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Spatial and Temporal Variation in the Diet Composition of Zooplankton in Mission Bay

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ABSTRACT

Analyses of quantitative data on zooplankton diets are vital for understanding the drivers of zooplankton abundance within an ecosystem. Such analyses also provide insight into trophic pathways within the lower planktonic food web, which support populations of higher trophic level species. This study used carbon and nitrogen stable isotope ratios of size-fractionated plankton in Mission Bay, San Diego, CA to examine the spatial and temporal variation in zooplankton trophic ecology and determine potential environmental drivers of zooplankton community structure. Carbon stable isotopes reflect primary production sources in an organism's diet, and nitrogen stable isotope ratios can be used to estimate the relative trophic positions of organisms. From April 2017 to April 2018, monthly sampling of environmental parameters and plankton tows were conducted at three sites, which varied in distance from the mouth of the bay. Plankton samples from each tow were divided into four size classes: 53-120 µm, 120-250 µm, 250-475 µm, and 475-1000 µm. Among the size classes, there was no significant variation in $\delta^{15}N$ values, suggesting that either the food web at this level is not strongly size-structured or that $\delta^{15}N$ values cannot delineate trophic structure in the lower planktonic portion of the food web. There were significant spatial differences in $\delta^{13}C$ in the two smallest size classes (53-120 and 120-250 µm). The comparison among sites also revealed a significant difference in $\delta^{15}N$ within the second largest size class (250-475 µm), which indicates

that this size class may be feeding on organisms at different trophic positions at each site. Additionally, positive correlations were found within each size class between $\delta^{15}N$ values and one or more environmental parameters, suggesting that there is an influence of environmental factors on stable isotope values of plankton in Mission Bay.

INTRODUCTION

Zooplankton are critical components of aquatic and marine systems, serving as trophic intermediaries (Stoecker and Capuzzo 1990, Turner 2004, Gifford *et al.* 2007). Accordingly, they are an important link in the transfer of carbon from the bottom of the food web to higher-level consumers (Cushing 1989, Saiz *et al.* 2007, Bănaru *et al.* 2014). Understanding the diets of zooplankton is therefore crucial for determining the mechanistic drivers of their population dynamics and the trophic pathways, or energy flow, in the lower planktonic food web (Bollens and Penry 2003). Previous research has demonstrated that zooplankton feed on a diversity of organisms with different trophic modes, including autotrophs, heterotrophs, and mixotrophs (Turner 2004, Gifford *et al.* 2007).

Through isotopic dietary analysis, it is possible to estimate the relative trophic position of zooplankton and the degree to which they consume heterotrophic or autotrophic prey (Peterson and Fry 1987, Grey and Jones 2001), which is important for understanding carbon flow through the food web (Fredriksen 2003, Phillips and Gregg 2003). Zooplankton community composition and diets may be influenced by local environmental conditions (Bollens and Penry 2003, Gifford *et al.* 2007). Furthermore, an examination of diet across a range of environmental conditions can indicate if and how zooplankton diets vary with differing parameters, e.g., temperature ranges and nutrient concentrations. Knowledge about these relationships is important for

a deeper understanding of food web structure and can facilitate predictions of changes in zooplankton as environmental conditions vary.

Trophic Ecology of Mesozooplankton

Insight into the trophic relationships between zooplankton and their prey is fundamental to our understanding of the structure of marine food webs (Landry 1982, Bollens and Penry 2003). Not only do zooplankton regulate phytoplankton by grazing, but also by the regeneration of nutrients through excretion (Hunt and Matveev 2005). For instance, differences in the coupling of primary production and grazing processes can result in substantial spatial and seasonal variation in phytoplankton standing stocks, nutrient utilization, and recycling efficiencies (Marine Zooplankton Colloquium 2001).

Understanding zooplankton community structure in estuaries is particularly important, as estuaries are transitional systems between land and sea (Marques *et al.* 2006, Menéndez *et al.* 2015) and serve as important fish nursery grounds by providing food and refuge for juveniles. During their larval stages, many fish species rely on zooplankton as sources of carbon and energy (Turner 2004). Within these ecosystems, tidal flux and freshwater input can create large variations in temperature, salinity, turbidity, and nutrient concentrations (Menéndez *et al.* 2015); mesozooplankton (0.2-2 mm; Sanders and Wickham 1993) feeding may be affected by this variation in water quality (Bollens and Penry 2003, Gifford *et al.* 2007). Previous studies have found seasonal variation in mesozooplankton feeding, particularly during phytoplankton blooms (Bollens and Penry 2003). During blooms,

mesozooplankton often feed on phytoplankton at higher rates than they do on microzooplankton, prokaryotes, and unicellular eukaryotes. During non-bloom periods, microzooplankton may become more important in the diet of mesozooplankton, because phytoplankton may not be able to fully sustain the mesozooplankton (Irigoien and Castel 1995). Changes in diet thus may be related to temporal variation in zooplankton abundance and community structure within estuarine ecosystems.

Biology and Ecology of Copepods

Copepods are small crustaceans that usually dominate mesozooplankton in numbers and biomass in marine waters (Miller and Wheeler 2012). Similarly to other crustaceans, copepod species have a complex life cycle, which includes larval stages. The life cycle of a copepod is made up of 5 to 6 naupliar stages, followed by five copepodite stages before maturity (Meunier *et al.* 2016). Copepod growth rate decreases during the transition from the naupliar stages to last copepodite stages (Sabatini and Kiørboe 1995) and the transition from the first to last copepodite stage (Meunier *et al.* 2016). Because the early stages of all copepods include nauplii, even copepods that are comparatively large as adults are small when young (Turner 2004). Ontogenetic shifts in feeding have been observed in a number of copepod species (Decho and Fleeger 1988, Falkenhaug *et al.* 1997).

Copepods are suspension feeders and use their second maxillae as paddles to push particles toward the mouth, in contrast to filtering particles

from the water. As they hunt and catch potential prey, copepods are capable of choosing to consume or not consume those captured items (Strickler 1982, Kleppel 1993). Based on strong evidence from open and coastal ocean studies, calanoid copepods often are omnivores (Kleppel 1993, Kleppel *et al.* 1996, Turner 2004). However, in estuaries, where fewer studies on copepod feeding preferences have been conducted, the results are less clear (Turner 2004). Many models of planktonic food web structure now include not only copepods transferring matter and energy along the traditional planktonic chain, but also participating in the microbial loop (Gifford 1991, Sanders and Wickham 1993) by ingesting heterotrophic flagellates and ciliates (Tiselius 1989). When there is a seasonal thermocline, the microbial loop becomes an important trophic pathway due to low nutrient concentrations favoring small flagellates (Sherr and Sherr 1988). However, in mixed coastal waters, the microbial loop is of less consequence since the phytoplankton biomass is generally dominated by larger organisms (Sahlsten *et al.* 1988).

The ability to consume a variety of foods increases the chances of copepods thriving in nutritionally poor environments, because it allows an organism to alter its diet as the composition of food in the surrounding environment changes. For example, copepods may respond to changes in available food composition by switching between herbivory and carnivory (Landry 1981). Copepods have also been known to feed size-selectively when food is abundant and non-selectively when food is scarce (Cowles 1979, Kleppel 1993). Based on results from past studies, copepod feeding activity

may also vary with physical environmental parameters (Kleppel 1993), such as water temperature (Kiørboe et al. 1982, 1985). Further examination of copepod feeding behavior in estuaries is especially meaningful, because estuarine copepods are an important food resource for larvae of many fish species that are commercially harvested as adults (Turner 2004).

Dietary Analysis

This study used bulk stable isotope ratios to analyze the diet composition of zooplankton. Stable isotopes have successfully been used to determine the relative contribution of various food sources to zooplankton diet (Fredriksen 2003, Phillips and Gregg 2003). In nature, elements (C, N, S, H, and O) occur in more than one isotopic form. Generally, the heavier form is less abundant in the environment than the lighter one (Parnell *et al.* 2013). A difference in the number of neutrons does not change most aspects of chemical reactivity, and different stable isotopes of the same element function the same in most reactions. However, since similar molecules with slightly different masses react at different rates, there are many biological and chemical reactions that modify the ratio of heavy to light isotopes in predictable ways (Peterson and Fry 1987).

Stable isotope analysis offers advantages over conventional diet evaluation methods, like gut content analysis, because it provides a more long-term representation of an organism's assimilated diet. In contrast, gut content analysis provides a snap shot of recently ingested material. Gut content analyses are more affected by temporal bias (Sholto-Douglas *et al.*

1991), often indicating only what was ingested in the last 24 hours (Kling *et al.* 1992, Bowes and Thorp 2015). Stable isotope analysis may even identify dietary sources that are not detectable by an examination of ingested material (Grey *et al.* 2000). It is also a relatively inexpensive method and can be completed in a matter of weeks at many laboratories across the globe (Bowes and Thorp 2015). Moreover, stable isotope analysis is advantageous for analyzing small organisms, like plankton. Due to the small size of plankton, they have the potential to turn over assimilated isotopes rapidly, in a matter of weeks. As a result, plankton may exhibit different isotopic signatures over a relatively short time scale. Repeated samplings throughout the year would be needed to analyze the seasonality of the isotopic signatures and to completely understand the dynamic diet of plankton (Grey *et al.* 2000).

A combination of carbon and nitrogen stable isotope signatures provides information on an animal's food sources and trophic position (Grey and Jones 2001). As carbon and nitrogen within organic matter are passed up a food web, the tissue that omnivores and carnivores develop from the elements becomes increasingly enriched in the less-abundant, heavier stable isotopes (^{13}C and ^{15}N) in relation to the dominant, lighter isotopes (^{12}C and ^{14}N). This occurs mainly because compounds containing the lighter isotopes are preferentially removed from the tissues since they fit slightly more readily into the active sites of metabolic enzymes (Miller and Wheeler 2012). The carbon and nitrogen stable isotopic values ($\delta^{13}C$ and $\delta^{15}N$) are based on the ratios of the heavier isotopes to lighter isotopes (Miller and Wheeler 2012).

These δ values can be used to express isotopic composition in terms of parts per thousand (ppt) differences from a standard reference:

$$\delta X = [(R_{sample}/R_{standard})\text{-}1] \times 10^3$$

The *X* represents ^{13}C or ^{15}N and the *R* represents the ratios, $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Peterson and Fry 1987).

The naturally occurring ratios of $\delta^{13}C$ and $\delta^{15}N$ in an organism's tissue provide a time-integrated measure of an organism's diet (Bănaru *et al.* 2014, Kopprio *et al.* 2015), ultimately reflecting the diet of the organism during the time in which its tissue was developed (Bearhop *et al.* 2002, 2004).

Carbon Stable Isotope Ratio

Because consumers generally have carbon compositions similar to their food, the carbon stable isotope ratio ($\delta^{13}C$) reflects the sources of primary production in a trophic network (Kling *et al.* 1992, McCutchan et al. 2003). The carbon ratio changes only slightly through the food web with an approximate 1‰ increase in $\delta^{13}C$ for each trophic level (Fry and Sherr 1989, Fry and Quiñones 1994). The large differences in $\delta^{13}C$ values between plants with either C_3 or C_4 photosynthetic pathways are reflected in the carbon isotopic ratios of organisms that derive carbon mostly from plants of one photosynthetic type of the other (DeNiro and Epstein 1978). Therefore, $\delta^{13}C$ offers information about the carbon sources utilized by primary producers and adjacent trophic levels (Kurten *et al.* 2015). For instance, the $\delta^{13}C$ values of organisms from freshwater, marine, and terrestrial environments are within the ranges of the $\delta^{13}C$ values of plants from those respective environments

(DeNiro and Epstein 1978). In marine ecosystems, an analysis of $\delta^{13}C$ values may also indicate inshore vs. offshore, or pelagic vs. benthic diet sources (Hobson *et al.* 1994).

Nitrogen Stable Isotope Ratio

Nitrogen stable isotope ratio ($\delta^{15}N$) can be used to estimate the relative trophic position of an organism by relating the organism's ratio to that of the components of its diet (Kling *et al.* 1992, Driscoll 2014). There is a consistent increase in ^{15}N in consumers with increasing trophic level. This results from the excretion of the lighter isotope, ^{14}N, as a byproduct of protein synthesis. This biochemical process leaves a consumer enriched in ^{15}N compared to the organism's dietary sources (Kling *et al.* 1992). Marine and aquatic studies use a general enrichment factor of 3.4‰ $\delta^{15}N$ for every trophic level. However, in natural systems, the value of this factor is contingent on the extent of omnivory. The enrichment of an omnivore, an organism that feeds at multiple trophic levels, should be greater than that of an herbivore but lower than that of a strict carnivore (Fry and Sherr 1989, Kling *et al.* 1992, Fry and Quiñones 1994).

Marine ecosystems are considered to have strongly size-structured food webs. The major primary producers are small, unicellular algae that support systems in which most consumers are larger than their prey (Sheldon *et al.* 1972, Cohen *et al.* 1993, Pope *et al*. 1994). Thus, body size may determine potential predators and prey, and trophic level is expected to increase with increasing size (Sholto-Douglas *et al.* 1991). Because trophic

relationships in food webs are chiefly ruled by organism size (Sholto-Douglas *et al.* 1991), a number of studies have assessed the diets and trophic level of organisms in an ecosystem in relation to size class. Past studies have used nitrogen stable isotope analysis to show that the trophic levels of plankton, invertebrates, and fishes increase progressively with increasing body size (Minagawa and Wada 1984, Jennings *et al.* 2002).

Sholto-Douglas *et al.* (1991) and Yang *et al.* (2017) reported a trend of ^{13}C and ^{15}N enrichment with increasing organism size within the plankton community, suggesting that larger plankton feed farther up the food web than smaller plankton. Rolff (2000) also found the enrichment of ^{15}N in plankton size classes to be a linear function of logarithmic organism size from 20 to 500 µm. Thus, size classes of plankton within a plankton community are expected to be representative of trophic groups (Rolff 2000). Other compelling arguments to classify by size rather than species in food web analyses include the increase in body size of marine species throughout their life cycle and the prevalence of cannibalism, cross-predation, and transient predator-prey relationships (Pope *et al.* 1994, Jennings *et al.* 2002). It is important to consider that size-based analyses assume that predator-prey relationships are mainly governed by body size and that there is a substantial occurrence of omnivory within the ecosystem. However, this size-based approach provides a strong foundation for analyzing the function and structure of a food web (Jennings *et al.* 2002).

Study Site: Mission Bay

A study, like this one, on spatial and temporal variation in the diets of copepods and other zooplankton would best be conducted in a location with a range of environmental conditions. Mission Bay, an estuary in San Diego, CA, is such a site. Over the past 150 years, the bay has been modified by river diversion, dredging, and filling. Consequently, it has become one of the most greatly altered coastal systems in southern California (Dexter and Crooks 2000). Due to the increased distance from the ocean and the presence of a large, artificial island, which creates two long, narrow channels, there is weak tidal influence in the inner parts of the bay (Figure 1.1). This lack of tidal flushing in the inner bay leads to long residence times and increases the potential for water quality problems (Largier *et al.* 2003).

Like other Mediterranean hypersaline estuaries, Mission Bay exhibits a distinct seasonal cycle. Mission Bay has been described as a shallow, vertically well-mixed estuary, although it can become horizontally stratified, particularly during the summer, when water in the back of the bay becomes hypersaline (Levin 1984). There is an absence of freshwater inflow during the warm, dry summers. Warm water dominates the inner basin, while cool water dominates near the mouth of the bay (Largier *et al*. 2003). During the late summer, the rate of evaporation exceeds the rate of fresh water supply via precipitation and runoff in the restricted areas of the bay, and, as a result, the back of the bay becomes hypersaline (Largier *et al*. 1997).

Plankton of Mission Bay

Past studies have examined variation in zooplankton community composition in Mission Bay in relation to tidal velocity, temperature, salinity, and rainfall (Kittinger 2006, Elliott and Kaufmann 2007, Griggs 2009, Shapiro 2018). Shapiro (2018), the most recent study, as well as Elliott and Kaufmann (2007), both observed copepods and tintinnids as the most predominant taxa in Mission Bay. During the two-year period of sampling conducted by Elliot and Kaufmann, there was an assemblage of commonly observed zooplankton taxa (*Acartia, Oithona, Euterpina, Tintinnopsis, Favella, Helicostomella,* bivalve veligers, and gastropod veligers). Out of the 37 differentiated taxa of zooplankton found regularly in Mission Bay during this study period, nine were copepods and eleven were tintinnid ciliates. The copepods *Acartia californiensis, Oithona similis,* and *O. oculata,* as well as the ciliates *Tintinnopsis lobiancoi, T. campanula, T. cornige, T. kofoidi, Favella* sp., *Steenstrupiella steenstrupii,* and *Stenosemella steini,* were the most common species, observed in at least 25% of all samples (Elliott and Kaufmann 2007).

As reported in Elliott and Kaufmann (2007), spatial variation in the zooplankton species composition of Mission Bay was less evident than temporal variation. As determined by canonical correlation analysis, 21% of variation was explained by site, while 34% of variance was explained by season and year. In 2003, high rainfall resulted in lowered salinities, high nutrient concentrations, and a number of copepod species that appeared

following rainfall and freshwater discharge. According to Elliott and Kaufmann (2007), the zooplankton composition of Mission Bay during this year was very similar to that of other shallow bays with seasonal freshwater inflow and restricted tidal flux. In years with much lower freshwater inflow, Mission Bay is similar to a typical Mediterranean coastal estuary with low freshwater discharge. Such environments are characterized by salinities at or slightly above seawater, low nutrient concentrations, and a summer zooplankton composition consisting of many tintinnid species and relatively few copepod taxa, with a prevalence of smaller species like *Oithona* spp. (Elliott and Kaufmann 2007).

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CHAPTER 2

2.1 ABSTRACT

Quantitative analyses on zooplankton diets are critical for determining the drivers of population dynamics of zooplankton and the trophic pathways in the lower planktonic food web, which ultimately support populations of higher trophic level species. This study analyzed carbon and nitrogen stable isotope ratios of size-fractionated plankton in Mission Bay, San Diego, CA to examine spatial and temporal variation in zooplankton trophic ecology and identify potential environmental drivers of zooplankton community structure. From April 2017 to April 2018, monthly plankton tows and measurements of environmental conditions (temperature, salinity, nutrients, chlorophyll *a* concentration) were taken at three different sites in the front, middle and back of the bay. There was no significant difference in $\delta^{15}N$ values among size classes, suggesting that either the food web at this level is not strongly size-structured or that $\delta^{15}N$ analysis cannot delineate trophic structure in the lower planktonic portion of the food web. In a comparison among sites, there were differences in $\delta^{13}C$ in the two smallest size classes (53-120 and 120-250 µm) and in $\delta^{15}N$ in the second largest size class (250-475 µm). Significant correlations were found between $\delta^{15}N$ values of each size class and one or more environmental parameters. These results suggest a relationship between environmental factors and stable isotope values of size-fractionated plankton in Mission Bay.

