorophyll-*a* concentration, phytoplankton elemental concentrations, and elemental ratios as a function of latitude fitted with linear regressions. There was a significant correlation with nitrate and nitrite concentration as a function of latitude (slope = -0.36 ; $R^2 = 0.27$; p-value = 0.02, figure A1). All phytoplankton elemental concentrations (mmol m^{-3}) were significantly correlated with latitude(p-value<0.05, figure A1). Phytoplankton TPC:TPN was significantly correlated with latitude (slope = 0.08; $R^2 = 0.35$; p-value = 0.01, figure A2). We also include our zooplankton molar C:N:P ratios and zooplankton C, N, and P concentration results with significant correlations as a function of environmental variables with 95 % confidence intervals (Figure A3-7).

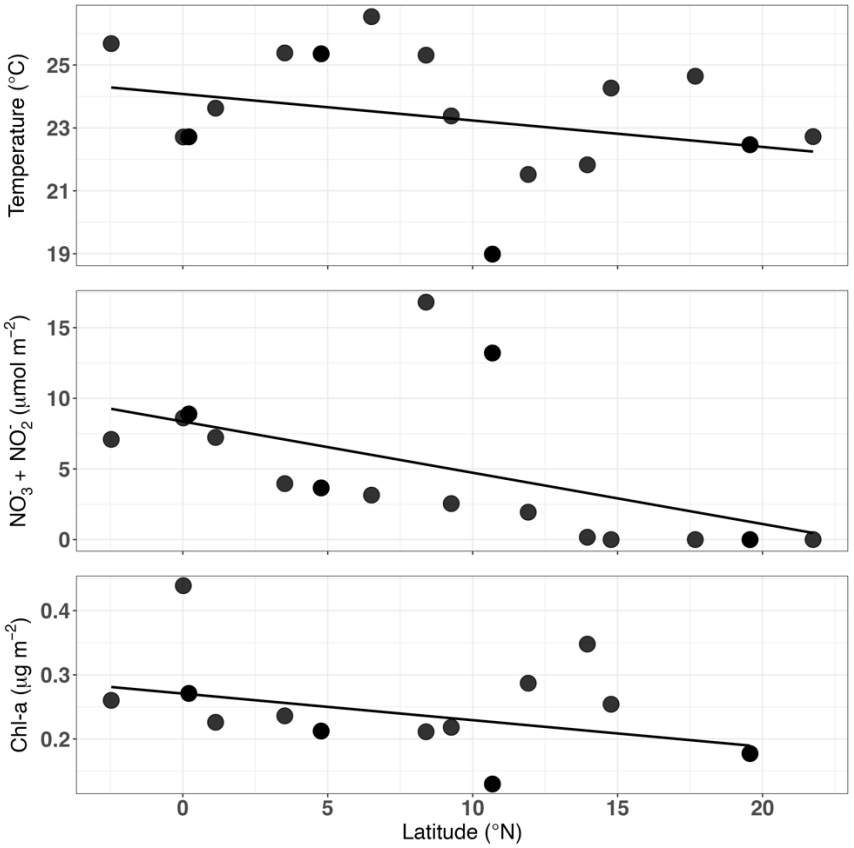

Figure A1. 100 m depth integrated temperature (ºC) slope = -0.08; R^2 =0.09; p-value= 0.20 (*top panel*), nitrate and nitrite concentration (µmol m^{-2}) slope = -0.36; R^2 = 0.27; p-value = 0.02 (*middle panel*), and chlorophyll-*a* concentration (µg m^{-2}) slope = -0.004; R^2 = 0.14; p-value = 0.13 (*bottom panel*) as a function of latitude (ºN). Measurement methods and protocols are described in the section 2.11 of the Chapter 2: methods and were obtained from datasets in Simon's CMAP database for the TN397 cruise.

