UTILIZING A TOWED SHADOWGRAPH IMAGING SYSTEM TO RESOLVE THE INFLUENCE OF VERTICAL STRUCTURE ON MESOZOOPLANKTON TRAITS IN THE SOUTH ATLANTIC BIGHT

by

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(Under the Direction of Adam Greer)

ABSTRACT

Mesozooplankton play a critical role in marine ecosystems, yet the oceanographic and ecological forces structuring their communities are not well resolved. Trait-based approaches are used to simplify complex marine systems and describe the oceanographic drivers of mesozooplankton community distributions. Conventional sampling techniques, however, typically do not measure distributions at the spatial scale of fine-scale oceanographic transitions. Using a towed shadowgraph imaging system, we measured mesozooplankton abundances along the mid-shelf in the South Atlantic Bight (SAB) in August 2021. The zooplankton community was parsed into key traits, and stratification intensity was used to identify discrete oceanographic zones. trait diversity was lower in smoothly stratified waters due to dominance of low carbon content zooplankton or passive swimmers. Bottom water intrusions with high chlorophyll-a generate sharp stratification resulting in high zooplankton abundances with a diverse set of traits, which has implications for trophic transfer and carbon flux on the shelf.

INDEX WORDS: Zooplankton, Trait-based, Imaging, Oceanography, Marine ecosystems, Ecological function, Generalized Linear Model, In situ plankton imaging, Gelatinous, Swimming speed, Feeding behavior, Stratification, Carbon content, High resolution sampling

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Mesozooplankton play a critical role in marine ecosystems as they transfer carbon from microzooplankton and primary producers to higher trophic levels (Steinberg and Landry, 2017). This intermediate link in the marine food web consists of thousands of species that have differing life histories, diets, and predators (Kiørboe, 2011). By better understanding the ecological linkages between meso and macro-zooplankton (>2.2 mm ESD) and their environment in changing conditions, we can resolve their influence on higher trophic levels, including economically valuable fisheries, and determine the influence of different zooplankton groups on broader ecosystem properties including vertical export of carbon, nutrient cycling or favorable habitat for fisheries production (Serra-Pompei et al., 2020).

The ecology and distribution of these larger zooplankton groups are sensitive to environmental factors, such as spatial variations in oceanographic conditions. Salinity, temperature, chlorophyll-a, and dissolved oxygen can influence the distribution and vertical structure of zooplankton (Coston-Clements et al., 2009; Greer et al., 2020; Treible et al., 2022). Although studies have quantified taxon-specific patch sizes and community structure in relation to oceanographic variables (Greer et al., 2016; Robinson et al., 2021), the variety of oceanographic and ecological forces influencing zooplankton patchiness, both vertically and horizontally, has yet to be explored thoroughly in different oceanographic contexts, especially on the fine spatial scales relevant to organism behavioral responses. Fine-scale (1-10 m) environmental parameters influence the structure of zooplankton communities and their ability to feed, sense prey, and migrate vertically in the water column (Prairie et al., 2012; Greer et al., 2020; Schmid et al., 2020). Because of the large number of species and trophic links, it can be difficult to resolve the functional roles of each organism in these complex marine systems (Kiørboe et al., 2018).

Trait-based approaches can reduce the complexity of taxonomically detailed marine food webs by collapsing taxonomic diversity into a smaller number of functional trait-based groups most relevant to ecosystem functioning (Kiørboe et al., 2018). Furthermore, in coastal marine systems, taxonomic groups can vary widely among regions, but functional traits are often consistent in similar habitats regardless of geographic location (Hemingson and Bellwood, 2018). Functional traits are defined as any heritable morphological or physiological characteristic that influences the fitness of an individual through growth, reproduction, or survival (Kiørboe et al., 2018).

Key traits are functional traits that best explain an individual’s fitness and have a large impact on how an organism interacts with its physical/chemical environment or other organisms. Understanding key traits and the associated trade-offs of energy cost or fitness potential helps determine how and why certain traits are favored under different conditions (Våge et al., 2014). Physiological traits including, for example, swimming ability and feeding strategy (i.e., sight or tactile sensing) transcend taxonomic distinctions (Kiørboe et al., 2018). Size and carbon content are also key traits that, when considered together, can indicate a plankter’s trophic level and provide information about its adaptations (Figueiredo et al., 2020; Martini et al., 2021). While high carbon organisms (e.g., shrimp or fish) are typically gape limited and consume prey in a relatively narrow size range, low carbon organisms (e.g., gelatinous zooplankton) can feed on a wider size range of prey (Acuña et al., 2011; Kiørboe, 2011). Trait-based approaches offer promise for understanding the functional role of different zooplankton groups and are increasingly being applied to marine ecosystems, but traits are rarely examined in the context of fine-scale oceanographic changes due to the commonly-used and relatively coarse net-based sampling techniques. Furthermore, plankton nets severely underestimate abundances of fragile gelatinous organisms that typically have low carbon content (Remsen et al., 2004; Kiørboe 2013).