2.2 INTRODUCTION

Zooplankton transfer carbon and energy from the bottom of the food web to higher-level organisms and, therefore, play an integral ecological role within aquatic ecosystems (Gifford *et al.* 2007, Bănaru *et al.* 2013). Zooplankton are composed of a diverse assemblage of species occupying different trophic levels and modes, consuming heterotrophs, autotrophs, and mixotrophs (Gifford *et al.* 2007). Quantitative data on the diet composition of zooplankton is crucial for understanding the mechanisms driving patterns in zooplankton abundance (Bollens and Penry 2003) and defining the energy pathways in the lower planktonic food web, which supports fishes and higher trophic level species.

This study focuses on an estuarine ecosystem, where the mesozooplankton community is primarily dominated by copepods. Most copepods are omnivorous, consuming a variety of autotrophs, heterotrophs, metazoa, protozoa, and organic material (Turner 2004). This trophic flexibility allows copepods to alter their diets as the composition of food in the surrounding environment changes, which can help populations survive in nutritionally variable environments. For example, copepods may respond to changes in available food composition by switching between herbivory and carnivory (Landry 1981). Copepods have also been known to feed size-selectively when food is abundant and non-selectively when food is scarce (Cowles 1979, Kleppel 1993). Copepod feeding activity may also vary with changes in physical environmental parameters (Kleppel 1993), including water temperature (Kiørboe et al. 1982, 1985).

Estimating the contributions of different prey resources to zooplankton is crucial for understanding carbon flow through the food web. Many previous studies have used carbon and nitrogen stable isotope analyses to examine the diets of organisms, including zooplankton (Fredriksen 2003, Phillips and Gregg 2003). This study aims to use bulk stable isotope values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to analyze the diet composition of zooplankton in an ecologically important estuarine environment. $\delta^{13}C$ reflects the sources of primary production in a trophic network (Kling *et al.* 1992, McCutchan *et al*. 2003), and in marine ecosystems, an analysis of $\delta^{13}C$ values may indicate inshore vs. offshore, or pelagic vs. benthic diet sources (Hobson *et al*. 1994). The nitrogen stable isotope ratio ($\delta^{15}N$) can be used to estimate the relative trophic position of an organism by relating the organism's ratio to that of the components of its diet (Kling *et al.* 1992, Driscoll 2014).

Since lower trophic levels of estuarine and marine food webs are poorly resolved and involve complex predator-prey interactions, some studies have used size fractions to simplify plankton food webs and more broadly characterize the trophic structure at the bottom of a food web (Rolff 2000, Sommer and Sommer 2004, Bănaru *et al.* 2013, Espinasse *et al.* 2014, Yang *et al.* 2017). For example, Bănaru *et al.* (2013) also found that the $\delta^{15}N$ signatures of plankton generally increased with size class in the Bay of Marseille, though not in a consistent pattern. Marine plankton have been frequently reported as opportunistic predators, with particle size as a major influential factor in prey selection (Chisholm 1992, Rolff 2000, Giering *et al.* 2018). Therefore, size classification

of plankton will likely reflect the trophic structure in the marine plankton community (Rolff 2000, Bănaru 2013). Size-related predation in marine plankton food webs has been reflected in studies that have found an increase in $\delta^{15}N$ values with increasing size classes in the Baltic Sea (Rolff 2000). The use of size fractions in food web studies makes the assumption that predator-prey relationships are determined by body size (Bănaru 2013). Therefore, these analyses represent approximations of trophic interactions rather than perfect portrayals of the intricacies within a food web (Jennings *et al.* 2002).

Variations in $\delta^{13}C$ and $\delta^{15}N$ have been related to local environmental conditions in previous studies (Rolff 2000, Bănaru *et al.* 2013, Yang *et al.* 2017). For example, seasonal variations in zooplankton $\delta^{13}C$ and $\delta^{15}N$ values in the northwest Mediterranean Sea were consistent with the fluctuations in local environmental factors throughout the year (Bănaru *et al.* 2013). An increase in zooplankton isotope signatures during the summer and fall were linked with low chlorophyll *a*, nitrate, and phosphate concentration. Zooplankton $\delta^{13}C$ and $\delta^{15}N$ in the western tropical North Pacific Ocean were also correlated, however positively, with local environmental factors, such as chlorophyll *a*, nitrate, and phosphate (Yang *et al.* 2017). This study concluded that there is an ecohydrographic influence on zooplankton production and stable isotopic composition at the base of the food web in the western North Pacific.

Since seasonal fluctuations in temperature and nutrient concentrations can influence zooplankton production (Wainright and Fry 1994, Rolff 2000, Calbet *et al.* 2001, Bănaru *et al.* 2013, Yang *et al.* 2017), it is imperative to understand how

seasonality influences zooplankton trophic ecology. Therefore, seasonal variation should be addressed in stable isotopic studies of plankton as seen in Wainright and Fry (1994), which examined the seasonal variation in carbon and nitrogen stable isotope signatures of plankton in a shallow coastal environment, Woods Hole Harbor, Massachusetts. Both carbon and nitrogen isotopic values of particulate organic matter (POM) and plankton varied temporally on time scales of weeks to months (Wainright and Fry 1994). Tracking seasonal variation in isotopic signatures can help examine how blooms and other environmental factors influence zooplankton diets. While a single sampling event only provides a snapshot of trophic interactions within a food web, a long-term examination of seasonal variation can provide a more comprehensive view of a food web.

In addition to seasonal variation, plankton exhibit spatial variation in their isotopic signatures (Mullin *et al*. 1984, Bănaru *et al.* 2013, Mompeán *et al.* 2013, Kürten *et al.* 2015, Yang *et al.* 2017). For example, copepods and chaetognaths from the Southern California Bight had higher $\delta^{15}N$ than those from the North Pacific Central Gyre (Mullin *et al.* 1984). Spatial differences in zooplankton isotopic signatures in the western tropical North Pacific Ocean were also reported, with generally higher isotopic values in the North Equatorial Counter Current and the North Equatorial Current and lower values in the Subtropical Counter Current (Yang *et al.* 2017). These spatial variations in $\delta^{13}C$ and $\delta^{15}N$ values were similar across all five size classes of zooplankton surveyed (100-200, 200-500, 500-1000, 1000-2000, >2000 μm) and were likely due to local differences in environmental factors (Yang *et al.* 2017). The relationship between environmental conditions

and isotopic values of plankton was also examined in this study. In particular, positive correlation was found between isotopic values and concentrations of nitrate, phosphate, and chlorophyll *a* (Yang *et al.* 2017).

The site for this study, Mission Bay, San Diego, California, like other seasonally hypersaline estuaries, exhibits spatial and temporal gradients of environmental parameters. During the warm, dry summers, the temperature and salinity increase in the inner regions of the bay, while during the cool, wet winters, the inner regions decrease in temperature and salinity compared to the mouth of the bay. Due to these spatial and temporal gradients, Mission Bay is a suitable study system in which to evaluate how zooplankton trophic ecology fluctuates across a range of environmental conditions. Variation in zooplankton community composition in Mission Bay has been examined previously in relation to tidal velocity, temperature, salinity, and rainfall (Kittinger 2006, Elliott and Kaufmann 2007, Griggs 2009, Shapiro 2018). During the two-year period from July 2002 to June 2004, significant spatial and temporal variation in the zooplankton species composition of Mission Bay was observed. Spatial variation was also determined to explain less of the variation in zooplankton community composition than temporal variation. Predator-prey interactions among plankton within Mission Bay, however, are largely unknown.

The main objectives of this study were to 1) characterize zooplankton community composition and taxonomic diversity and 2) examine spatial and temporal variation in the diets of zooplankton species in Mission Bay using carbon and nitrogen stable isotope analysis. Specifically, the spatial and temporal

variation in $\delta^{13}C$ and $\delta^{15}N$ values of different zooplankton size classes in Mission Bay were examined. Environmental parameters (temperature, salinity, nutrient concentrations, and chlorophyll *a* concentration) were also analyzed to examine the drivers of zooplankton isotope values and trophic ecology.

2.3 MATERIALS AND METHODS

2.3.1 STUDY SITE

This study was focused in Mission Bay, an estuary in San Diego, CA. Mission Bay has been modified over the past 150 years through river diversion, dredging, and filling (Levin 1984, Dexter and Crooks 2000, Largier *et al.* 2003). As a result, the bay has become one of the most altered coastal systems in southern California (Dexter and Crooks 2000). These modifications have resulted in lower water exchange in the back of the bay due to the distance from the ocean and the presence of a large, artificial island, Fiesta Island, which creates two long, narrow, un-branched channels (Figure 1.1). Due to the restricted connection between Mission Bay and the ocean, there is weak tidal flushing within the inner bay, leading to long residence times (Largier *et al.* 2003).

Similar to other hypersaline estuaries in Mediterranean climates, Mission Bay exhibits a distinct seasonal cycle. Mission Bay is a shallow, vertically well-mixed estuary, but can become horizontally stratified particularly in the summer. During the warm, dry summers, there is an absence of freshwater inflow, with warmer water dominating the inner bay, and cooler water near the mouth (Largier *et al*. 2003). Evaporation exceeds the supply of fresh water via precipitation and

runoff in the restricted parts of the bay, and consequently, the back of the bay becomes hypersaline in the late summer (Levin 1984, Largier *et al*. 1997).

2.3.2 FIELD METHODS

Three sites within Mission Bay (Figure 1.1) were sampled at monthly intervals for one year (April 2017 – April 2018). Sampling occurred at monthly intervals to capture trends in zooplankton population dynamics and changes in diet. The maximum generation time (egg to adult) in a laboratory setting of the copepod, *Oithona similis*, the most commonly found species in Mission Bay, has been reported as 19.7 days (Sabatini and Kiørboe 1994). Although this estimate was derived from a laboratory setting, it is likely that the growth rates of the local zooplankton are slow enough to capture trends in zooplankton populations with monthly sampling. Additionally, monthly sampling allowed for the identification of seasonal changes in environmental conditions, as trends in temperature and salinity show strong seasonal patterns. Three sites were chosen because they vary in distance from the mouth of the bay and degree of tidal influence: Ventura Point in the front of the bay with strong tidal flushing; Fiesta Bay, farther from the mouth and with weaker tidal flushing; and Hilton Dock in the back of the bay, with very little tidal flushing. The three sites also vary in depth. Although dependent on tidal phase, the approximate depths of each site are as follows: Ventura Point (5.5 m), Fiesta Bay (4 m), and Hilton Dock (3 m).

Zooplankton tows and environmental conditions were measured at each sampling event. A GPS unit was used to locate each sampling site. Water depth was measured with an acoustic gauge, and turbidity was measured using a Secchi

disk. Water samples were taken at the surface and 0.5 m above the seafloor. Three liquid-in-glass thermometers were used to measure the temperature of each water sample, while a refractometer was used to measure salinity. A 1 L water sample taken at each depth was then stored in brown Nalgene bottles on ice for later chlorophyll and nutrient analyses. A remote sonde with a digital multimeter was used to create water column profiles of temperature (°C), salinity (PSU), dissolved oxygen (mgL^{-1}), conductivity ($S\ m^{-1}$), and turbidity (Ntu) at 0.5 m increments.

Once the water sampling was completed at each site, two plankton tows were conducted. A 0.5 m diameter net with 50 μm mesh was used and each tow lasted for 5 minutes, while the speed of the boat was recorded from a speedometer. The numerical reading on the counter of a flowmeter, as well as GPS coordinates, were also recorded before and after each tow. The sample collected in the cod end of the net was then stored in a labeled Nalgene bottle and placed in a cooler with ice. Samples from both net tows were later combined for further analysis.

2.3.3 LABORATORY METHODS

Plankton Sieving and Stable Isotope Analysis

On the day of collection, samples were sieved in the laboratory into 53-120, 120-250, 250-475, 475-1000 μm size classes using stackable plankton sieves and deionized water for rinsing. Approximately, half of the sample in each size class was placed in a weigh boat and dried for stable isotope analysis. The remaining half of the sample was rinsed with deionized water into a 3.7%

buffered formaldehyde solution in a 500 mL jar for later identification. The weigh boats containing the size-sorted samples were air dried for two days. A dried subsample of ~1 mg was placed in tin capsules and sent to the University of California Davis Stable Isotope Facility for carbon and nitrogen stable isotope analysis.

Dried samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ isotopes using a PDZ Europa ANCA-GSL elemental analyzer and a PDZ Europa 20-20 isotope ratio mass spectrometer. Samples were combusted at 1000°C in a reactor packed with chromium oxide and silvered copper oxide. Oxides were then removed from the samples in a reduction reactor (reduced copper at 650°C). Next, and CO_2 and N_2 were separated on a Carbosieve GC column (65°C, 65 mL min^{-1}), followed by isotope-ratio mass spectrometry (IRMS). During IRMS analysis, replicates of at least two different laboratory standards were used as controls. The laboratory standards had been previously calibrated against NIST Standard Reference Materials and chosen to be compositionally comparable to the samples. The isotope ratio of each sample was first measured relative to the reference gases analyzed with each sample. These values were then corrected based on the known values of the laboratory standards. The standard deviation was 0.2 permil for $\delta^{13}C$ and 0.3 permil for $\delta^{15}N$. The ultimate delta values were calculated relative to the international standards, VPDB (Vienna PeeDee Belemnite) for carbon and Air for nitrogen (University of California Davis Stable Isotope Facility; https://stableisotopefacility.ucdavis.edu/13cand15n.html).

Chlorophyll a *Analysis*

Water samples from each site were analyzed to determine concentrations of chlorophyll *a* and phaeopigments. Within 6 hours of water collection, 250 mL of each water sample were filtered through a GF/F glass fiber filter. The filter was then placed in aluminum foil and stored at -80°C for later analysis. Twenty-four hours before analysis, each filter was placed in a centrifuge tube with 10 mL of 90% acetone. The centrifuge tubes were placed back in the freezer for approximately 24 hours. Then, 1 mL of the liquid was transferred to a glass vial by pipet. Each vial was wiped with a Kimwipe before being placed into a fluorometer (Turner Biosystems Modulus Fluorometer 9200-000) for analysis. Samples were run against a recently created standard curve. After the first reading, the same vial was removed and 30μL of 0.1 N HCl was added. The vial was gently shaken for 90 seconds before being put back into the fluorometer for a second reading. The first reading determined the concentration of phaeo-pigments in the sample, while the difference between the two readings determined the concentration of chlorophyll *a* in the sample.

Nutrient Analysis

Water samples from each site were analyzed to determine nutrient concentration. Two hundred and fifty mL of each water sample was filtered through a 47 mm diameter, 0.43 μm filter (GE Healthcare 1005042). The filtered water samples were stored in labeled 50 mL tubes at -20 °C for later analysis. Flow injection analysis was performed with a QuikChem 8000 FIA+ nutrient analyzer to determine the concentration of silicate, phosphate, nitrate, and

ammonia in each sample. All samples were run against a standard curve. Reagent sets were purchased from Lachat Instruments for analysis of nitrate, phosphate, and ammonia (Product No. 52903, 52902, 52904). The silicate reagents were prepared in the lab following standard procedures provided by Lachat Instruments.

Plankton Identification

Approximately, half of the plankton samples were stored in 3.7% buffered formaldehyde in 500mL jars. Plankton were identified using a compound microscope at 100x magnification. A subsample of 1 mL from each plankton sample was placed on a Sedgewick-Rafter slide. Three slides were examined from each sample, and 10 non-overlapping, randomly-selected fields of view were viewed per slide. All organisms were identified to the lowest possible taxon. Rarefaction curves were constructed for each sample by plotting the number of species identified against the number of individuals identified in the sample. Once the curve reached a clear asymptote, the sample was considered to be an effective representation of the species composition of the community (Simberloff 1978, Gotelli and Colwell 2011).

2.3.4 DATA ANALYSIS

Statistical analyses were performed using R. Stable isotope ratios across size classes, sites, months, and seasons were compared using a Kruskal-Wallis test. In order to determine which sample group was significantly different, a *post hoc* Dunn's test was performed. A comparison of surface environmental parameters among sites and among months was also conducted using Kruskal-

Wallis and post-hoc Dunn's tests. Significant correlations between $\delta^{15}N$ or $\delta^{13}C$ values and environmental parameters at the surface were determined by a non-parametric bivariate analysis, Spearman rank correlation analysis. A multivariate analysis, canonical correlation analysis, was also performed to determine correlation coefficients between $\delta^{15}N$ or $\delta^{13}C$ values and surface environmental parameters.

The relationship between stable isotopic values and taxonomic percent composition of the plankton assemblage was examined using the Spearman's correlation coefficient. The correlation between the density of organisms and surface environmental parameters within each size class was also tested by a Spearman rank correlation analysis. Finally, species diversity at each site within each size class was calculated using the Shannon-Wiener Index. The species diversity, evenness (Shannon's equitability), and richness were all reported.

2.4 RESULTS

2.4.1 WATER QUALITY

Sea surface temperature (SST) measurements from April 2017 through April 2018 ranged from 14-23°C at Ventura Point, 15-25°C at Fiesta Bay, and 15-27°C at Hilton Dock (Figure 2.1.A). The highest mean SST measurement of all three sites occurred in July (mean ± SD = 24.9 ± 1.9°C) and the lowest mean temperature of all sites occurred in February (14.6 ± 0.2°C). For each sampling event, with the exception of December and February, the lowest SST was measured at Ventura Point and the highest at Hilton Dock. In general, the range of SST values among sites across Mission Bay decreased from the beginning of

the study, April 2017 (7.6°C), until December 2017 (0.73°C), followed by an increase in range through April 2018 (6.2°C).

Sea surface salinity (SSS) ranged between 32.3 and 34.5 PSU at Ventura Point and between 32.8 and 34.5 PSU at Fiesta Bay (Figure 2.1.B). There was a greater range in SSS at Hilton Dock, where values ranged from 32.9 to 35.6 PSU. The highest SSS measurement at Hilton Dock occurred in July (35.6 PSU). At all three sites, SSS generally decreased from August through December or January, depending on the site. The greatest range in salinity among sites occurred from July through October, with lower SSS at Ventura Point and higher SSS at Hilton Dock.

The highest chlorophyll *a* concentrations were measured at the surface in July at Ventura Point (65.5 μg L^{-1}), the bottom depth in February at Fiesta Bay (46.5 μg L^{-1}), and the bottom depth in September at Hilton Dock (120.1 μg L^{-1}; Figure 2.2). At Ventura Point, the total pigment concentration was highest from April 2017 through July. In August, the concentration started to decrease, reaching a minimum in December, and then increased from January through April 2018. The total pigment concentrations followed a different pattern throughout the year at Fiesta Bay. The lowest concentrations at Fiesta Bay occurred in June and January, while the highest concentrations occurred in September, February, and March. The total pigment concentrations at Hilton Dock were generally the highest and the most variable among those of the three sites, with an exceptionally high concentration in the near-bottom sample in September.