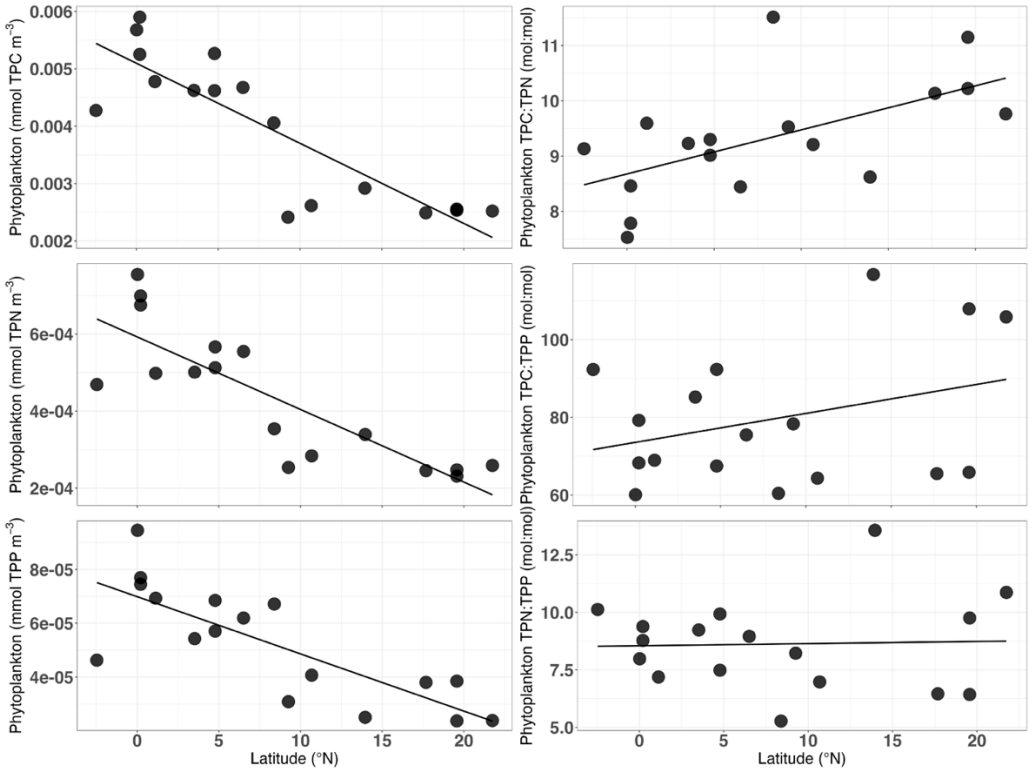

Figure A2. Phytoplankton TPC (slope = -1.4e-4; $R^2 = 0.74$; p-value = 9.5e-6), TPN (slope = -1.9e-5; $R^2 = 0.70$; p-value = 2.4e-5), and TPP (slope = -2.1e-6 ;$R^2 = 0.61$; p-value = 1.8e-4) concentration (mmol m^{-3}) (*left column panels*), and phytoplankton TPC:TPN (slope = 0.08; $R^2 = 0.35$; p-value = 0.01), TPC:TPP (slope =0.74; $R^2 = 0.10$; p-value = 0.20), and TPN:TPP (slope = 0.009; $R^2 = 0.001$; p-value = 0.89) ratio (mol:mol) (*right column panels*) as a function of latitude (ºN). Phytoplankton measurements are described in section 2.15 of the Chapter 2: Methods.

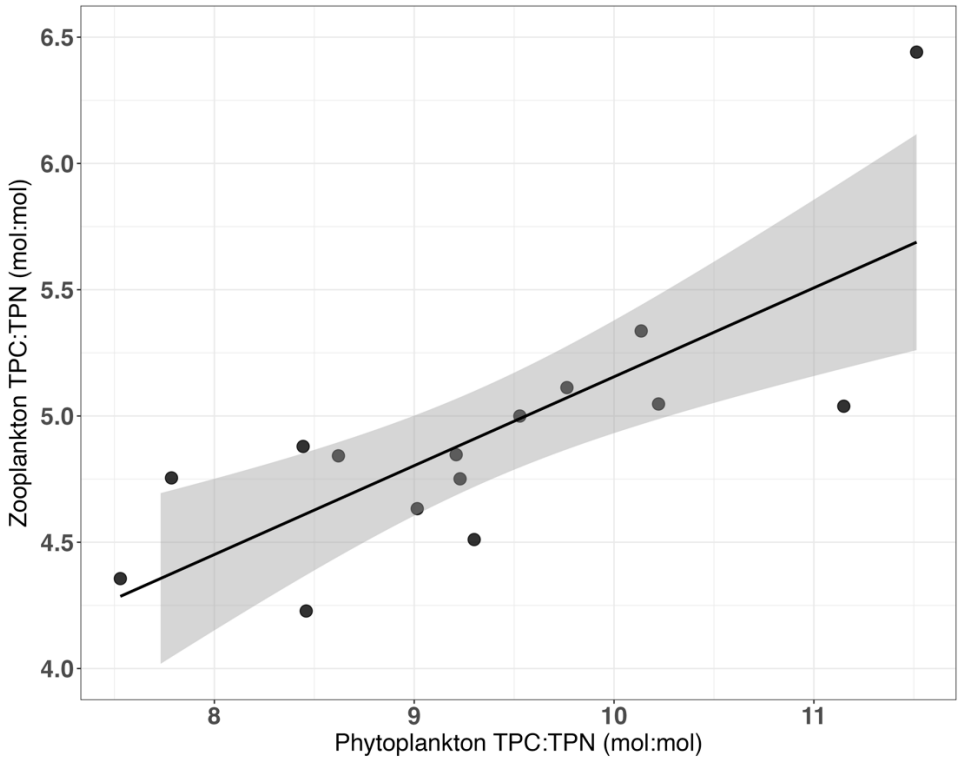

Figure A3. Molar C:N ratio (mol:mol) in >2000 µm size fraction as a function of phytoplankton molar C:N ratio (mol:mol). Shaded region represents 95 % confidence interval. Linear regression model coefficients are given in Table IX.

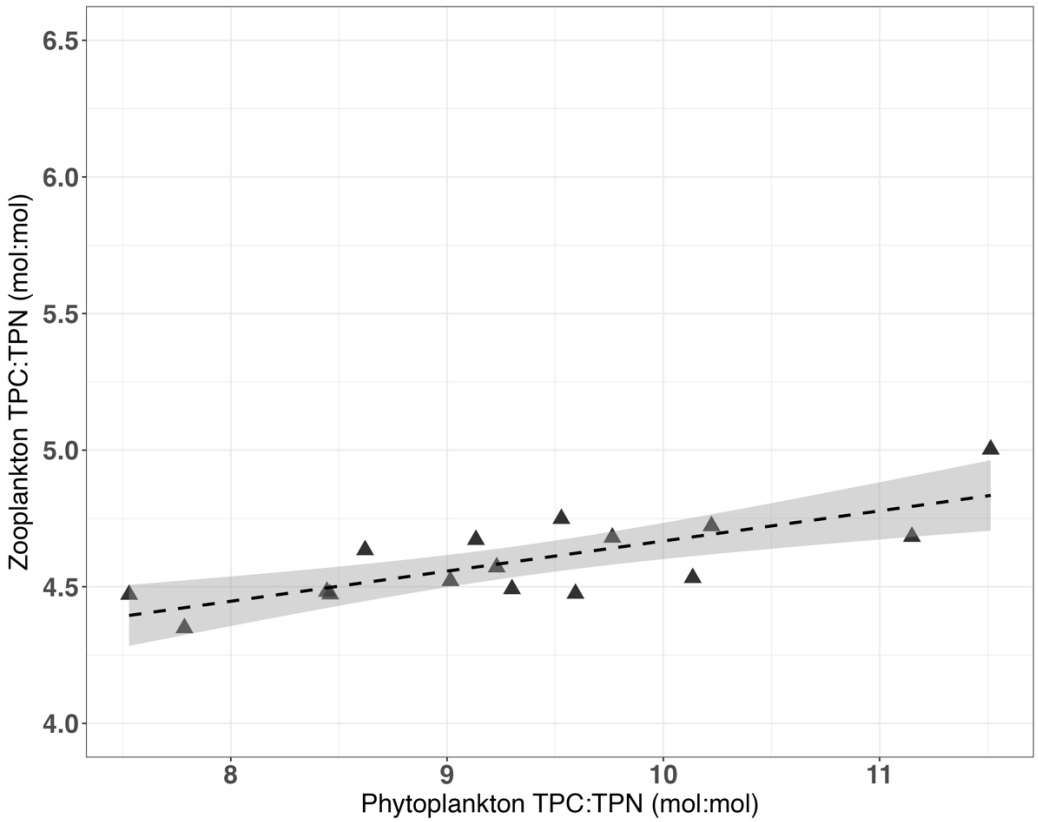

Figure A4. Molar C:N ratio (mol:mol) in 500-2000 µm size fraction as a function of phytoplankton molar C:N ratio (mol:mol). Shaded region represents 95 % confidence interval. Linear regression model coefficients are given in Table IX.