For all three sites, the concentration of ammonia at the surface was lowest during July (Figure 2.3). Highest ammonia concentrations occurred at the surface in December at Hilton Dock (45.3 μM), January at Ventura Point (43.0 μM), and February at Fiesta Bay (41.5 μM). Surface nitrate concentrations were highest in April 2017 at Fiesta Bay (4.22 μM) and Hilton Dock (2.02 μM), but were highest at Ventura Point in June (5.35 μM; Figure 2.3). The lowest concentrations of nitrate at the surface occurred at Ventura Point in July (0.50 μM), at Fiesta Bay in June (0.41 μM), and at Hilton Dock in July (0.33 μM). The highest concentration of phosphate occurred during April 2017 at the surface for all three sites (VP: 1.67 μM, FB: 1.67 μM, HD: 1.65 μM; Figure 2.3). For two sites, Ventura Point and Hilton Dock, the lowest phosphate concentration occurred at the surface during May (0.37 and 0.05 μM, respectively), while the lowest concentration at Fiesta Bay occurred in July (0.44 μM). The highest concentration of silicate occurred at the surface in March at Ventura Point (78.3 μM), in July at Fiesta Bay (75.4 μM), and in June at Hilton Dock (70.7 μM), while the lowest occurred at Ventura Point in February (26.7 μM), at Fiesta Bay in June (9.80 μM), and at Hilton Dock in April 2017 (33.6 μM; Figure 2.3).

Differences in surface environmental parameters (temperature, salinity, concentrations of nutrients, and chlorophyll *a*) among the three sites and among monthly sampling events were assessed using a Kruskal-Wallis test. Among the sites, significant differences were found in chlorophyll *a* concentration ($p = 0.020$). Chlorophyll *a* concentrations at Ventura Point were significantly lower than those at Hilton Dock. Among the monthly events, significant differences

were found in temperature, salinity, phosphate, and ammonia ($p = 0.002$, $p = 0.015$, $p = 0.023$, $p = 0.006$, respectively). No significant temporal differences were found in nitrate, silicate, or chlorophyll *a* concentration. As expected, surface temperature was highest in the summer and early fall and became cooler in winter and spring (Figure 2.1A). Surface salinity at all three sites decreased from August through December or January, depending on the site, and gradually returned to higher values through April 2018 (Figure 2.1B). Over the sampling period, ammonia concentrations increased at all three sites (Figure 2.3). There was no temporal trend in the concentration of phosphate throughout the sampling period; however, the highest phosphate concentration for all three sites was measured in April 2017, the first sampling event of the study (Figure 2.3).

2.4.2 PLANKTON COMMUNITY COMPOSITION

Plankton samples primarily consisted of copepods, tintinnids, and dinoflagellates. Copepods comprised the largest proportion of zooplankton across all months (Figure 2.4). As seen in Figure 2.4, the most common copepod species each month was *Oithona similis*. Another common taxon of zooplankton observed during the study period was tintinnids. Throughout the study, the number of each tintinnid species, including *Favella* spp., *Helicostomella endentala*, and *Tintinnopsis campanula*, changed markedly from month to month (Figure 2.4). Additionally, as depicted in Figure 2.4, there was a great rise in the percentage of bivalve veligers during the months of August and September.

In a comparison across size classes, copepods were observed to be the most common taxon of zooplankton within all four size classes (Table 2.1). The

percent composition of copepods increased with increasing size class was as follows: 53-120 µm (60.1%), 120-250 µm (75.7%), 250-475 µm (82.2%), 475-1000 µm (91.9%). Various copepod life stages (eggs, nauplii, juveniles, and adults) were found in all size classes, but only juveniles and adults were found in the largest size class (475-1000 µm). Tintinnids and dinoflagellates were also found in all size classes, except the largest one. The two smallest size classes contained phytoplankton, while the two largest did not. The second largest size class (250-475 µm) contained the mixotrophic dinoflagellate *Ceratium* spp., while the largest size class was the only class entirely consisting of heterotrophs.

At all three sites, the smallest size class (53-120 µm) had the highest mean Shannon-Wiener diversity and taxonomic richness, while the largest size class (475-1000 µm) had the lowest taxonomic richness (Table 2.2). Among sites, the highest mean taxonomic richness occurred at Ventura Point, decreasing with increasing distance from the mouth to the back of the bay. Based on percent composition, the dominant taxon at all three sites throughout the year was the cyclopoid copepod *Oithona similis*, which comprised 27.9-30.3% of all zooplankton at each site (Table 2.3). The next two dominant groups at all sites were copepod nauplii and copepod eggs. Both Fiesta Bay and Hilton Dock also had the same fourth and fifth most dominant taxa, the tintinnids, *Tintinnopsis campanula* and *Favella* spp. By contrast, at Ventura Point, the fourth and fifth most dominant species were *Acartia californiensis* and *Ceratium lineatum,* respectively (Table 2.3).

In each size class, a significant correlation was found between organismal density and one or more environmental parameters (Table 2.4). Organismal density correlated significantly and positively with ammonia concentration across all four size classes: 53-120 µm (rho: 0.52; *p*-value: 0.00083), 120-250 µm (rho: 0.53; *p*-value: 0.0012), 250-475 µm (rho: 0.53; *p*-value: 0.00061), 475-1000 µm (rho: 0.51; *p*-value: 0.00095). There was also a significant negative correlation between organismal density and salinity among the following three size classes: 53-120 µm (rho: -0.40; *p*-value: 0.014), 250-475 µm (rho: -0.34; *p*-value: 0.036), 475-1000 µm (rho: -0.46; *p*-value: 0.0036). Organismal density correlated significantly and negatively with temperature in the two largest size classes: 250-475 µm (rho: -0.39; *p*-value: 0.015), 475-1000 µm (rho: -0.40; *p*-value: 0.012). Additionally, there was a significant positive correlation between organismal density and chlorophyll *a* in the smallest size class: 53-120 µm (rho: 0.39; *p*-value: 0.017).

2.4.3 STABLE ISOTOPE VALUES OF PLANKTON

Although $\delta^{15}N$ values in larger size classes were slightly higher than those in smaller size classes, no significant difference in $\delta^{15}N$ values among size classes were found using a Kruskal-Wallis one-way analysis of variance. However, there was a significant difference in $\delta^{13}C$ values among size classes at Ventura Point (Kruskal-Wallis test, $p = 0.04$; Table 2.5). A *post hoc* Dunn's test indicated that $\delta^{13}C$ was significantly different between two specific size classes: 53-120 µm and 250-475 µm, ($p = 0.04$). The $\delta^{13}C$ values were greater in the smallest size class (53-120 µm; mean = -19.1‰; median = -21.3‰) compared to the second largest

size class (250-475 µm; mean = -21.5‰; median = -22.6‰). Means and standard deviations for both carbon and nitrogen stable isotope values for each size class can be found in the appendix (Table A.1).

A test for spatial variation in stable isotope values found significant differences in $\delta^{13}C$ in two size classes: 53-120 and 120-250 µm ($p = 0.006$ and $p = 0.001$, respectively; Table 2.6). For both of these size classes, a *post hoc* Dunn's test indicated that $\delta^{13}C$ values at Ventura Point were significantly higher compared to the other sites. The size class 53-120 µm at Ventura Point had a median $\delta^{13}C$ of -18.9 ± 1.6‰, while Fiesta Bay and Hilton Dock had medians of -22.7 ± 1.8 and -24.1 ± 0.9‰, respectively. The size class 120-250 µm at Ventura Point had a median $\delta^{13}C$ of -19.9‰ compared to medians of -23.8 and -24.4‰ at Fiesta Bay and Hilton Dock, respectively. There was also a significant difference in $\delta^{15}N$ among sites in the second largest size class (250-475 µm). A *post hoc* Dunn's test did not identify any individual site that was significantly different from the other two. However, a *post hoc* Mann-Whitney U test was performed to determine significant differences in $\delta^{15}N$ among the sites, and the $\delta^{15}N$ values at Ventura Point were found to be significantly lower than those at the other two sites (Table 2.6).

Values of $\delta^{13}C$ and $\delta^{15}N$ were analyzed statistically across both months and seasons (spring: Mar-May, summer: Jun-Aug, fall: Sep-Nov, winter: Dec-Feb, to examine temporal variation. A Kruskal-Wallis test indicated significant differences in $\delta^{13}C$ values among months ($p = 8.04E-08$; Table 2.7), as well as a significant difference in $\delta^{15}N$ values among months ($p = 4.24E-06$; Table 2.7).

Additionally, there was a significant difference in $\delta^{13}C$ values among seasons (Kruskal-Wallis test; $p = 0.0012$; Table 2.7). According to a *post-hoc* Dunn's test, $\delta^{13}C$ values during the summer were significantly higher than those during the spring ($p = 0.0022$), and those during the fall were also significantly higher than those during the spring ($p = 0.0068$). There was also significant variation in $\delta^{15}N$ values among seasons (Kruskal-Wallis test; $p = 0.0079$; Table 2.7). A *post-hoc* Dunn's test revealed that the $\delta^{15}N$ values during the winter were significantly higher than those during the fall ($p = 0.0055$) and significantly higher than those during the summer ($p = 0.046$).

An association between stable isotopic values and taxonomic percent composition of the plankton assemblage was examined using the Spearman's correlation coefficient. At Ventura Point, there was significant positive correlation between $\delta^{13}C$ and *Ceratium lineatum* within the smallest size class (53-120 µm) and significant negative correlation between $\delta^{13}C$ and *Acartia californiensis* within the largest size class (475-1000 µm; Table 2.8). At Fiesta Bay, there was a significant negative correlation between $\delta^{13}C$ and *Oithona similis* within the size class, 250-475 µm. Additionally, within the largest size class (475-1000 µm) at Fiesta Bay, there was significant negative correlation between $\delta^{13}C$ and *Acartia californiensis* and significant positive correlation between $\delta^{15}N$ and *Acartia californiensis* (Table 2.8). Hilton Dock, there was significant negative correlation within the smallest size class, 53-120 µm, between $\delta^{15}N$ and *Oithona similis*, as well as positive correlation within the

largest size class, 475-1000 μm, between $\delta^{15}N$ and *Acartia californiensis* (Table 2.8).

Finally, the relationship between stable isotope values of plankton and environmental variables was explored using two approaches. First, a Spearman's rank correlation test was run to examine correlation between the stable isotope values of each size class and measured environmental parameters at the surface. No significant correlation was found between $\delta^{13}C$ values and environmental parameters. However, $\delta^{15}N$ values of each size class correlated significantly with one or more environmental parameters (Table 2.9). There was a significant positive correlation between $\delta^{15}N$ and ammonia concentration for two of the size classes, 53-120 μm ($p = 0.0066$) and 250-475 μm ($p = 0.036$). There was also a significant negative correlation between $\delta^{15}N$ and nitrate concentration in the two size classes, 120-250 μm ($p = 0.0013$) and 250-475 μm ($p = 0.00035$). Additionally, the smallest size class (53-120 μm) had a significant negative correlation between $\delta^{15}N$ and salinity ($p = 0.035$) and the largest size class (475-1000 μm) had a significant negative correlation between $\delta^{15}N$ and silicate concentration ($p = 0.0042$).

The second approach to examine correlation between the isotopic signatures and surface environmental parameters was a canonical correlation analysis (CCA) for each size class of plankton. In a visual representation of a CCA analysis, the length of each vector reflects the strength of correlation and the direction of each vector reflects the direction of the correlation. The canonical correlation analysis for the smallest size class (53-120 μm) identified a positive

correlation between $\delta^{15}N$ and ammonia ($\rho = 0.45$), a positive correlation between $\delta^{13}C$ and chlorophyll *a* ($\rho = 0.23$), and a negative correlation between $\delta^{15}N$ and salinity ($\rho = -0.40$; Figure 2.5). The CCA for the next larger size class (120-250 μm) also revealed a positive correlation between $\delta^{15}N$ and ammonia ($\rho = 0.27$) and a negative correlation between $\delta^{15}N$ and salinity ($\rho = -0.27$; Figure 2.5). The second largest size class (250-475 μm), according to CCA, had a positive correlation between $\delta^{15}N$ and ammonia ($\rho = 0.22$) and a negative correlation, albeit weak, between $\delta^{15}N$ and nitrate ($\rho = -0.076$; Figure 2.6). The largest size class (475-1000 μm) had a negative correlation between $\delta^{15}N$ and silicate ($\rho = -0.42$; Figure 2.6).

2.5 DISCUSSION

During this study, spatial and temporal changes were observed in the stable isotopic composition of plankton species in Mission Bay, as well as variation with size class and correlation with environmental conditions. At Ventura Point, significant differences were found in $\delta^{13}C$ values among size classes, with the $\delta^{13}C$ of the smallest size class (53-120 μm) significantly higher than in the second largest size class (250-475 μm). For $\delta^{15}N$, there was a slight increase in larger size classes, though this increase was not significant. This result is surprising, as we expected to see measurable changes in isotopic composition that would reflect trophic structure among size classes. Across sites, significant differences were detected in isotopic values: the two smallest size classes, 53-120 and 120-250 μm, had the highest $\delta^{13}C$ at Ventura Point, and the second largest size class (250-475 μm) had significantly lower $\delta^{15}N$ values at

Ventura Point. Temporal variation was also detected, as both $\delta^{13}C$ values and $\delta^{15}N$ values showed significant changes across months and seasons. Additionally, significant correlations were found between $\delta^{15}N$ values of each size class and one or more environmental parameters, suggesting that changes in environmental conditions may explain trends in $\delta^{15}N$. Most notably, $\delta^{15}N$ values were strongly related to ammonia and nitrate concentration, suggesting that nitrogenous nutrient utilization by phytoplankton was a major mechanism influencing the isotopic values of the zooplankton. Overall, this study provides insight into the trophic structure of the zooplankton population in Mission Bay and the environmental parameters that drive the stable isotope values of the zooplankton.

2.5.1 PLANKTON COMMUNITY COMPOSITION

The greatest taxonomic richness among the three sites occurred at Ventura Point and decreased with increasing distance from the mouth to the back of the bay. These spatial differences in diversity were also observed in recent studies conducted in Mission Bay (Swope 2005, Elliott and Kaufmann 2007, Shapiro 2018). Additionally, in accordance with recent studies (Elliott and Kaufmann 2007, Shapiro 2018), copepods and tintinnids were the most predominant taxa in the samples collected. Copepods made up the largest proportion of zooplankton across all size classes (Table 2.1). Elliott and Kaufmann (2007) observed copepods and tintinnids in at least 25% of the plankton samples collected biweekly over a 2-year period at six different sites in Mission Bay. *Oithona similis* and *Acartia californiensis* were the most common copepod species observed in our study. *Oithona similis,* in particular, had the highest percent

occurrence of all species across the three sites. Previous studies found this species to be the most abundant copepod species throughout the bay as well (Kittinger 2006, Kaufmann and Elliot 2007, Shapiro 2018).

Tintinnids (ciliates), a common prey source for *Oithona similis* and *Acartia californiensis* (Bollens and Penry 2003, Nishibe *et al.* 2010), were observed at all three sites during the study, and two tintinnid taxa were ranked in the five most dominant taxa at Fiesta Bay and Hilton Dock. However, tintinnids were not as abundant in the front of the bay at Ventura Point. Also, the abundance of tintinnid species was not consistent across the bay and varied temporally from month to month. The spatial and temporal variation in the abundance of these tintinnid species likely had an effect on the isotopic values of their potential predators, *Oithona similis* and *Acartia californiensis* (see section 2.5.4). At Ventura Point, one of the most prevalent species was *Ceratium lineatum*, a mixotrophic marine dinoflagellate, which was the fifth most dominant taxon at this site. It was not surprising that a greater abundance of this marine species was observed at Ventura Point, where there is the most coastal oceanic influence, as well as the least fresh water input of the three sites.

2.5.2 ZOOPLANKTON TROPHIC STRUCTURE

Although there were significant differences in $\delta^{13}C$ values among size classes, there was no increase in $\delta^{13}C$ with increasing size class. The $\delta^{13}C$ values of the smallest size class (53-120 µm) were significantly higher than those of the second largest size class (250-475 µm) at Ventura Point (median $\delta^{13}C$ of -18.9 vs. -21.3‰; Table 2.5). Unlike our findings, other studies reported a general increase

in $\delta^{13}C$ with increasing size class (Bănaru *et al.* 2013, Yang *et al.* 2017). Bănaru *et al.* (2013) found that $\delta^{13}C$ followed a general, though not consistent, increasing trend with increasing size. The lowest $\delta^{13}C$ values measured by Bănaru *et al.* (2013) were observed in the 200-300 μm size class, the second smallest size class. Yang *et al.* (2017) found a small increase in $\delta^{13}C$ with increasing size class, although this increase was not significant. In isotopic studies, an increase of 1‰ in $\delta^{13}C$ is thought to reflect an increase in trophic position (Rolff 2000, Jennings *et al.* 2002, Yang *et al.* 2017).

The difference in $\delta^{13}C$ between the two size classes, 53-120 and 250-475 μm (median $\delta^{13}C$ of -18.9‰ vs. -21.3‰) in this study is likely due to differences in carbon sources for these two size classes. Mission Bay is an estuarine ecosystem, with both marine and fresh water inputs, which should provide different carbon sources and distinct $\delta^{13}C$ values at the base of the food web. Previous studies have observed that temperate marine phytoplankton have higher $\delta^{13}C$ values (-18 to -24‰; Haines and Montague 1979, Gearing *et al.* 1984, Fry and Sherr 1989) compared to estuarine phytoplankton (-24 to -30‰; Sherr 1982, Tan and Strain 1983, Fry and Sherr 1989). Within the smallest size class (53-120 μm), there was a significant positive correlation between $\delta^{13}C$ and the percentage of *Ceratium lineatum*, a mixotrophic marine dinoflagellate (Barton *et al.* 2013), at Ventura Point (Table 2.8). The strong presence of this marine dinoflagellate in the 53-120 μm size class and its absence in the 250-475 μm size class may help to explain the difference in $\delta^{13}C$ between the two size classes.

Overall, there was a small increase in $\delta^{15}N$ in larger size classes compared to the smaller size classes. However, these differences in $\delta^{15}N$ among size classes were not significant (Table 2.5), unlike results from similar plankton studies (Bănaru *et al.* 2013, Yang *et al.* 2017). This is a surprising result, as SIA is frequently used to examine food web structure. Previous studies have shown a consistent increase in ^{15}N in consumers with increasing trophic level due to the excretion of the lighter isotope, ^{14}N, as a byproduct of protein synthesis (Kling *et al.* 1992, Post 2002). The absence of significant differences in $\delta^{15}N$ values among size classes suggests that the base of the Mission Bay planktonic food web, as represented by plankton within these size classes, is not strongly size-structured, or that $\delta^{15}N$ values cannot delineate trophic structure in the lower planktonic portion of this food web.

If this community were strongly size-structured, then the size classes would be expected to include taxa at different trophic levels (Pope *et al.* 1994) and have significantly different $\delta^{15}N$ values (Owens 1987). One possible explanation for the lack of significant differences in $\delta^{15}N$ among classes is bias within size class selection. The size classification parameters used in this study were based on previous studies, but may not be indicative of local trophic groups. If the organisms were categorized into different size ranges, then perhaps significant variation in $\delta^{15}N$ among size classes may have been observed. However, since there was a large range (53 to 1000 µm) between the smallest and largest size classes, significant differences in $\delta^{15}N$ values were expected between these two size classes if the planktonic food web is size-structured between 53

and 1000 μm. Another possible explanation for the homogeneity of $\delta^{15}N$ among size classes in this study is a prevalence of omnivory. The most abundant taxon across all three sites (Table 2.3) and in all four size classes in either juvenile or adult form (Table 2.1) was the cyclopoid copepod *Oithona similis*, which has been shown to feed preferentially on ciliates, but also on dinoflagellates, diatoms, and other nano-microplankton (Castellani *et al.* 2005). *Oithona similis* may be consuming organisms at different trophic levels, including autotrophs with lower $\delta^{15}N$ values and heterotrophs with higher $\delta^{15}N$ values. Therefore, it is likely that either the trophic interactions among the organisms in these size classes are not size dependent or that $\delta^{15}N$ analysis cannot define the trophic levels within the lower planktonic portion of the food web due to a prevalence in omnivory.

2.5.3 SPATIAL VARIATION OF PLANKTON STABLE ISOTOPE VALUES

There were significant spatial differences in $\delta^{13}C$ in the two smallest size classes, 53-120 and 120-250 μm, with highest values measured at Ventura Point (Table 2.6). The spatial disparity within these two size classes may be attributed to differences in carbon sources among the sites. Since higher $\delta^{13}C$ values have been reported for temperate marine phytoplankton (-18 to -24‰; Haines and Montague 1979, Gearing *et al.*1984, Fry and Sherr 1989) compared to river-estuarine phytoplankton (-24 to -30‰; Sherr 1982, Tan and Strain 1983, Fry and Sherr 1989), we would expect that plankton from the site closest to the mouth of the bay (Ventura Point) would have higher $\delta^{13}C$ values than plankton from the back of the bay (Hilton Dock). Ventura Point is the site closest to the mouth of

the bay and experiences the most tidal influence and least fresh water input. Therefore, zooplankton at Ventura Point may be sourcing carbon more from marine phytoplankton and less from estuarine phytoplankton than those at the other two sites, leading to higher $\delta^{13}C$ values at this site.

Ceratium lineatum, a marine dinoflagellate, was found to be one of the most dominant taxa in the smallest size class (53-120 μm) at Ventura Point, but not at Fiesta Bay or Hilton Dock (Table 2.1). A significant positive correlation was also found between $\delta^{13}C$ and the percentage of *Ceratium lineatum* at Ventura Point within the 53-120 μm size class (Table 2.8). The strong presence of this marine dinoflagellate at Ventura Point could explain the significantly higher $\delta^{13}C$ values within the 53-120 μm size class at this site. It could also explain the significantly higher $\delta^{13}C$ values within the 120-250 μm size class at Ventura Point, if the organisms in this size class are sourcing carbon from the 53-120 μm size class. However, future research is needed to explicitly test this hypothesis. Future studies that examine differences in $\delta^{13}C$ values between carbon sources (*e.g.* fresh water vs. marine) in Mission Bay would be helpful for answering this question.

There was also significant spatial variation in $\delta^{15}N$ values in the second largest size class (250-475 μm), with significantly lower $\delta^{15}N$ values at Ventura Point than at Fiesta Bay or Hilton Dock (Table 2.6). This size class (250-475 μm) was mostly composed of copepods (Table 2.1), particularly *Oithona similis.* *Oithona similis* has been observed to feed on dinoflagellates, diatoms, and other nano-micorplankton, but it preferentially feeds on ciliates, which include tintinnid

species (Castellani *et al.* 2005). According to community composition analysis conducted during this study, tintinnids were abundant at Fiesta Bay and Hilton Dock, but not Ventura Point. If the lack of tintinnids at Ventura Pont led to diet switching by *Oithona similis*, these abundant copepods could be feeding at a lower trophic position at Ventura Point, perhaps explaining the relatively lower $\delta^{15}N$ values in this size class at Ventura Point compared to the other two sites.

2.5.4 TEMPORAL VARIATION OF PLANKTON STABLE ISOTOPE VALUES

The isotopic values of plankton within Mission Bay also showed significant temporal differences, in accordance with previous plankton studies in other regions (Wainright and Fry 1994, Bănaru *et al.* 2013, Yang *et al.* 2017). In a comparison across months, the highest mean $\delta^{13}C$ values were measured in January, while the lowest values were measured in February (Figure 2.7). It's possible that the variation in isotopic values may be explained by changes in the taxonomic composition of plankton in the different size classes throughout time. Thus, we examined correlations between isotopic values and dominant taxa over the study period. We found that $\delta^{13}C$ values showed a significant negative correlation with percent composition of *Oithona similis* (Table A.2). However, we found no significant differences in percent composition of *Oithona similis* among months or seasons (Figure 2.4) and could not conclude that the significant temporal variation in $\delta^{13}C$ values (Table 2.7) was due to changes in percent composition of *Oithona similis.*

Rainfall was also considered as a potential explanation for temporal changes in $\delta^{13}C$ values during the sampling period. In our study, one of the first and largest rainfall events during the sampling period was followed by a large increase in carbon isotopic values at all three sites in January 2018 (Figure 2.7). This rainfall occurred on January 8th, 2018 with 4 cm of rain, and samples were collected on January 21st, 2018. The mean increase of $\delta^{13}C$ in January across all size classes at each site was as follows: Ventura Point 2.9 ± 1.0‰, Fiesta Bay 6.4 ± 1.7‰, and Hilton Dock 8.5 ± 1.0‰. However, previous studies have concluded that freshwater inflow results in ^{13}C depleted terrestrial input, leading to lower $\delta^{13}C$ values of zooplankton (Kibirige *et al.* 2002, Bănaru *et al.* 2013), and rainfall events have been shown to result in terrestrial inputs into Mission Bay (Largier *et al.* 2003). Nevertheless, the only rainfall event greater than 1.5 cm during the study period and the greatest change in $\delta^{13}C$ values occurred at Hilton Dock, the site closest to the fresh water inputs, and the change in $\delta^{13}C$ decreased away from those fresh water sources. Therefore, this first flush of rainfall may have been the driver of this change in $\delta^{13}C$, though not through depletion of ^{13}C, as would be expected.

To examine patterns on time scales broader than months, stable isotope values were grouped into seasons (spring: Mar-May, summer: Jun-Aug, fall: Sep-Nov, winter: Dec-Feb), and significant variation was found in $\delta^{13}C$ values among seasons (Table 2.7). At two of the sites, Ventura Point and Fiesta Bay, higher $\delta^{13}C$ values were observed in summer and autumn. Two other similar plankton studies, Bănaru *et al.* (2013) and Kibirige *et al.* (2002), which were conducted in

the Mediterranean Sea and a South African estuary, respectively, also observed higher $\delta^{13}C$ in the summer and fall. The increase in $\delta^{13}C$ during the summer and fall in those two studies was considered to be related to a lack of rain, which lowered ^{13}C-depleted terrestrial inputs and elevated sea surface temperatures (Bănaru *et al.* 2013 and Kibirige *et al.* 2002). Goericke and Fry (1994) found a significant correlation between $\delta^{13}C$ of phytoplankton and sea surface temperature using data compiled from the open ocean at latitudes ranging from the Arctic to the Antarctic. Goericke and Fry (1994) suggested that this correlation resulted from the temperature dependence of $\delta^{13}C$ on dissolved CO_2, a probable source of inorganic carbon for phytoplankton. However, our study found no significant correlation between $\delta^{13}C$ and SST. The temperature range in Mission Bay may not be large enough to see this effect. Goericke and Fry (1994) analyzed a data set with an SST range from -5 to 35°C, while the SST in our study ranged from 14 to 28°C. The higher $\delta^{13}C$ in the summer and fall in our study could also be related to the lack of rain during summer and fall. The sampling for this study occurred during a historically dry year with less than 0.1 cm of total rainfall during the summer and fall, from June 2017 to November 2017. As previously mentioned, less freshwater inflow could result in a decrease in ^{13}C-depleted terrestrial input as a carbon source for plankton (Kibirige *et al.* 2002, Bănaru *et al.* 2013), which could accordingly lead to an increase in plankton $\delta^{13}C$.

The results of this study indicated that $\delta^{15}N$ values varied temporally on time scales of months and seasons (Table 2.7). In a comparison among seasons, the highest $\delta^{15}N$ values were detected in the winter and the lowest $\delta^{15}N$ values

were observed in the fall. As stated previously, we analyzed relationships between changes in isotopic values over time and changes in dominant taxa. We found that $\delta^{15}N$ values had significant positive correlation with percent composition of *Acartia californiensis* (Table A.2), but we found no significant variation in percent composition of *Acartia californiensis* from month to month or season to season. However, *Acartia californiensis* is known to have a diverse diet, preferentially feeding on ciliates during periods of high food abundance (Bollens and Penry 2003). Throughout the study period, the abundance of tintinnids (ciliates), including *Helicostomella endentala*, *Favella* spp., and *Tintinnopsis campanula*, changed substantially from month to month (Figure 2.4). These temporal changes in the abundance of tintinnids may have led to temporal changes in the feeding activity of *Acartia californiensis*. Consequently, through diet switching, the feeding activity of *Acartia californiensis* may have influenced the nitrogen stable isotope values of the sampled plankton throughout the study period.

2.5.5 ENVIRONMENTAL DRIVERS OF PLANKTON STABLE ISOTOPE VALUES

There was no significant correlation between $\delta^{13}C$ values and environmental parameters in Mission Bay during this study. It is likely that we did not see significant correlations because there may not be a single mechanism driving carbon isotope values in this estuary. Multiple factors, such as light, nutrients, rate of photosynthesis, and temperature, may be affecting the $\delta^{13}C$ of these organisms. As mentioned before, previous studies have found correlations between $\delta^{13}C$ and sea surface temperature, likely due to temperature dependence

of $\delta^{13}C$ in $[CO_2]_{aq}$ (Goericke and Fry 1994). The lack of correlation between $\delta^{13}C$ and SST observed in this study may be attributed to the smaller temperature range (14 to 28°C) compared to the range observed in Goericke and Fry (1994; -5 to 35°C).

There were, however, significant correlations between $\delta^{15}N$ values of each size class and one or more environmental parameters. A significant negative correlation was found between $\delta^{15}N$ and nitrate within the intermediate size classes (120-250 μm and 250-475 μm). Nitrate is one of the major nutrients required by phytoplankton (Sigman *et al.* 2009, Timmermans *et al.* 2004), and previous research has determined that ^{14}N, rather than ^{15}N, is preferentially assimilated by phytoplankton (Waser *et al.* 1998, Sigman *et al.* 2009, Somes *et al.* 2010). So, as nutrient utilization increases, the $\delta^{15}N$ of the remaining pool of nitrate increases, and phytoplankton will eventually begin to assimilate greater amounts of ^{15}N (Waser *et al.* 1998, Sigman *et al.* 2009, Somes *et al.* 2010). Ohman *et al.* (2012) also found a negative correlation between $\delta^{15}N$ of copepod species and nitrate concentration in the Southern California region. Since the $\delta^{15}N$ of phytoplankton can be inversely associated with nitrate concentration by means of nitrate utilization by phytoplankton and isotopic fractionation processes (Waser *et al.* 1998, Ohman *et al.* 2012), enrichment in ^{15}N in plankton in Mission Bay should be consistent with greater nitrate utilization and lower nitrate concentrations. This mechanism may help to explain the relationship between nitrate concentrations and $\delta^{15}N$ values of the plankton in Mission Bay.

A significant negative correlation was also found between $\delta^{15}N$ and silicate within the largest size class (475-1000 μm). Silicate is one of the major nutrients required by phytoplankton (Timmermans *et al.* 2004, Sigman *et al.* 2009), specifically by diatoms (Tsunogai and Watanabe 1983, Egge and Aksnes 1992). Even though our plankton samples were dominated by dinoflagellates, there may be diatoms in Mission Bay that are utilizing silicate but getting grazed down too quickly to be prevalent in our samples. If this assumption is correct, then greater silicate utilization by phytoplankton in the bay would likely coincide with greater nitrate utilization. Accordingly, if greater nitrate utilization causes an increase in $\delta^{15}N$ in plankton, then we should also expect to see a negative correlation between $\delta^{15}N$ and silicate concentration.

Within two size classes (53-120 μm and 250-475 μm), there was a significant positive correlation between $\delta^{15}N$ and ammonia concentration. It is well-known that ammonia is a nitrogenous waste product of zooplankton and other heterotrophs (Jawed 1973, Ikeda and Motoda 1978, Alcaraz *et al.* 1994). Therefore, we would expect that as the number of heterotrophic organisms increases at our sites, the concentration of ammonia would also increase. The significant positive correlation between ammonia concentration and density of organisms within all four size classes supports this hypothesis. Furthermore, as stated previously, an increase in phytoplankton density and the resulting greater nitrate utilization should be consistent with higher $\delta^{15}N$ in plankton (Rau *et al.* 2003, Ohman *et al.* 2012). We may be able to conclude then, that as the density of organisms increases, we would likely see both an increase in ammonia

production and an increase of $\delta^{15}N$ in plankton. These processes would result in the positive correlation we found between ammonia concentration and $\delta^{15}N$ values. However, it is important to note that the $\delta^{15}N$ values are only representative of plankton at the surface and does not reflect other organisms within the rest of the water column. Since the study sites within Mission Bay, a well-mixed estuary (Levin 1984, Largier *et al.* 2003), did not show significant stratification, the ammonia detected at the surface may not be a product of the plankton sampled, but that of other organisms throughout the water column as well as benthic organisms.

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Fig. 1.1 Aerial view of Mission Bay showing the three sampling sites. Image courtesy of Google Maps.

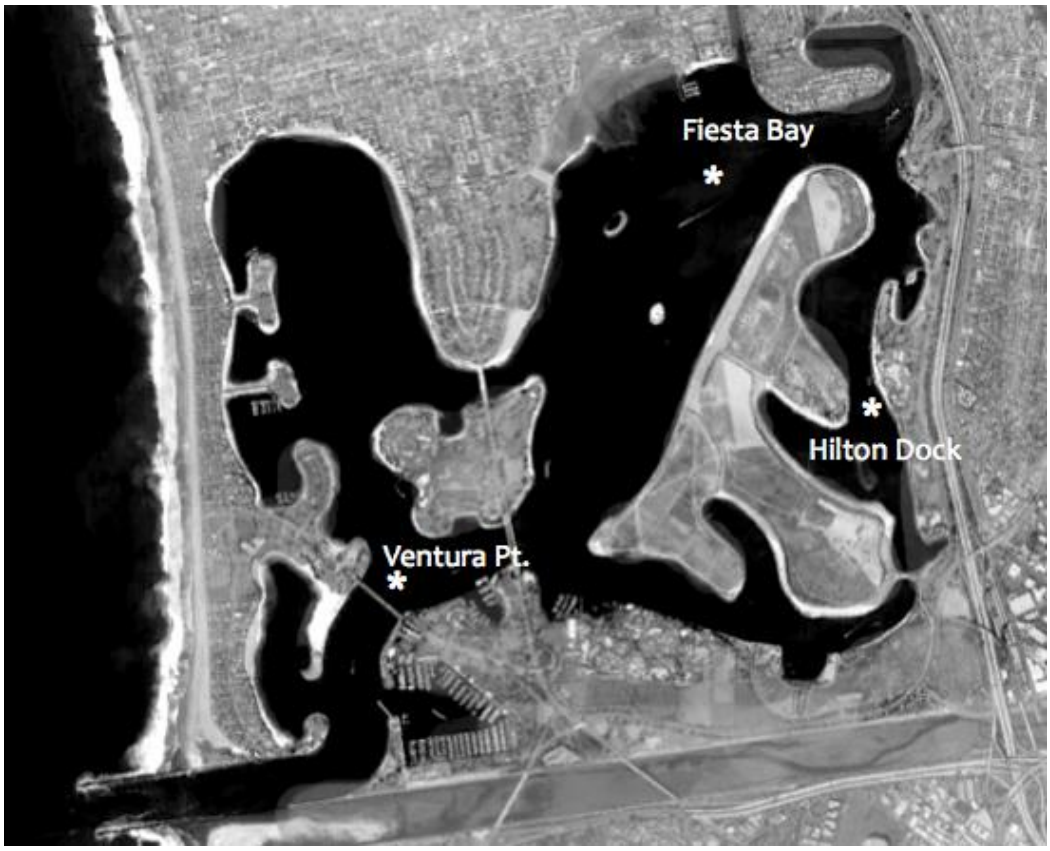
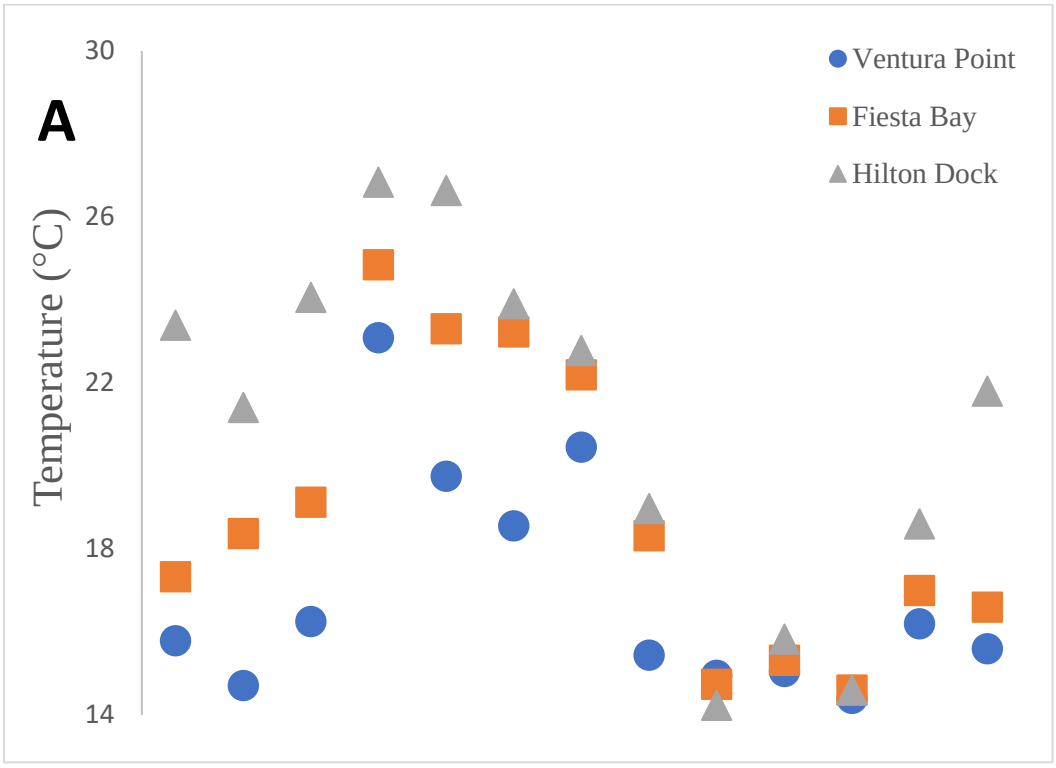
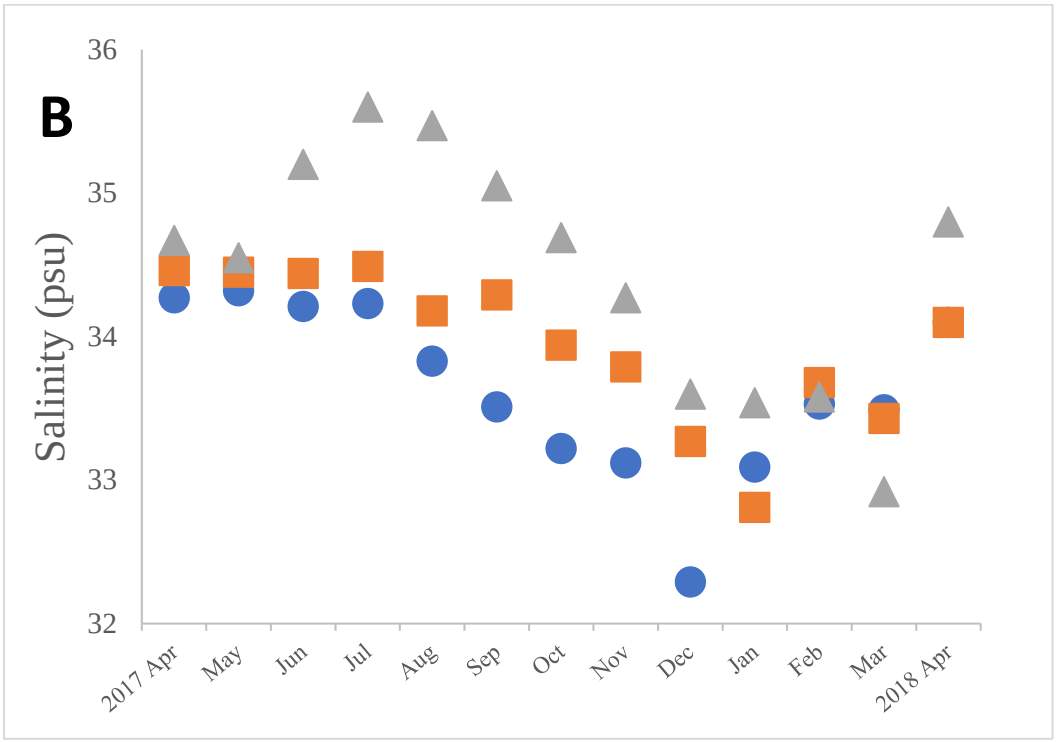

Fiesta Bay
Hilton Dock
Ventura Pt.