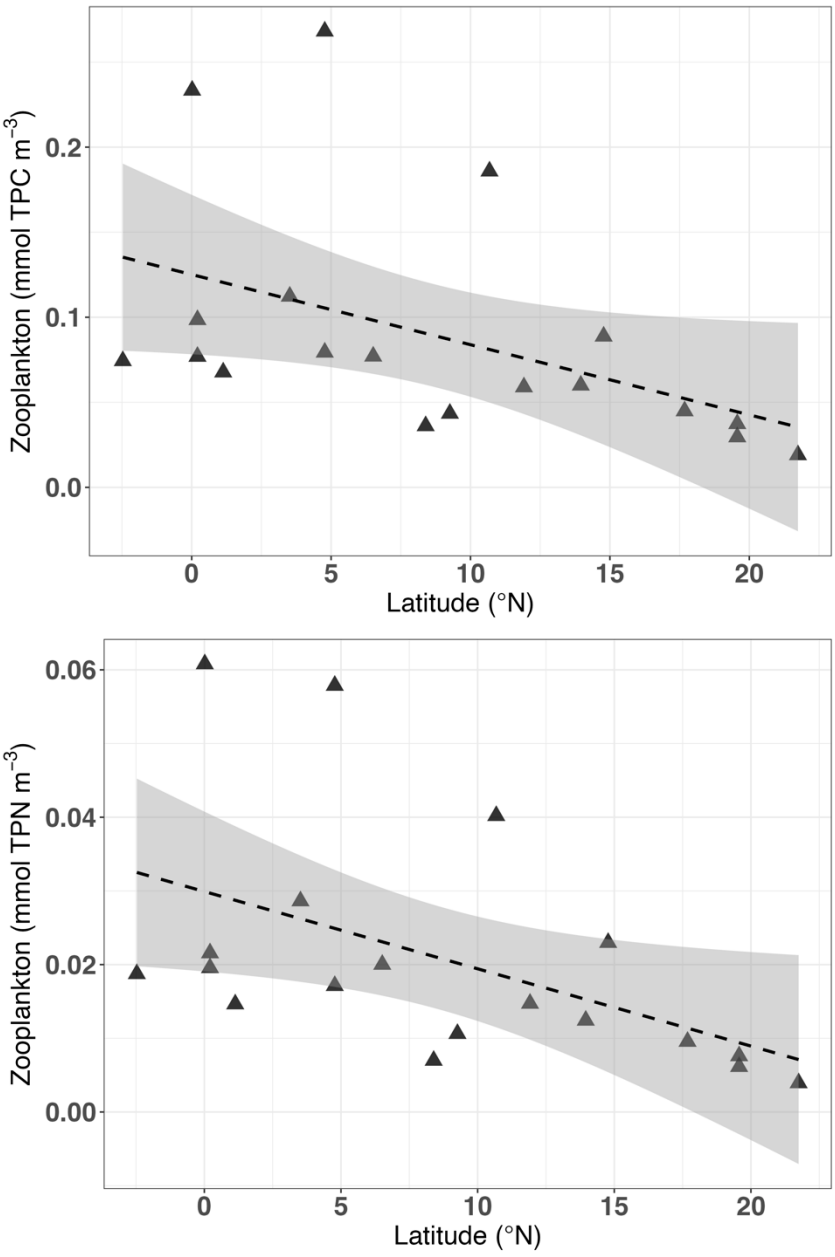

Figure A5. C and N concentration (mmol m^{-3}) in the 500-2000 µm as a function of latitude (ºN). Shaded regions represent 95 % confidence intervals. Linear regression model coefficients are given in Table VIII.