Figure 2.1 Sea surface measurements at each station from April 2017 through April 2018. Blue circles represent samples from Ventura Point, orange squares represent samples from Fiesta Bay, and gray triangles represent samples from Hilton Dock. A. Sea surface temperature (°C). B. Sea surface salinity (psu)


A
Temperature (°C)
30
26
22
18
14
Ventura Point
Fiesta Bay
Hilton Dock


B
Salinity (psu)
36
35
34
33
32
2017 Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
2018 Apr

.

Figure 2.2 Chlorophyll *a* and phaeopigment concentrations (μg L^{-1}) at the sea surface (Surf) and 0.5 m above bottom (Btm) at all three sites.

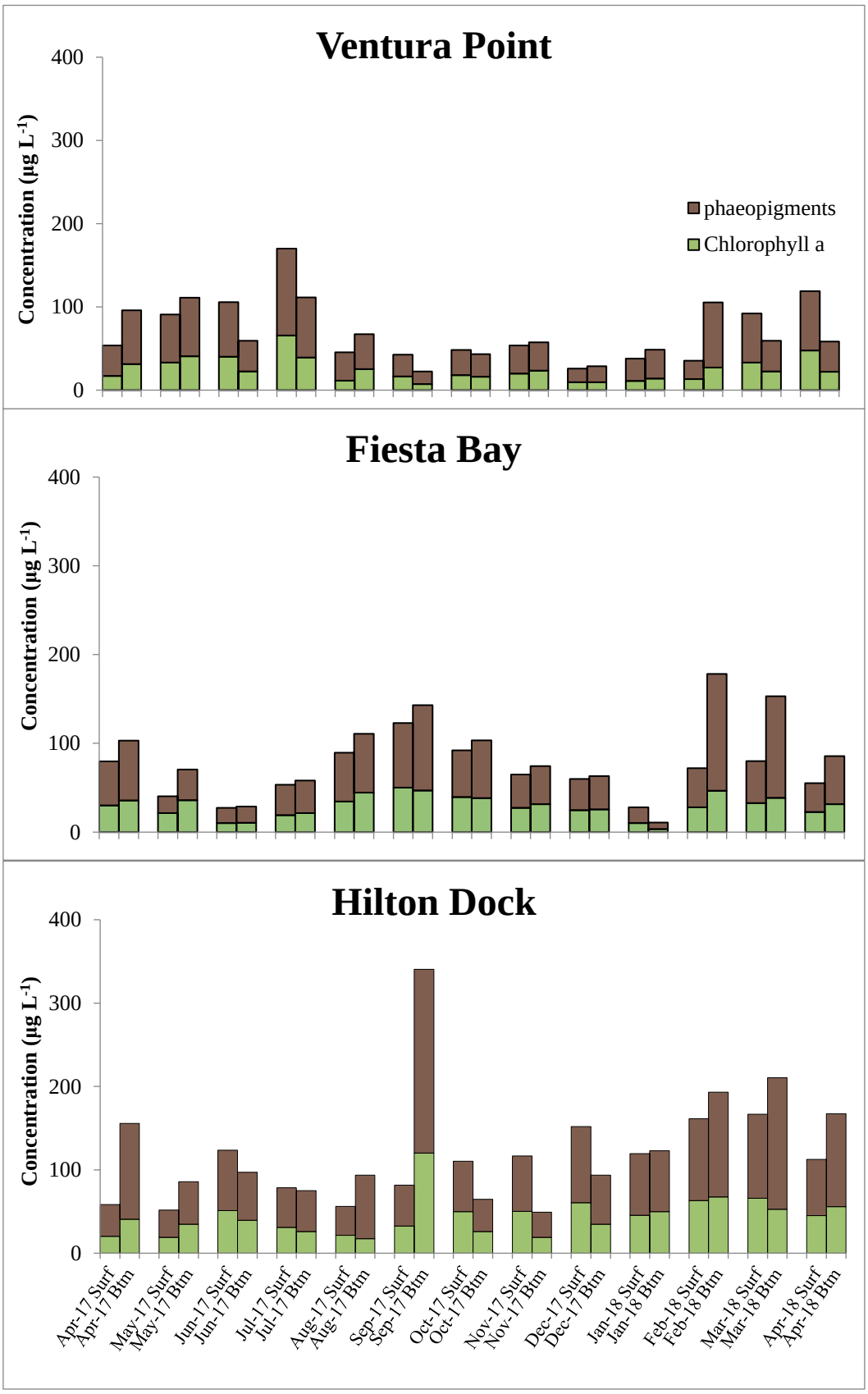
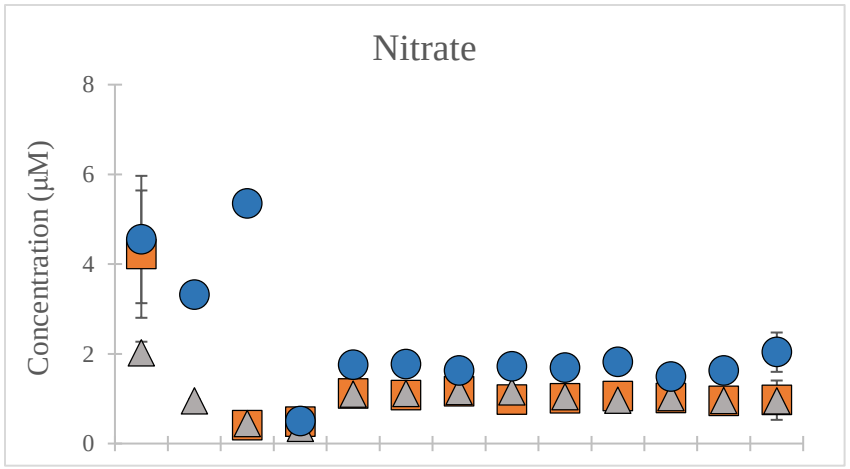
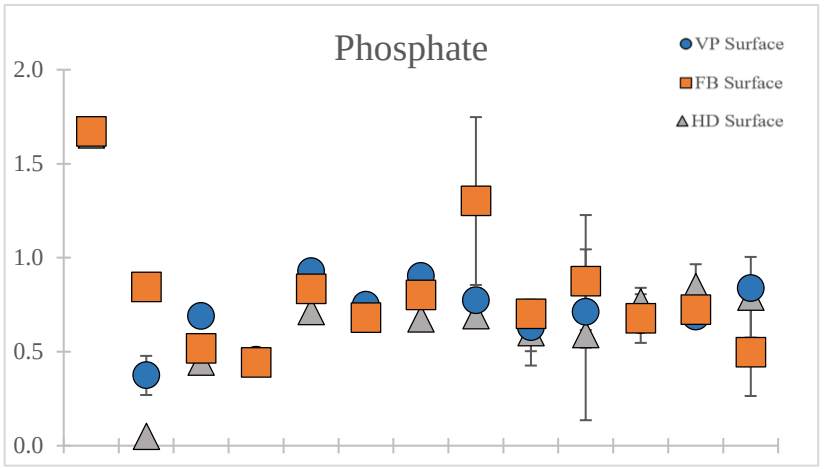
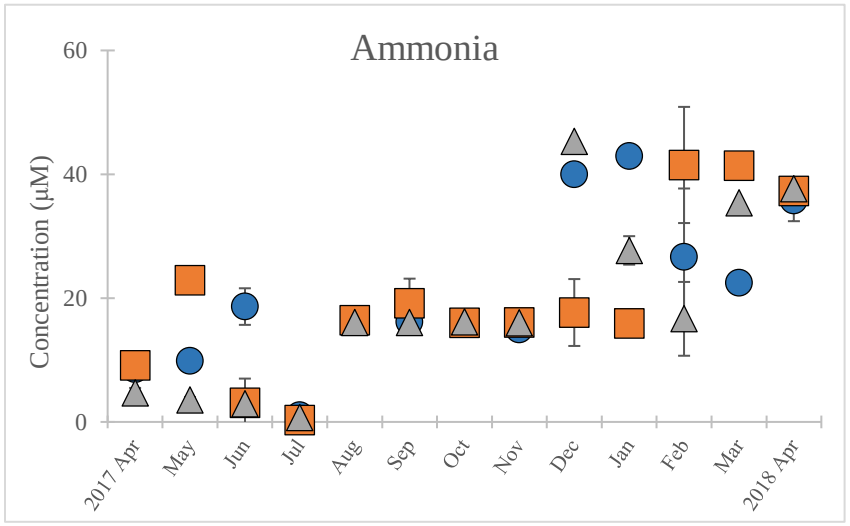

Ventura Point
Concentration (µg L-1)
400
300
200
100
0
phaeopigments
Chlorophyll a
Fiesta Bay
Concentration (µg L-1)
400
300
200
100
0
Hilton Dock
Concentration (µg L-1)
400
300
200
100
0
Apr-17 Surf
Apr-17 Btm
May-17 Surf
May-17 Btm
Jun-17 Surf
Jun-17 Btm
Jul-17 Surf
Jul-17 Btm
Aug-17 Surf
Aug-17 Btm
Sep-17 Surf
Sep-17 Btm
Oct-17 Surf
Oct-17 Btm
Nov-17 Surf
Nov-17 Btm
Dec-17 Surf
Dec-17 Btm
Jan-18 Surf
Jan-18 Btm
Feb-18 Surf
Feb-18 Btm
Mar-18 Surf
Mar-18 Btm
Apr-18 Surf
Apr-18 Btm

Figure 2.3 Surface nutrient concentrations from April 2017 to April 2018 at three sampling sites: Ventura Point (VP; blue circles), Fiesta Bay (FB; orange squares), and Hilton Dock (HD; gray triangles). Error bars represent $\pm$ one standard deviation.


Nitrate
Concentration (µM)
8
6
4
2
0


Phosphate
VP Surface
FB Surface
HD Surface
2.0
1.5
1.0
0.5
0.0


Ammonia
Concentration (µM)
60
40
20
0
2017 Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
2018 Apr

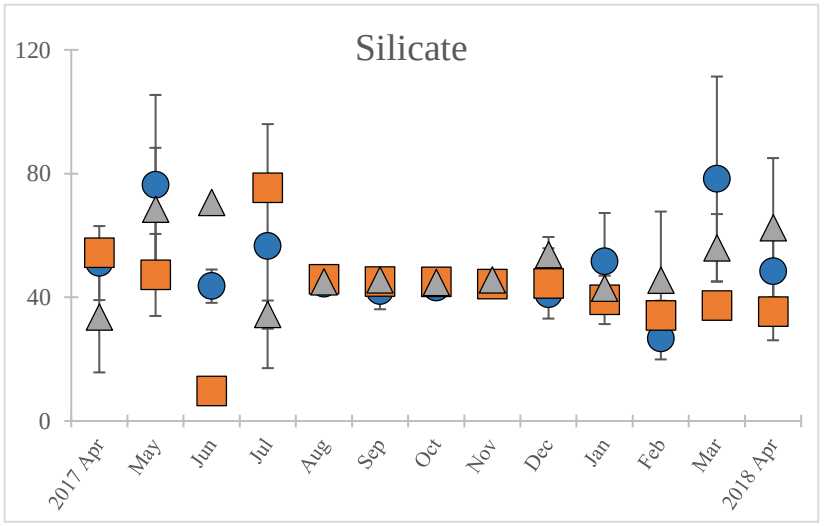

Silicate
120
80
40
0
2017 Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
2018 Apr

Figure 2.4 Percent composition of major taxa of plankton observed at all three sites from April 2017 to April 2018.

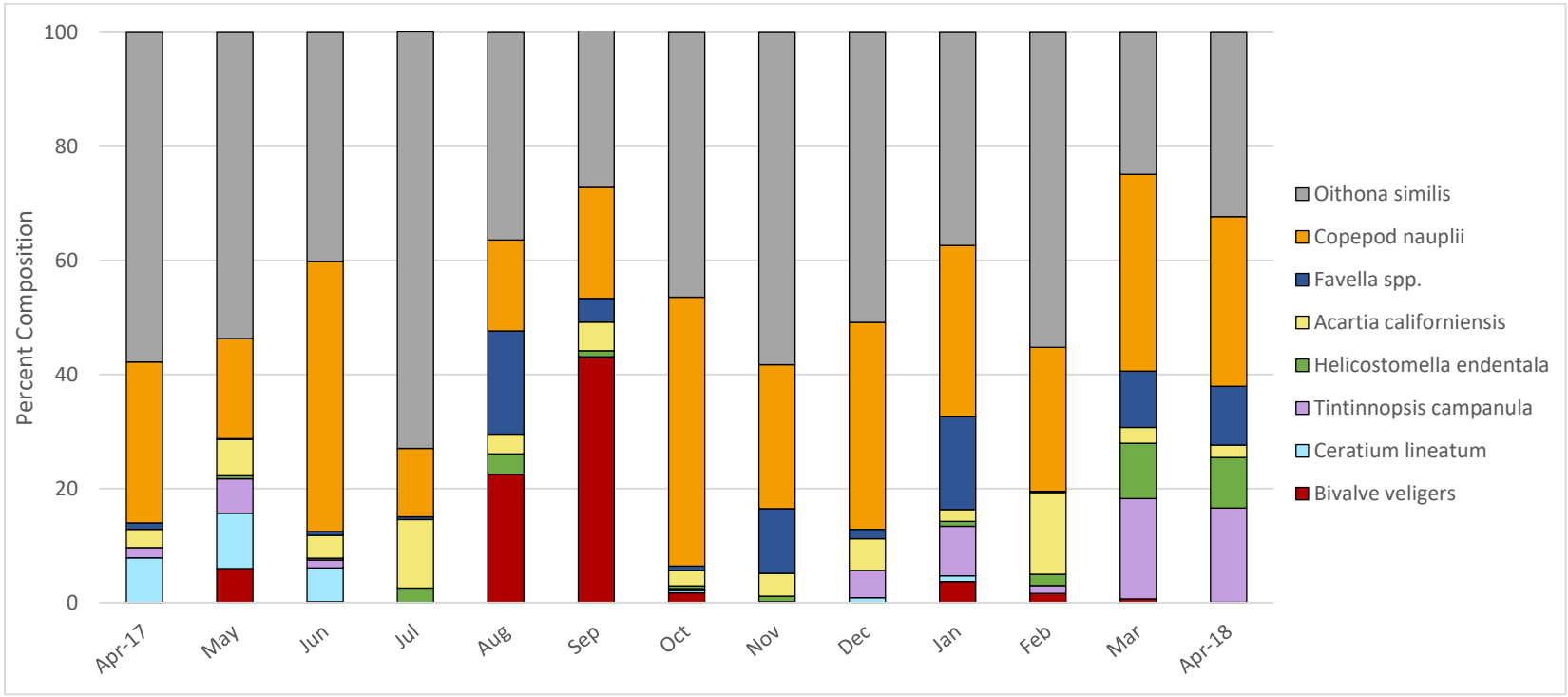
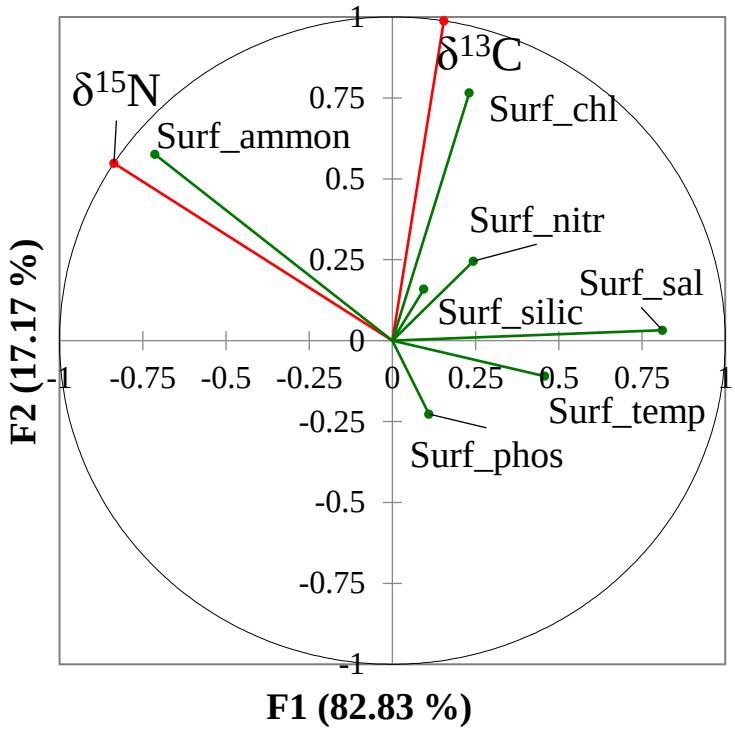
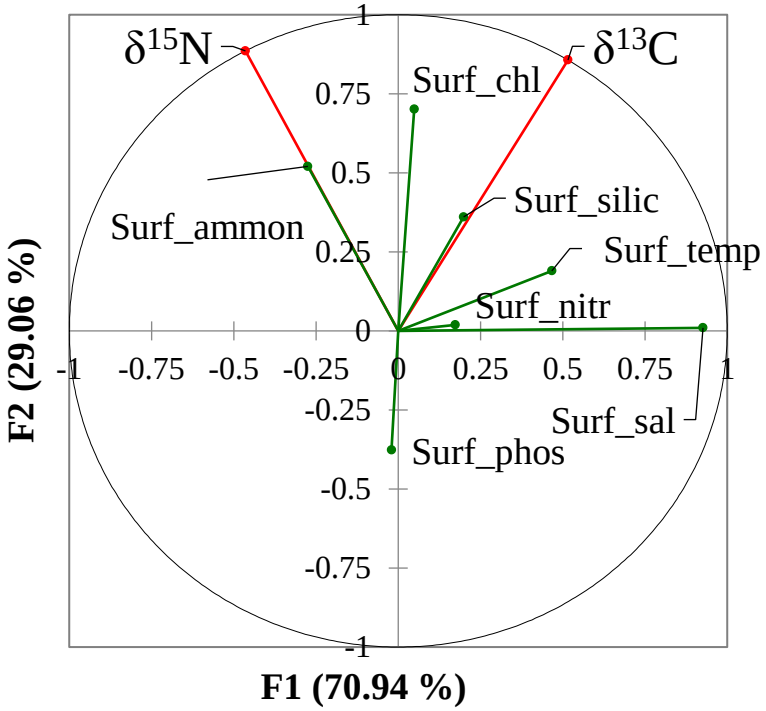

Percent Composition
100
80
60
40
20
0
Apr-17
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr-18
Oithona similis
Copepod nauplii
Favella spp.
Acartia californiensis
Helicostomella endentala
Tintinnopsis campanula
Ceratium lineatum
Bivalve veligers

Figure 2.5 Canonical correlation analyses of stable isotope signatures, $\delta^{15}N$ and $\delta^{13}C$ (red vectors), and environmental parameters (green vectors) for size classes (53-120 µm) (top) and (120-250 µm) (bottom). Surf_ammon (surface ammonia concentration), surf_chl (surface chlorophyll *a* concentration), Surf_nitr (surface nitrate concentration), Surf_phos (surface phosphate concentration), Surf_sal (surface salinity), Surf_silic (surface silicate concentration), Surf_temp (surface temperature).

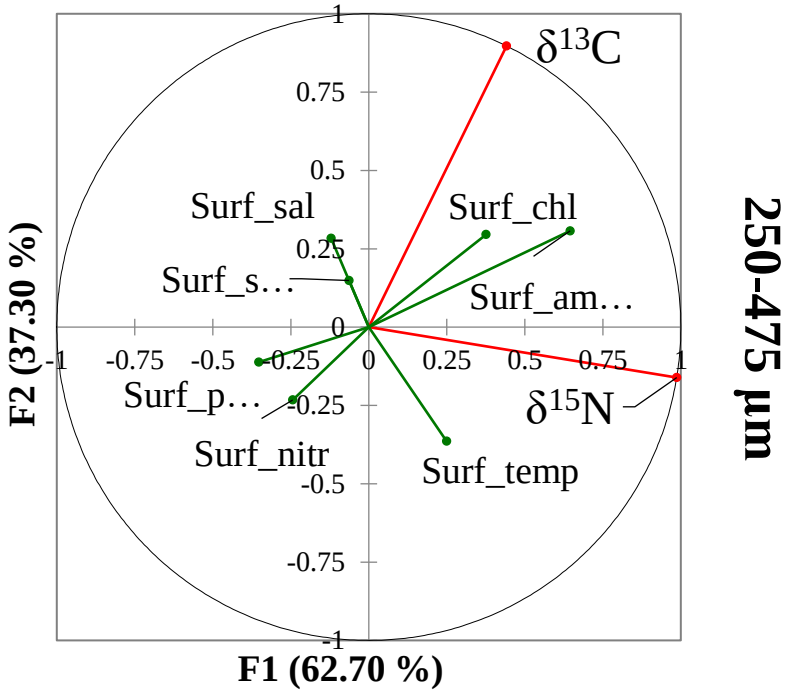


53-120 µm



120-250 µm

Figure 2.6 Canonical correlation analyses of stable isotope signatures, $\delta^{15}N$ and $\delta^{13}C$ (red vectors), and environmental parameters (green vectors) for size classes (250-475 µm) (top) and (475-1000 µm) (bottom). Surf_ammon (surface ammonia concentration), Surf_chl (surface chlorophyll *a* concentration), Surf_nitr (surface nitrate concentration), Surf_phos (surface phosphate concentration), Surf_sal (surface salinity), Surf_silic (surface silicate concentration), Surf_temp (surface temperature).


250-475 µm
F2 (37.30 %)
F1 (62.70 %)
δ13C
Surf_sal
Surf_chl
Surf_s…
Surf_am…
Surf_p…
δ15N
Surf_nitr
Surf_temp
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-0.75
-0.5
-0.25
0.25
0.5
0.75

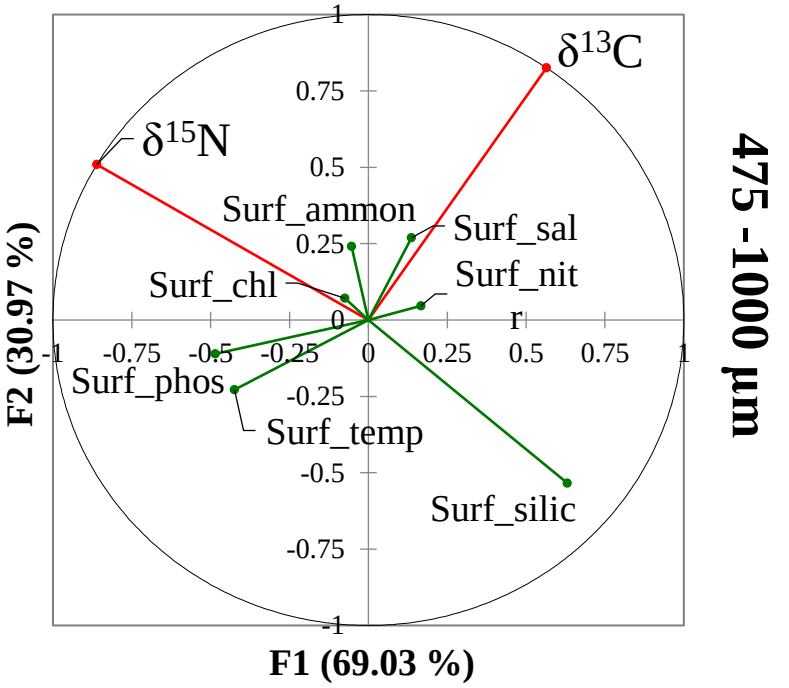

475 - 1000 µm
F2 (30.97 %)
F1 (69.03 %)
δ13C
δ15N
Surf_ammon
Surf_sal
Surf_chl
Surf_nit
r
Surf_phos
Surf_temp
Surf_silic
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-0.75
-0.5
-0.25
0.25
0.5
0.75

Figure 2.7 Carbon stable isotope ratios at Ventura Point (A), Fiesta Bay (B), and Hilton Dock (C) throughout the annual cycle from April 2017 to April 2018 for all four size classes: 53-120 μm (blue diamonds), 120-250 μm (red triangles), 250-475 μm (green squares), 475-1000 μm (purple circles).

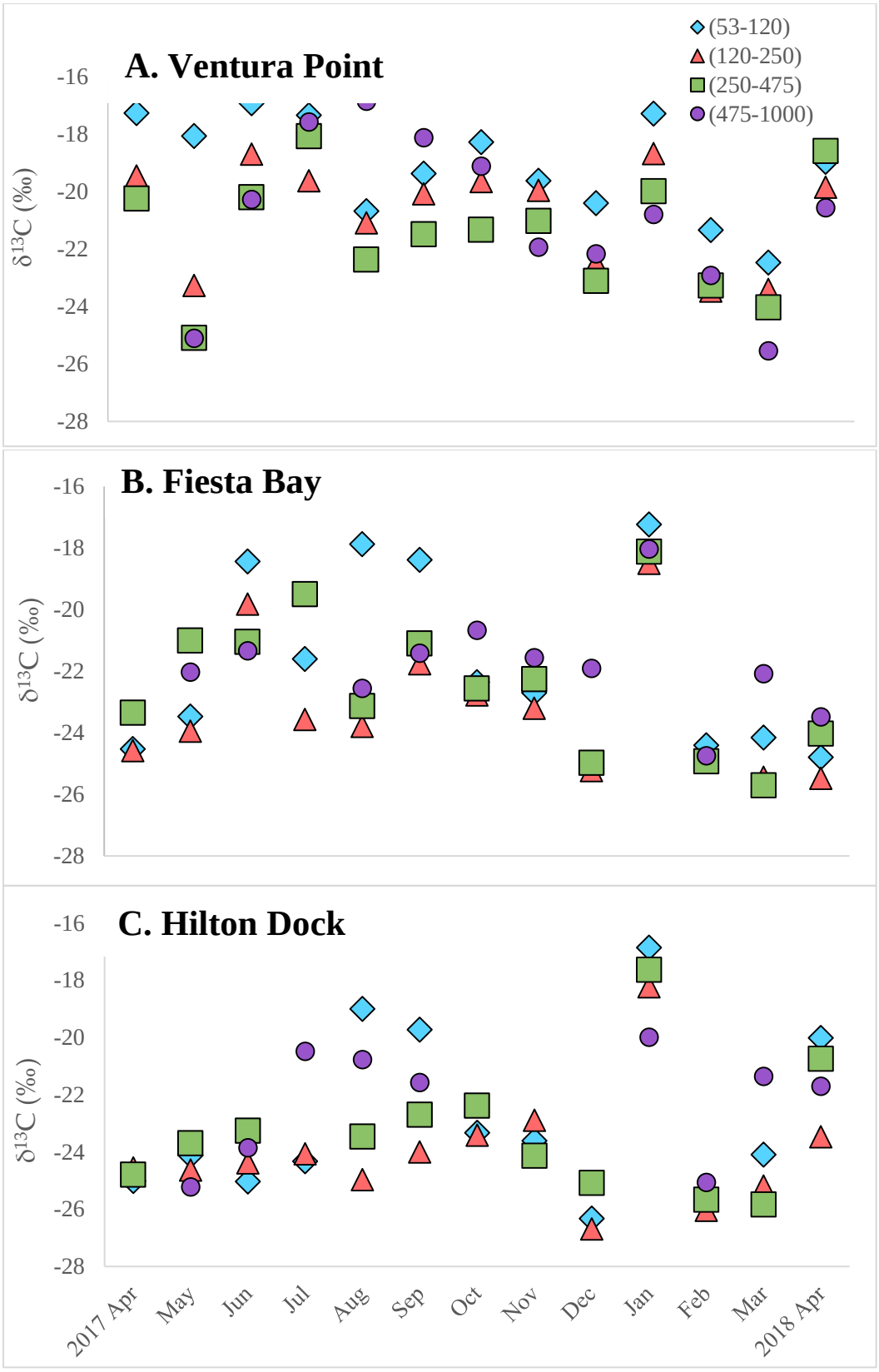

A. Ventura Point
B. Fiesta Bay
C. Hilton Dock
(53-120)
(120-250)
(250-475)
(475-1000)
δ13C (‰)
-16
-18
-20
-22
-24
-26
-28
2017 Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
2018 Apr

Figure 2.8 Nitrogen stable isotope ratios at Ventura Point (A), Fiesta Bay (B), and Hilton Dock (C) throughout the annual cycle from April 2017 to April 2018 for all four size classes: 53-120 μm (blue diamonds), 120-250 μm (red triangles), 250-475 μm (green squares), 475-1000 μm (purple circles).

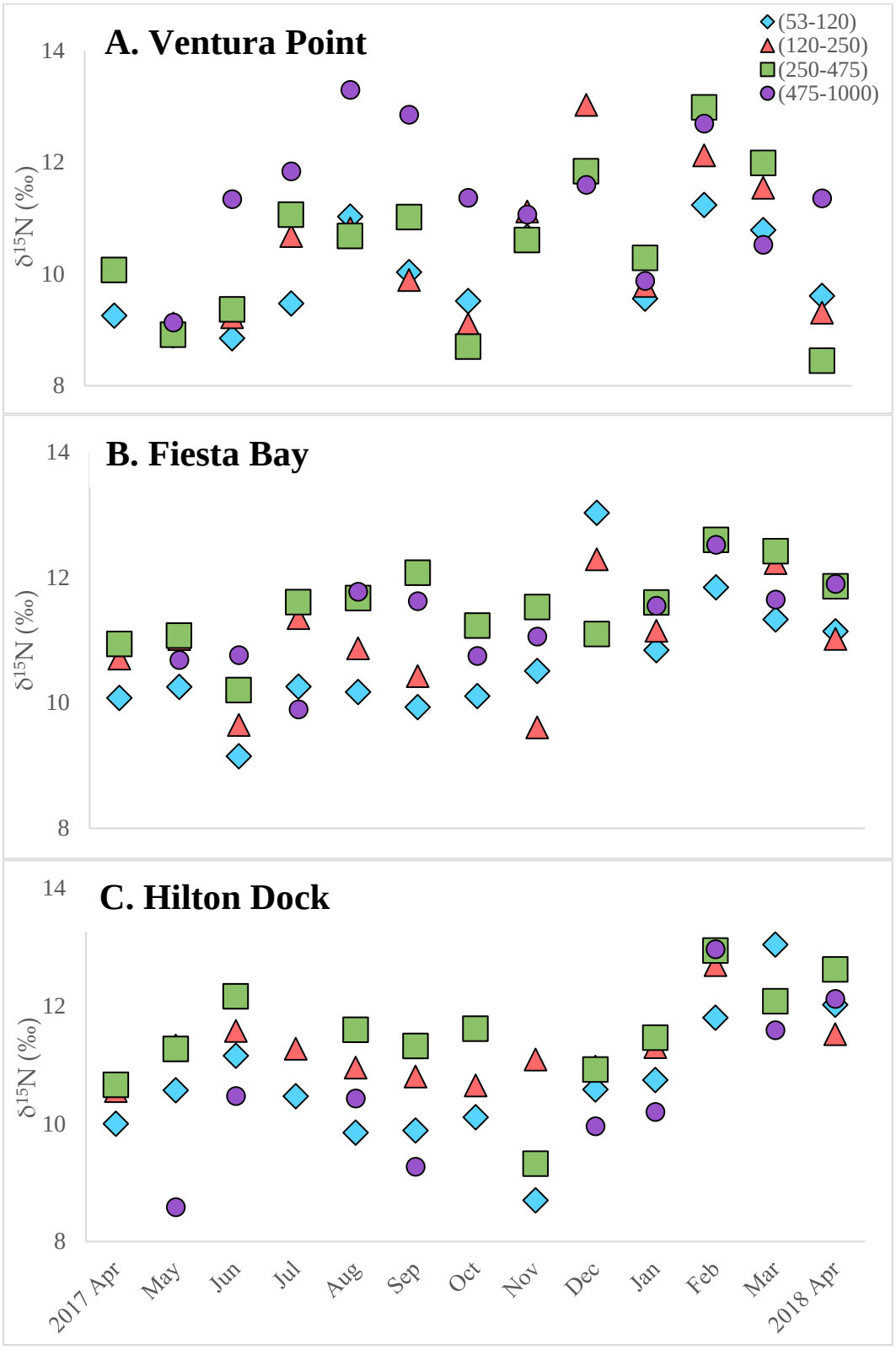

A. Ventura Point
B. Fiesta Bay
C. Hilton Dock
(53-120)
(120-250)
(250-475)
(475-1000)
δ15N (‰)
14
12
10
8
2017 Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
2018 Apr

Table 2.1 Percent composition of the five most dominant plankton taxa identified within each size class, where taxa are ranked from the most to least common.

Site	Rank	53-120		120-250		250-475		475-1000	
		Taxa	% Occurrence	Taxa	% Occurrence	Taxa	% Occurrence	Taxa	% Occurrence
Ventura Point	1	copepod nauplii	22.97	*Oithona similis*	49.91	*Oithona similis*	56.25	*Acartia californiensis*	72.35
	2	copepod eggs	22.36	copepod nauplii	20.82	*Acartia californiensis*	26.68	bivalve veligers	12.74
	3	*Ceratium lineatum*	11.86	*Favella* spp.	8.76	*Acartia* juvenile	7.16	*Oithona similis*	7.91
	4	*Protoperidinium conicum*	9.84	fish eggs	6.96	copepod nauplii	3.33	zoea	3.47
	5	*Stenosemella steini*	7.18	*Acartia californiensis*	4.66	zoea	2.76	fish eggs	2.71
Fiesta Bay	1	copepod nauplii	33.71	*Oithona similis*	62.62	*Oithona similis*	49.08	*Oithona similis*	58.4
	2	copepod eggs	29.37	copepod nauplii	16.47	copepod nauplii	18.74	*Acartia californiensis*	31.51
	3	bivalve veligers	9.53	*Favella* spp.	4.81	*Acartia californiensis*	7.35	*Acartia* juvenile	7.21
	4	*Tintinnopsis campanula*	6.96	*Tintinnopsis campanula*	4.44	*Tintinnopsis campanula*	6.58	zoea	2.4
	5	*Favella* spp.	5.91	*Helicostomella endentala*	4.09	*Helicostomella endentala*	4.63	ostracods	0.25
Hilton Dock	1	copepod eggs	36.58	*Oithona similis*	58.69	*Oithona similis*	54.39	*Acartia californiensis*	50.65
	2	copepod nauplii	29.58	copepod nauplii	13.93	copepod nauplii	12.01	*Oithona similis*	47.74
	3	*Tintinnopsis campanula*	9.08	*Tintinnopsis campanula*	8.31	*Acartia* juvenile	6.74	zoea	0.88
	4	bivalve veligers	7.04	*Favella* spp.	6.7	*Tintinnopsis campanula*	5.65	fish eggs	0.74
	5	*Oithona similis*	5.85	*Tintinnopsis cylindrica*	5.01	*Acartia californiensis*	4.88	-	-

Table 2.2 Shannon-Wiener diversity index, evenness (H/H_{max}), and taxonomic richness for each size class at each site from April 2017 through April 2018.

Site	Size	Diversity	Evenness	Richness
Ventura Point	53-120 µm	0.75	0.26	19
	120-250 µm	0.56	0.2	16
	250-475 µm	0.66	0.28	11
	475-1000 µm	0.69	0.36	7
Fiesta Bay	53-120 µm	1.26	0.44	18
	120-250 µm	0.94	0.36	14
	250-475 µm	1.14	0.42	15
	475-1000 µm	0.84	0.47	6
Hilton Dock	53-120 µm	1.1	0.41	15
	120-250 µm	0.99	0.39	13
	250-475 µm	0.82	0.32	13
	475-1000 µm	0.4	0.29	4

Table 2.3 Percent composition of the five most dominant plankton taxa identified at each sampling site, where taxa are ranked from most to least common.