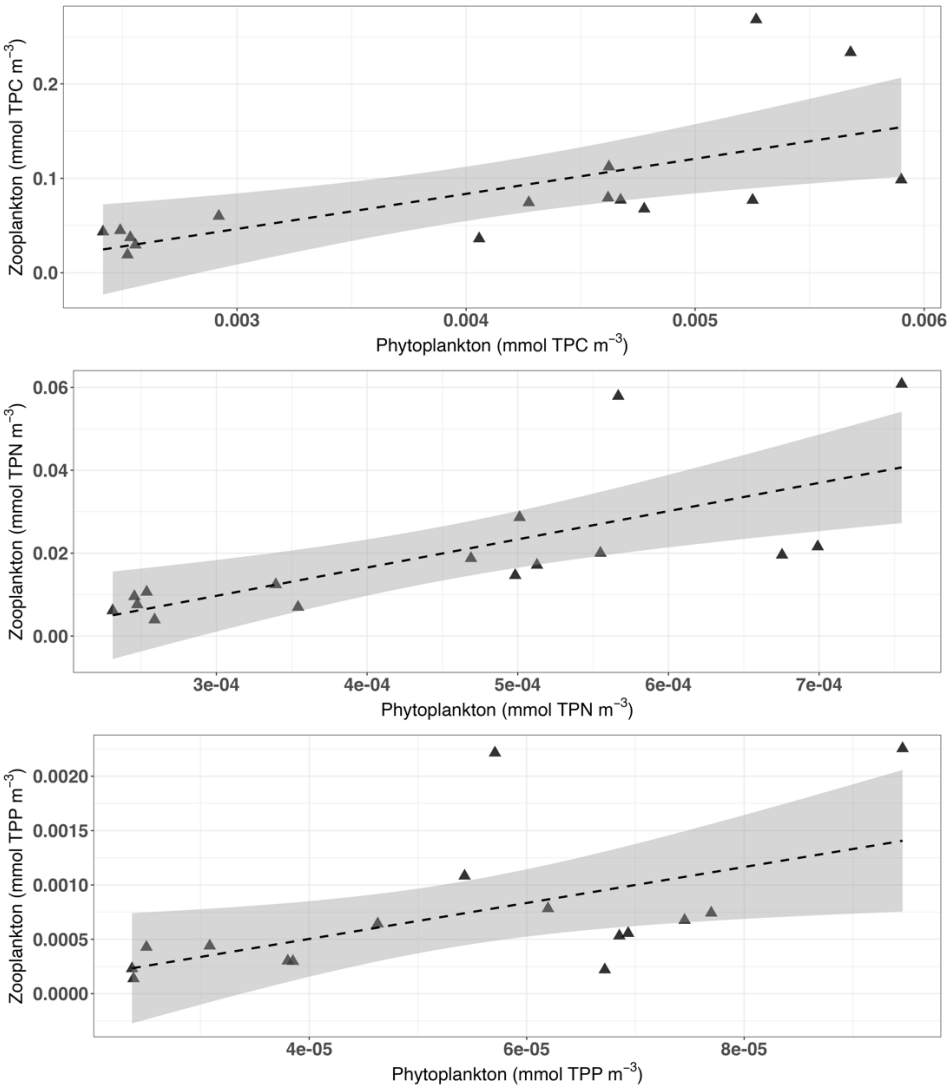

Figure A6. C, N, and P concentration (mmol m^{-3}) in the 500-2000 µm as a function of phytoplankton C, N, and P concentration (mmol m^{-3}). Shaded regions represent 95 % confidence intervals. Linear regression model coefficients are given in Table VIII.

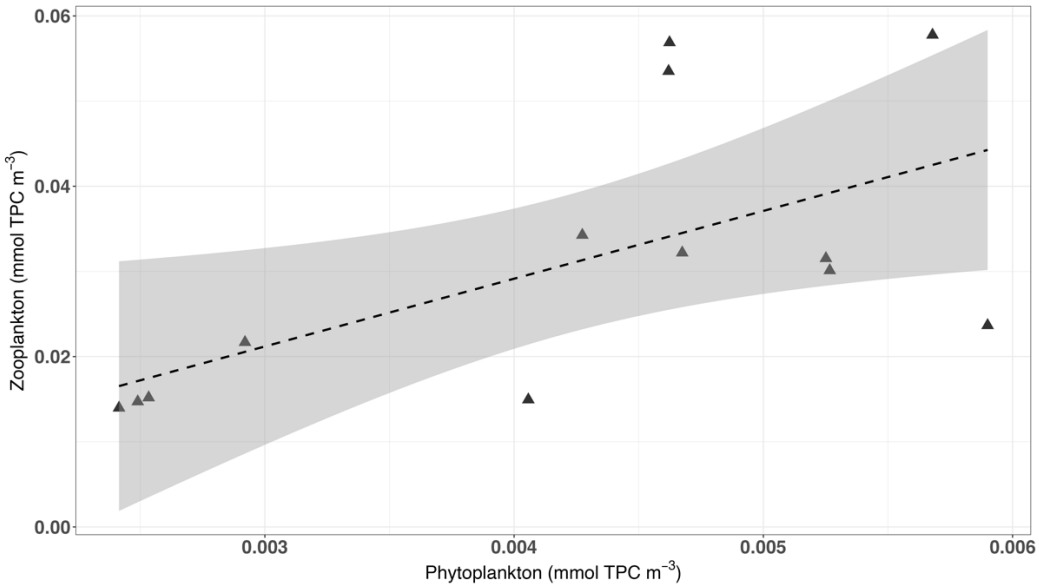

Figure A7. C concentration (mmol m^{-3}) in the 64-250 µm size fraction as a function of phytoplankton C concentration (mmol m^{-3}). Shaded region represents 95 % confidence interval. Linear regression model coefficients are given in Table VIII.