Site	Rank	Taxa	% Occurrence
Ventura Point	1	*Oithona similis*	27.88
	2	copepod nauplii	16.31
	3	copepod eggs	10.64
	4	*Acartia californiensis*	10.42
	5	*Ceratium lineatum*	5.24
Fiesta Bay	1	*Oithona similis*	29.45
	2	copepod nauplii	24.70
	3	copepod eggs	15.70
	4	*Tintinnopsis campanula*	5.93
	5	*Favella* spp.	4.89
Hilton Dock	1	*Oithona similis*	30.30
	2	copepod nauplii	21.61
	3	copepod eggs	20.87
	4	*Tintinnopsis campanula*	8.28
	5	*Favella* spp.	5.45

Table 2.4 Results of Spearman Rank Correlation analysis between density of organisms (no. m^{-3}) and surface environmental parameters for all size classes. Asterisks indicate results with a p-value greater than 0.05.

Size Class	Rho value	*P*-value	Factor
53-120 µm	-0.40	0.014	Salinity
	0.39	0.017	Chlorophyll
	0.52	0.00083	Ammonia
	0.31*	0.058*	Nitrate*
120-250 µm	0.53	0.0012	Ammonia
250-475 µm	-0.39	0.015	Temperature
	-0.34	0.036	Salinity
	0.53	0.00061	Ammonia
475-1000 µm	-0.40	0.012	Temperature
	-0.46	0.0036	Salinity
	0.51	0.00095	Ammonia

Table 2.5 Results of a Kruskal-Wallis test comparing $\delta^{13}C$ and $\delta^{15}N$ values of plankton among size classes (53-120, 120-250, 250-475, 475-1000 µm) for each site. Value in bold is statistically significant ($p \leq 0.05$). Results of a *post-hoc* Dunn's test are shown in superscript (< indicates significantly higher than other size class).

Site	Isotopic Ratio	Chi-squared	*P*-value
Ventura Point	$\delta^{13}C$	8.04	(250-475)<(53-120) **0.045**
	$\delta^{15}N$	6.76	0.080
Fiesta Bay	$\delta^{13}C$	6.76	0.15
	$\delta^{15}N$	9.09	0.059
Hilton Dock	$\delta^{13}C$	5.31	0.26
	$\delta^{15}N$	6.51	0.16

Table 2.6 Results of a Kruskal-Wallis test comparing $\delta^{13}C$ and $\delta^{15}N$ values of plankton among the three sampling sites for each size class. Values in bold are statistically significant ($p \leq 0.05$). Results of a *post-hoc* Dunn's test are shown in superscript (< indicates significantly higher than other two sites; > indicates significantly lower than other two sites).

Size Class (µm)	Isotopic Ratio	Chi-squared	*P*-value
53-120	$\delta^{13}C$	10.39	**0.0056**$^{<VP}$
	$\delta^{15}N$	2.60	0.27
120-250	$\delta^{13}C$	13.47	**0.0012**$^{<VP}$
	$\delta^{15}N$	4.98	0.083
250-475	$\delta^{13}C$	4.67	0.097
	$\delta^{15}N$	6.61	**0.037**$^{>VP}$
475-1000	$\delta^{13}C$	0.34	0.84
	$\delta^{15}N$	0.77	0.68

Table 2.7 Results of the Kruskal-Wallis test comparing $\delta^{13}C$ and $\delta^{15}N$ values across months and seasons (spring: Mar-May, summer: Jun-Aug, fall: Sep-Nov, winter: Dec-Feb). Values in bold are statistically significant ($p \leq 0.05$).

Isotopic Ratio	Month		Season	
	Chi-squared	*P*-value	Chi-squared	*P*-value
$\delta^{13}C$	56.96	**8.04E-08**	15.90	**0.0012**
$\delta^{15}N$	47.24	**4.24E-06**	11.87	**0.0079**

Table 2.8 Significant results of Spearman Rank Correlation analysis between stable isotopic values and percent composition of taxa for all size classes at all three sites.

Site	Size class	Isotopic Value	Rho value	p-value	Taxon
Ventura Point	53-120	$\delta^{13}C$	0.59	3.54E-02	*Ceratium lineatum*
	475-1000	$\delta^{13}C$	-0.70	1.45E-02	*Acartia californiensis*
Fiesta Bay	250-475	$\delta^{13}C$	-0.71	8.83E-03	*Oithona similis*
	475-1000	$\delta^{13}C$	-0.74	6.40E-03	*Acartia californiensis*
		$\delta^{15}N$	0.87	2.46E-04	*Acartia californiensis*
Hilton Dock	53-120	$\delta^{15}N$	-0.73	4.22E-03	*Oithona similis*
	475-1000	$\delta^{15}N$	0.67	3.38E-02	*Acartia californiensis*

Table 2.9 Significant results of Spearman Rank Correlation analysis between $\delta^{15}N$ and surface environmental parameters for all size classes.

	Size Class	Rho	*P*-value	Parameter
$\delta^{15}N$	53-120 µm	-0.34	0.035	Salinity
		0.43	0.0066	Ammonia
	120-250 µm	-0.51	0.0013	Nitrate
	250-475 µm	-0.55	0.00035	Nitrate
		0.34	0.036	Ammonia
	475-1000 µm	-0.48	0.0042	Silicate

Chapter 3

Conclusion

Studies on the trophic interactions among plankton are crucial for understanding energy and carbon flow at the base of aquatic food webs. Furthermore, an examination of zooplankton diet across a range of environmental conditions can help predict changes in zooplankton populations as environmental conditions vary. Through isotopic dietary analysis, particularly carbon and nitrogen stable isotopes, it is possible to determine the carbon sources and relative trophic position of zooplankton. Stable isotope analysis is more advantageous than conventional diet analyses, like gut content analysis, because it provides a more time-integrated measurement of an organism's diet. This study used bulk carbon and nitrogen stable isotope ratios to examine the diets of size-fractionated plankton in Mission Bay, San Diego, CA.

Through stable isotope analysis, we now have a better understanding of the trophic pathways in the lower planktonic food web of Mission Bay and the environmental factors that influence those pathways. Since stable isotope analysis is frequently used to examine food web structure, it was surprising to find no significant differences in $\delta^{15}N$ among size classes of plankton within Mission Bay. One possible explanation for the lack of variation was a prevalence of omnivory. As in previous studies conducted in Mission Bay (Elliott and Kaufmann 2007, Shapiro 2018), the copepod, *Oithona similis*, was the most abundant species during the sampling period. *Oithona similis* has

been shown to have an omnivorous diet (Castellani *et al.* 2005). Although *O. similis* has been reported to preferentially feed on ciliates (tintinnids), this species has also been reported to feed on dinoflagellates, diatoms, and other nano-microplankton (Castellani *et al.* 2005). Another explanation for the homogeneity in $\delta^{15}N$ is that the trophic interactions within these size classes are not strongly size-dependent.

In an examination of spatial variation in the $\delta^{15}N$ of zooplankton in Mission Bay, lower $\delta^{15}N$ values were observed within the 250-475 μm size class at Ventura Point compared to the other two sites. The most abundant taxon in this size class at Ventura Point was *Oithona similis.* As stated above, *O. similis* preferentially feeds on ciliates (tintinnids), and tintinnids were less abundant at Ventura Point compared to the other two sites. Due to the lack of abundance of its preferred prey, *O. similis* could have been preying on organisms at a lower trophic position and of lower $\delta^{15}N$ values than tintinnids, possibly phytoplankton, at Ventura Point.

Analyses of the temporal variation in $\delta^{15}N$ detected significant differences from month to month and season to season. This finding may be explained in part by the positive correlation between $\delta^{15}N$ values and the abundance of the calanoid copepod, *Acartia californiensis* throughout the study period. Previous literature has shown that *A. californiensis* has a diverse diet and, similarly to *O. similis,* preferentially feeds on ciliates during periods of high food abundance. The abundance of tintinnids (ciliates), fluctuated notably throughout the study from month to month and season to season.

Therefore, through diet switching, the feeding activity of *A. californiensis* may be influencing nitrogen stable isotope values in Mission Bay.

Moreover, we concluded that nitrate utilization by phytoplankton may be a major mechanism influencing the $\delta^{15}N$ values of zooplankton after finding a strong negative correlation between $\delta^{15}N$ and nitrate. Nitrate is a major nutrient required by phytoplankton, and past studies have determined that ^{14}N, rather than ^{15}N, is preferentially assimilated by phytoplankton. Consequently, as nutrient utilization increases, the $\delta^{15}N$ of the remaining pool of nitrate increases and the phytoplankton will eventually begin to assimilate greater amounts of ^{15}N. Enrichment in ^{15}N in plankton should be consistent with greater nitrate utilization and lower nitrate concentrations. We also found a negative correlation between $\delta^{15}N$ and silicate concentration. Silicate is another major nutrient for phytoplankton, particularly diatoms. Silicate utilization likely coincides with nitrate utilization, which is thought to drive the increase in nitrogen values. Our results suggest that the changes in the isotopic values of zooplankton are consistent with changes in the supply of nutrients for the bottom of the food web.

There was also a positive correlation between $\delta^{15}N$ and ammonia concentration. Ammonia is a nitrogenous waste product of zooplankton and other heterotrophs; and therefore, we would expect an increase in ammonia as the number of heterotrophic organisms multiplies. This assumption was supported by the significant positive correlation between ammonia concentration and density of organisms observed within all four size classes.

Moreover, a rise in the density of plankton and accordingly, greater utilization of nitrate should result in a greater amount of ^{15}N assimilated in plankton. Therefore, as the density of organisms increases, we would likely see both an increase in ammonia production and an increase of $\delta^{15}N$ in plankton.

Since other studies have reported a general increase in $\delta^{13}C$ with increasing size class, it was surprising to find higher carbon ratios in the smallest size class (53-120 μm) compared to the second largest size class (250-475 μm) at Ventura Point. Within the smallest size class at Ventura Point, there was a positive correlation between $\delta^{13}C$ and the percentage of *Ceratium lineatum*, a mixotrophic marine dinoflagellate. This finding suggests that this species had a substantial influence on $\delta^{13}C$ of zooplankton in Mission Bay. Additionally, previous literature has reported higher $\delta^{13}C$ in temperate marine phytoplankton versus estuarine phytoplankton. The strong presence of this marine dinoflagellate in the 53-120 μm size class and its absence in the 250-475 μm size class may help to explain the difference in $\delta^{13}C$ between the two size classes. The abundance of *C. lineatum* at Ventura Point may also explain the significantly higher $\delta^{13}C$ ratios within the two smallest size classes at Ventura Point compared to the other two sites.

Our analyses also detected temporal variation in $\delta^{13}C$ values, which was likely driven by changes in rainfall. Higher carbon ratios were observed in the summer and fall, likely due to the lack of rainfall during these seasons. Less freshwater inflow could result in a decrease in terrestrial input, which generally has lower carbon ratios. A decrease in terrestrial input could

consequently lead the plankton to source carbon with higher $\delta^{13}C$ values. This seasonal pattern has also been seen in similar plankton studies (Kibirige *et al.* 2002, Bănaru *et al.* 2013). It should be noted that this study occurred during a historically dry year in San Diego. Since precipitation extremes are possible outcomes of climate change, studies like this one may help to predict the outcome of future environmental changes, like drought years.

Overall, this study provides insight into the trophic structure of the zooplankton population in Mission Bay and the environmental parameters that may drive the stable isotope values of the zooplankton. The results of this study provide a baseline of stable isotopic values and provoke more questions about the trophic structure of Mission Bay's plankton community, offering opportunities for future studies to expand upon this project. Those potential studies in Mission Bay may be able to address interannual variation in the diets of this plankton community. In addition, the carbon sources of the plankton could be furthered examined by comparing the isotopic signatures of particulate organic matter from different parts of the bay, as well as the bay's fresh water inputs, to the isotopic signatures of the plankton. Further research on the plankton community of Mission Bay, one of the few remaining wetlands along the California coastline, will continue to help monitor the stability of its food web and provide resources for the management of this vulnerable ecosystem.

Figure A.1 Density of organisms in the 53-120 μm size class at all three sites over the annual cycle from April 2017 to April 2018.

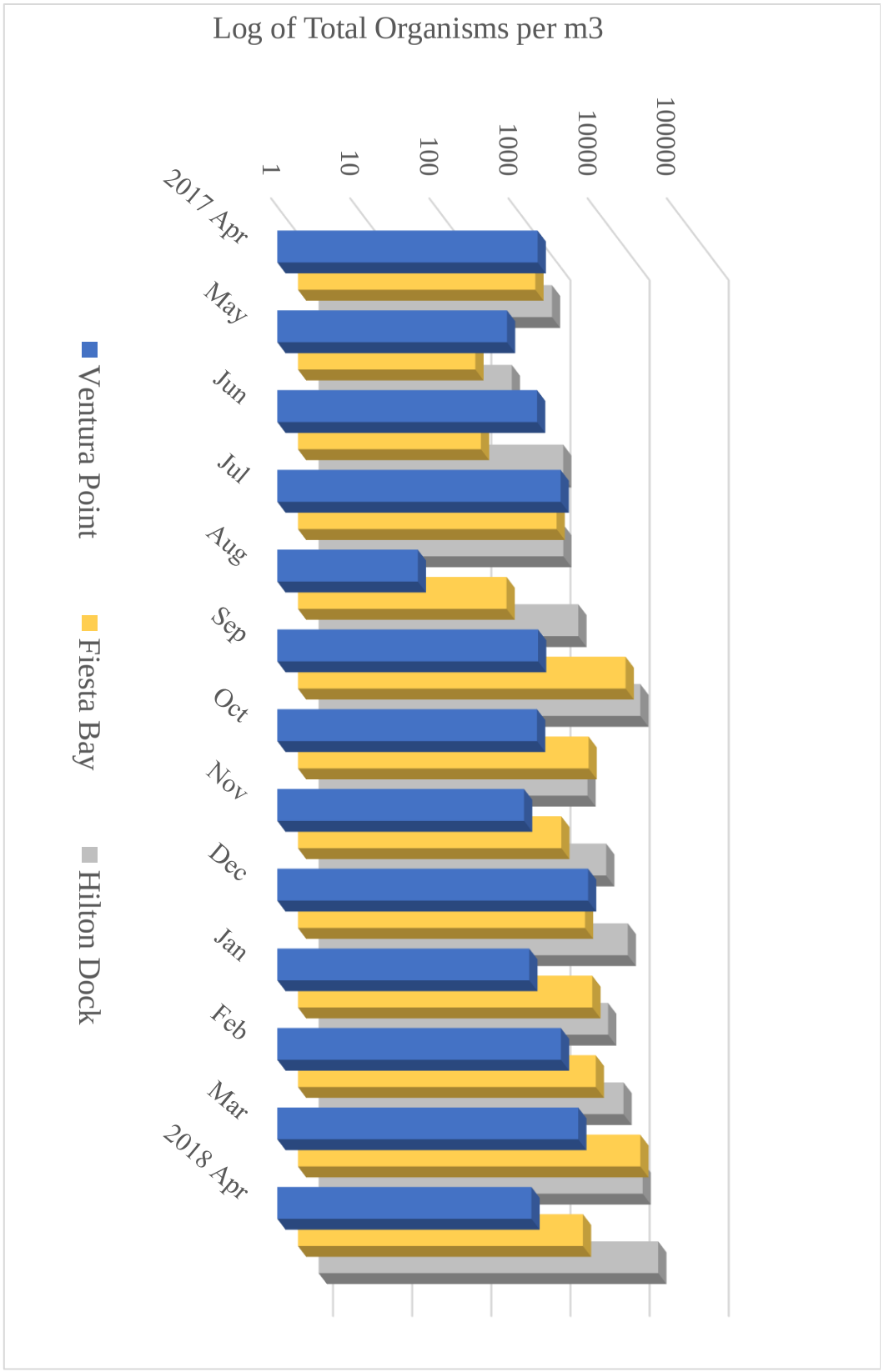

Log of Total Organisms per m3
1
10
100
1000
10000
100000
2017 Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
2018 Apr
Ventura Point
Fiesta Bay
Hilton Dock

Figure A.2 Density of organisms in the 120-250 μm size class at all three sites over the annual cycle from April 2017 to April 2018.

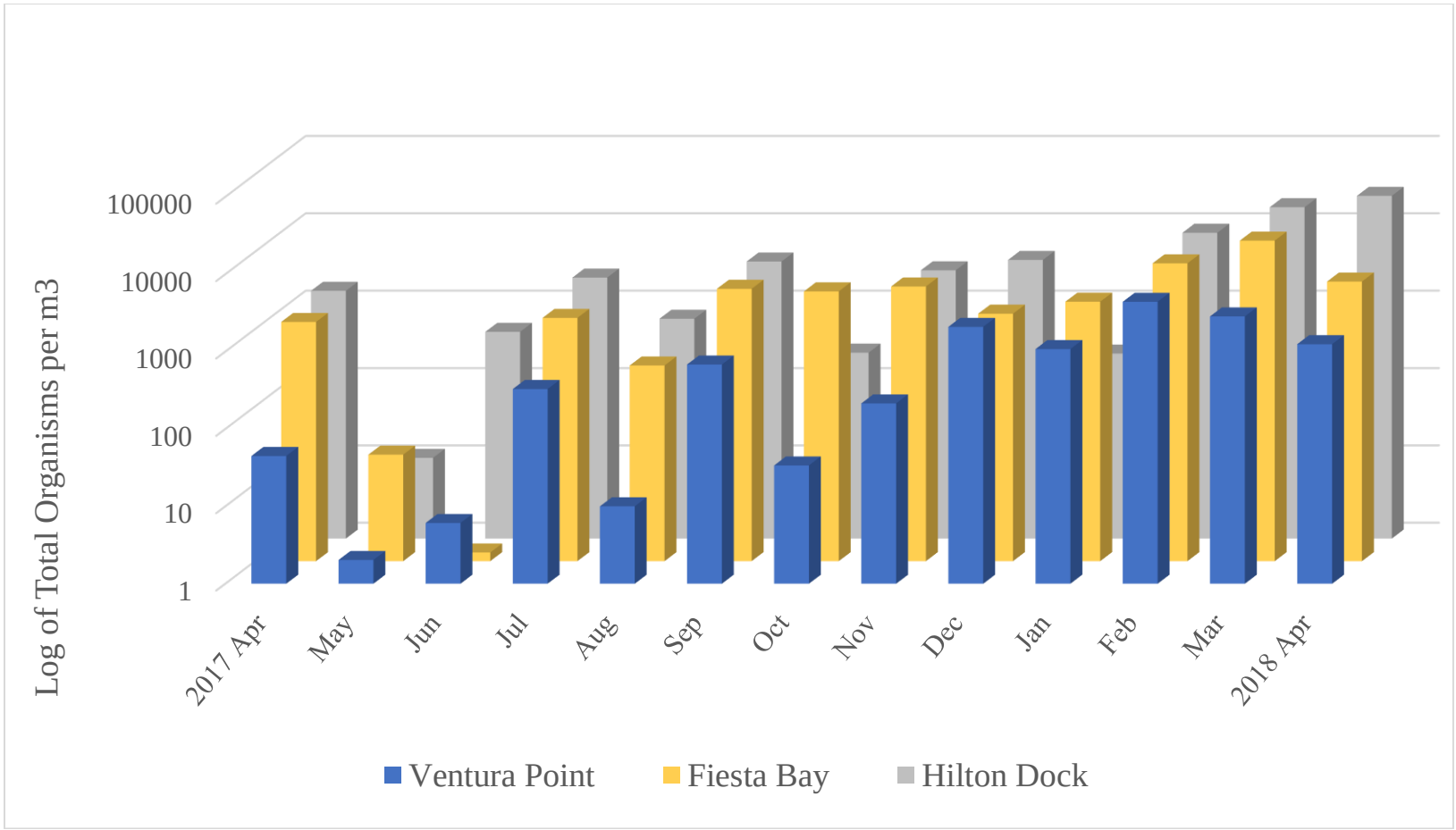

Log of Total Organisms per m3
100000
10000
1000
100
10
1
2017 Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
2018 Apr
Ventura Point
Fiesta Bay
Hilton Dock

Figure A.3 Density of organisms in the 250-475 μm size class at all three sites over the annual cycle from April 2017 to April 2018.

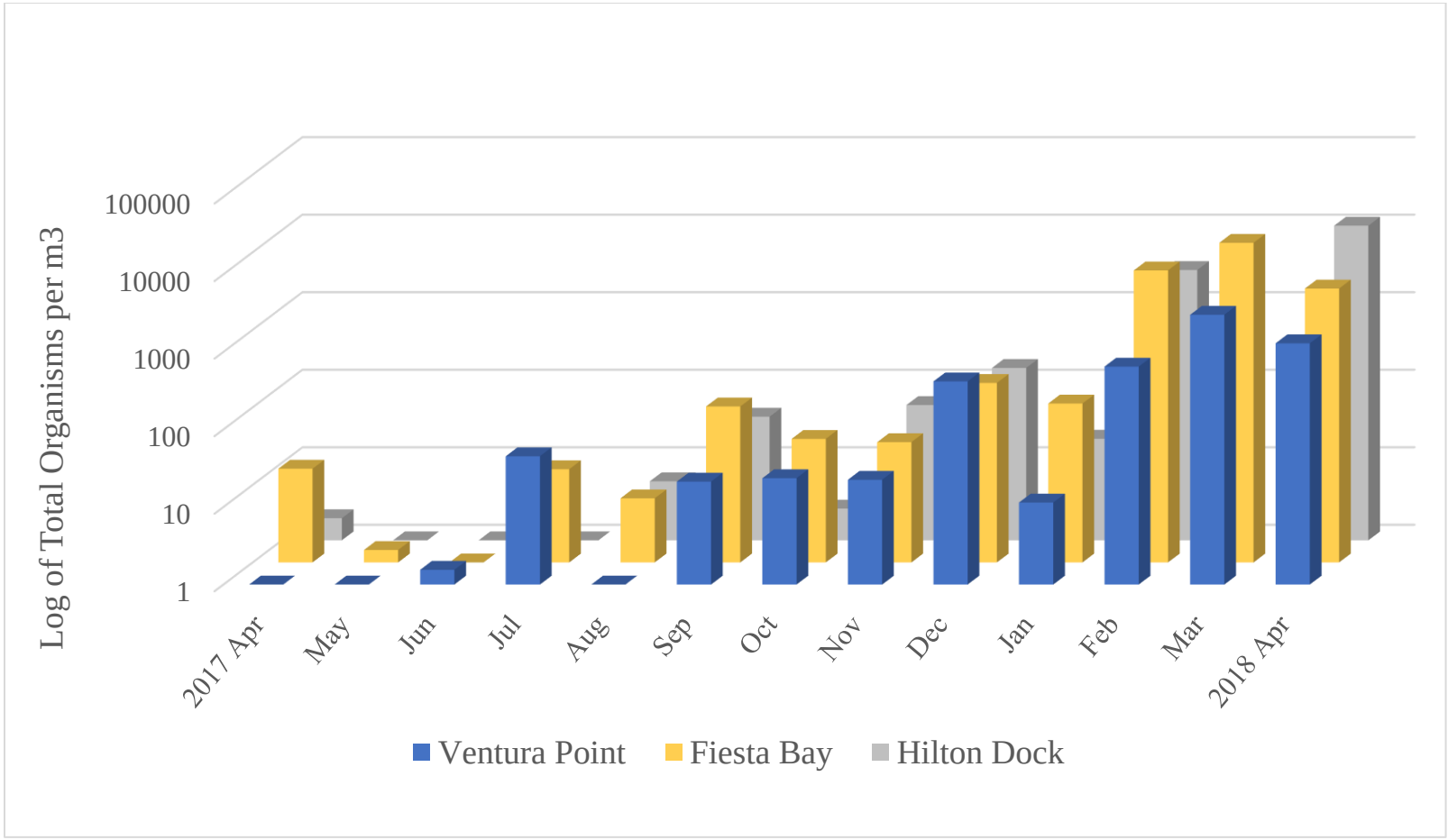

Log of Total Organisms per m3
100000
10000
1000
100
10
1
2017 Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
2018 Apr
Ventura Point
Fiesta Bay
Hilton Dock

Figure A.4 Density of organisms in the 475-1000 μm size class at all three sites over the annual cycle from April 2017 to April 2018.

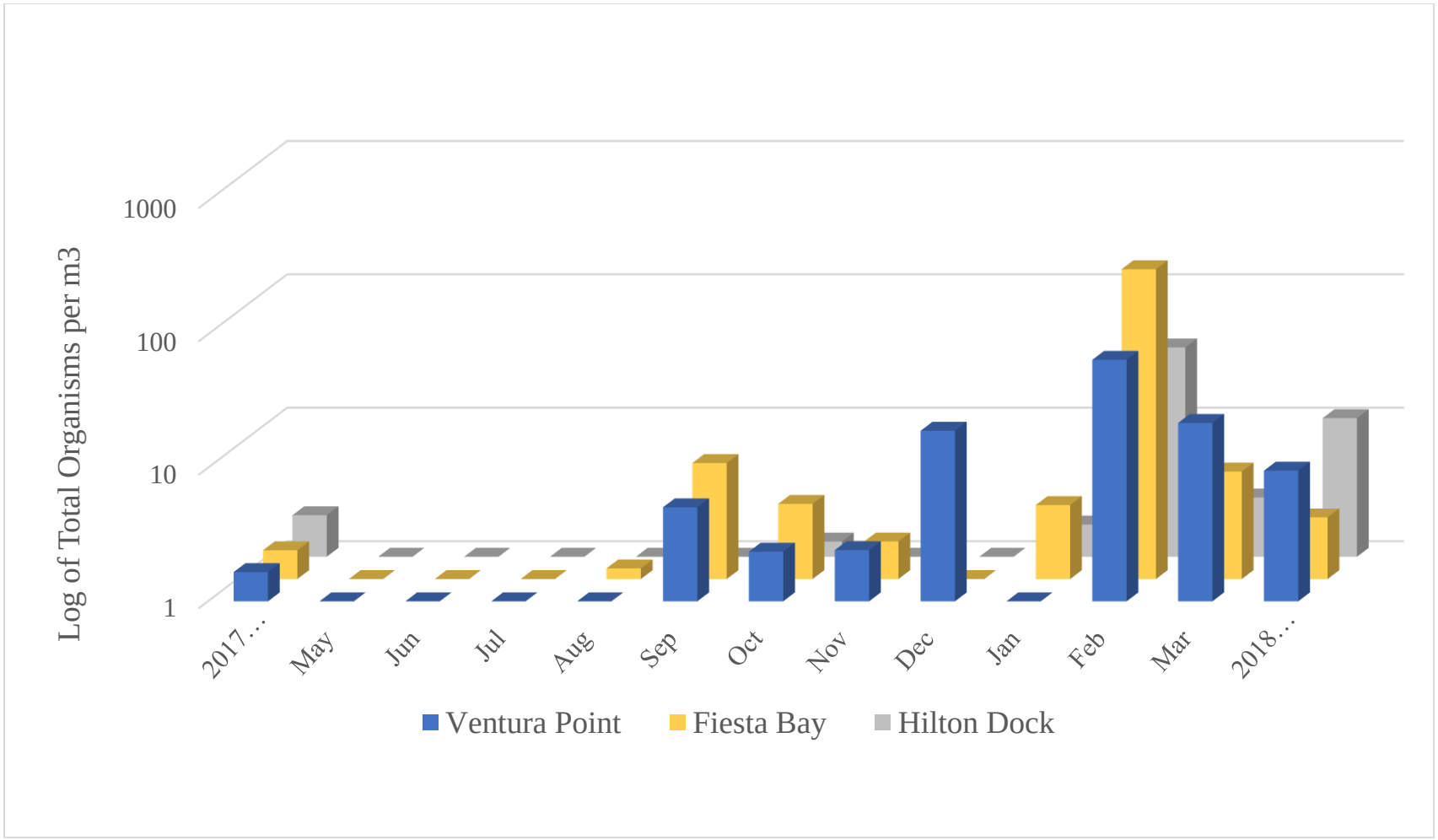

Log of Total Organisms per m3
1000
100
10
1
2017…
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
2018…
Ventura Point
Fiesta Bay
Hilton Dock

Figure A.5 Comparison of carbon and nitrogen stable isotope ratios among the four size classes for each site: 53-120 μm (blue diamonds), 120-250 μm (red triangles), 250-475 μm (green squares), and 475-1000 μm (purple circles).

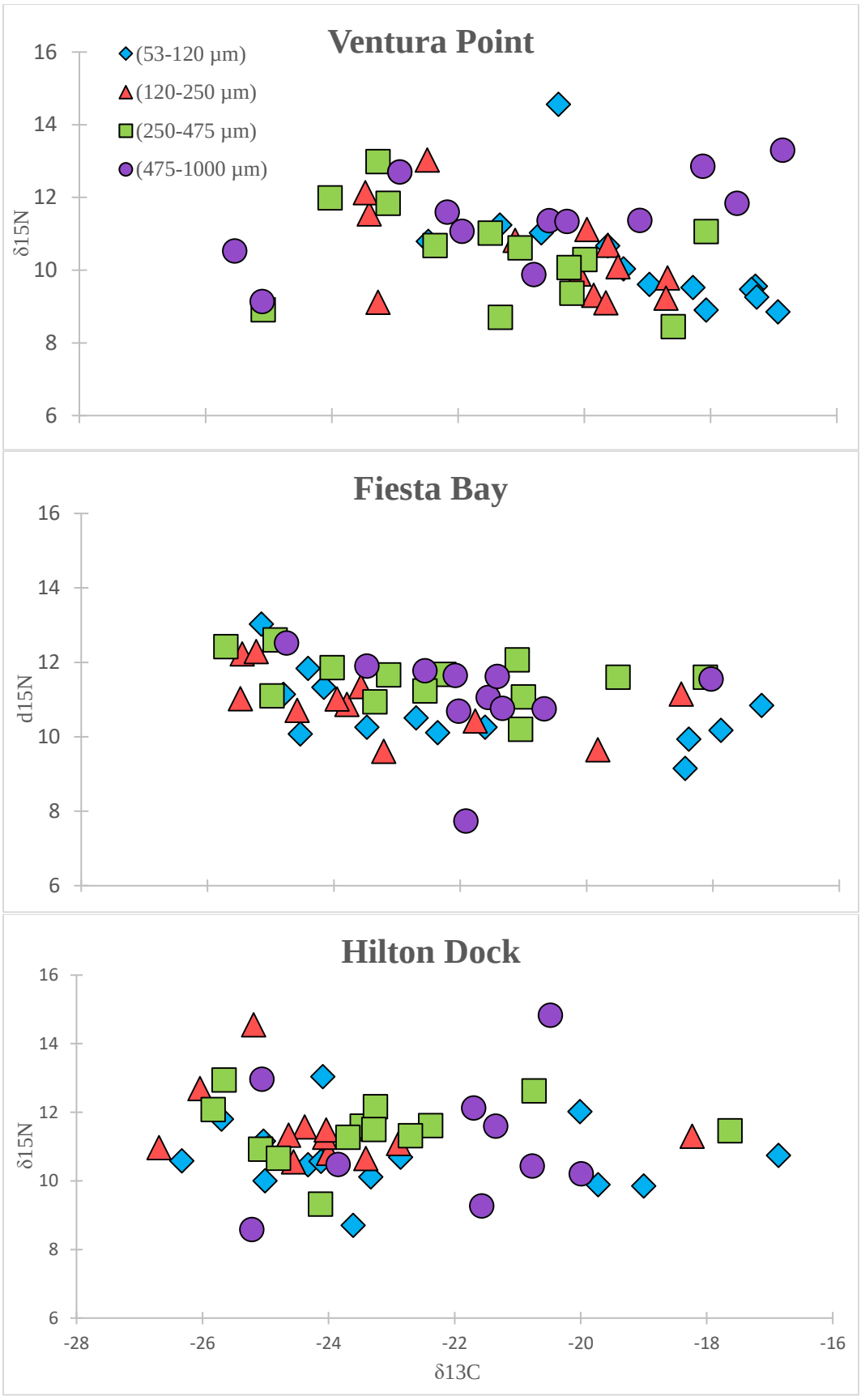

Ventura Point
(53-120 µm)
(120-250 µm)
(250-475 µm)
(475-1000 µm)
δ15N
16
14
12
10
8
6
Fiesta Bay
d15N
16
14
12
10
8
6
Hilton Dock
δ15N
16
14
12
10
8
6
-28
-26
-24
-22
-20
-18
-16
δ13C

Figure A.6 Carbon and nitrogen stable isotope ratios for each size class at all three sites: Ventura Point (blue circles), Fiesta Bay (orange squares), and Hilton Dock (gray triangles).

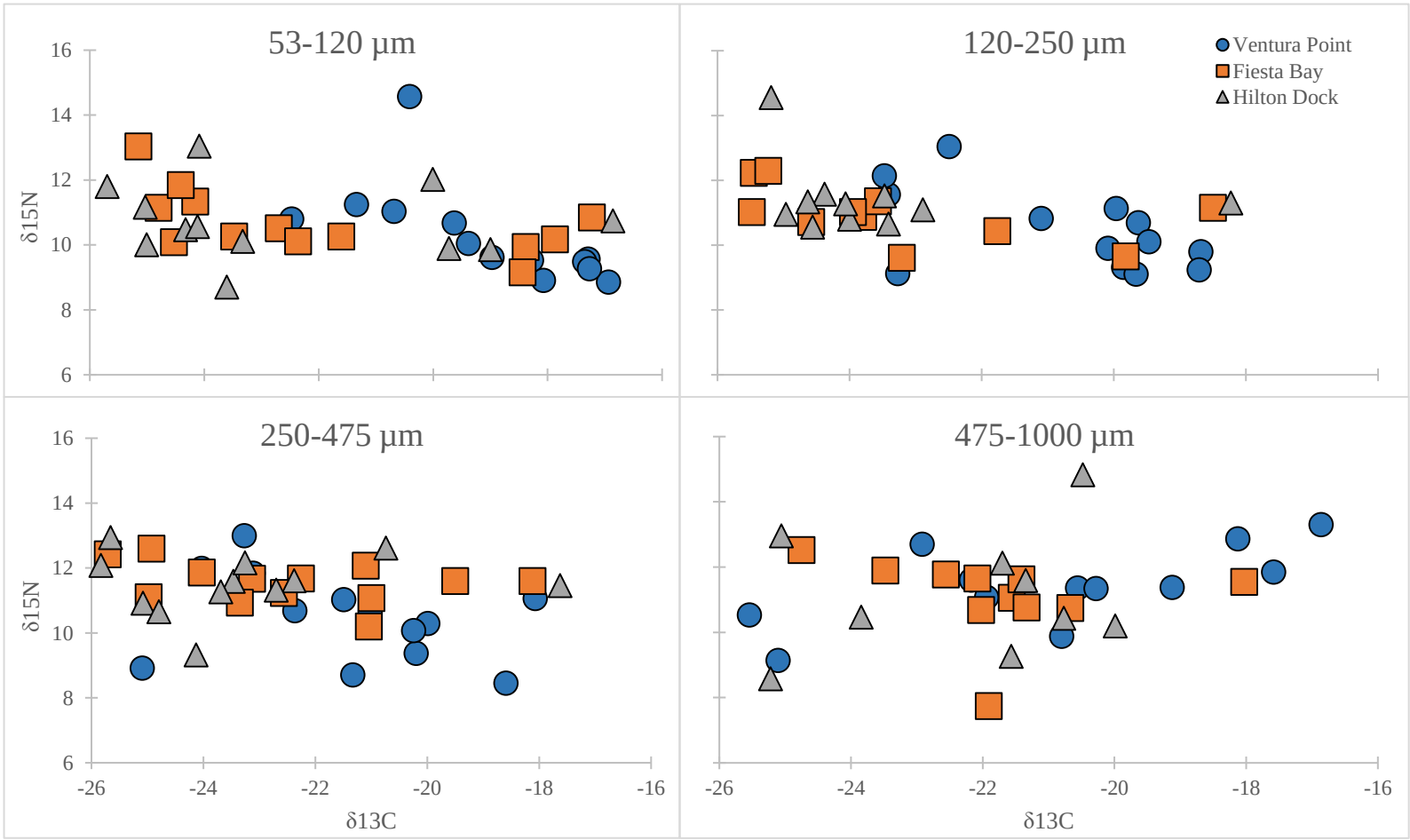

53-120 µm
120-250 µm
250-475 µm
475-1000 µm
Ventura Point
Fiesta Bay
Hilton Dock
δ15N
δ13C
16
14
12
10
8
6
-26
-24
-22
-20
-18
-16

Table A.1 Mean ± standard deviation of carbon and nitrogen stable isotope values for each size class (53-120 µm, 120-250 µm, 250-475 µm, and 475-1000 µm) across all sites.

Size Class (µm)	Mean ± SD	
	Carbon (‰)	Nitrogen (‰)
53-120	-21.3 ± 2.8	10.3 ± 1.1
120-250	-23.4 ± 2.0	10.9 ± 1.0
250-475	-22.6 ± 2.2	11.3 ± 0.9
475-1000	-21.5 ± 3.2	11.4 ± 1.8

Table A.2 Significant results of Spearman Rank Correlation analysis between stable isotopic values and percent composition of taxa for all size classes at all three sites.

Site	Size class	Isotopic Value	Rho value	p-value	Taxon
Ventura Point	53-120	$\delta^{13}C$	0.59	3.54E-02	*Ceratium lineatum*
	475-1000	$\delta^{13}C$	-0.70	1.45E-02	*Acartia californiensis*
Fiesta Bay	250-475	$\delta^{13}C$	-0.71	8.83E-03	*Oithona similis*
	475-1000	$\delta^{13}C$	-0.74	6.40E-03	*Acartia californiensis*
		$\delta^{15}N$	0.87	2.46E-04	*Acartia californiensis*
Hilton Dock	53-120	$\delta^{15}N$	-0.73	4.22E-03	*Oithona similis*
	475-1000	$\delta^{15}N$	0.67	3.38E-02	*Acartia californiensis*