Over the last 100 years, zooplankton sampling technologies have been developed to acquire increasingly detailed observations to closely match the observational scales of physical or environmental sampling, cm to m, (Wiebe and Benfield, 2003; Wiebe et al., 2017). Net-based systems and high-speed samplers have played an important role in oceanography, but they lack capabilities for fine-scale analysis of zooplankton communities and collection of synoptic environmental data. Additionally, microscopy-based analysis of plankton samples is laborious to process and analyze. Optical-based systems have been developed to address these problems, each with different tradeoffs and advantages for different size classes of organism (Lombard et al. 2019). While most of these imaging systems sample through a constrained or small volume that limits the size of organisms that can be imaged to microzooplankton or phytoplankton, many towed imaging systems provide the capabilities needed for fine-scale data collection, automated sizing, and fully or semi-automated analysis, making them a useful tool for investigating the fine-scale distribution of zooplankton with respect to in situ water column conditions (Bi et al., 2015; Luo et al., 2018; Lombard et al., 2019; Bochinski et al., 2019)

Shadowgraph imagers, such as the Ichthyoplankton Imaging System (ISIIS, Cowen and Guigand 2008), allows for the imaging of large and relatively rare meso and macrozooplankton by capturing images of plankton passing through an unconstrained volume in front of a light source. The optical technique uses a small point source and plano-convex lenses to produce collimated light, allowing the plankton appear the same size regardless of their distance from the camera within the imaged volume (Cowen and Guigand, 2008). Shadowgraph imagers like these have been increasingly used to study how zooplankton communities are structured (Greer et al., 2013; Robinson et al., 2021; Briseño-Avena et al., 2020). They are ideal for use in continental shelf regions where a large portion of the water column can be sampled and the effects of oceanographic anomalies such as intrusions, internal waves, and eddies can be observed (Greer et al., 2014; Schmid et al., 2020; Swieca et al., 2020).

One location that provides an ideal environment for investigating the role of water column structure in zooplankton communities is the South Atlantic Bight (SAB). This continental shelf region of the North American east coast extends from Cape Hatteras, North Carolina to Cape Canaveral, Florida and is primarily influenced by the Gulf Stream western boundary current. The shelf width is at a minimum of 30 km off Cape Hatteras, increases to a max of 120 km off Georgia, and then decreases down to 50 km on the Florida coast. Wind varies in direction throughout the year in the SAB, tending to be more consistent and higher velocity in the winter months (Weber and Blanton, 1980). The SAB can be split into three shelf zones, the inner shelf (up to-20m), mid-shelf (20-

45m), and outer shelf (45m-shelf edge); each of which have different oceanographic influences. The inner shelf dynamics are driven by river output, wind patterns and tidal fluctuations, while the outer shelf is mostly influenced by changes in the Gulf Stream (Lee et al., 1991). Variability on the mid shelf is primarily driven by seasonal winds and tides (Michel, 2013).

The SAB continental shelf has a large mid-shelf region of with relatively long residence times for water (~41 days) in the summer months (Castelao, 2011; Savidge and Savidge, 2014). Residence times can be longer in the winter months, but the water column is typically well mixed. Several interrelated processes including heat flux, bottom water intrusions, and wind forcing cause stratification on the mid-shelf, structuring the water column and creating different environments that lead to particular groups of primary producers and broader ecological properties (Yoder et al., 1983; Blanton and Atkinson, 1983; Atkinson et al., 1983). Additionally, nutrient-rich upwelled water that moves onto the shelf through bottom intrusion stimulates productivity because of the availability of light in the relatively shallow mid-shelf zone (Yoder et al., 1985; Pelegrí and Csanady, 1991; Castelao, 2011). Deep water intrusion or river output and seasonal oceanographic conditions on the mid-shelf can influence zooplankton distributions and increase abundances of certain taxa with the influx of nutrients (Paffenhöfer et al., 1984; Paffenhöfer et al., 1987; Paffenhofer, 1992; Lopez-Figueroa, 2017).

Due to infrequent mixing or input from the Gulf Stream and rivers, water on the shallow, broad mid-shelf of the South Atlantic Bight (SAB) has relatively constrained nutrient sources and long residence times that have similar time scales to the life cycle durations of many shelf zooplankton species. Thus, the mid-shelf region of the SAB provides the opportunity to observe the dynamics of many zooplankton groups in situ without requiring a Lagrangian study. Additionally, summer months cause more stratification in shelf regions due to solar heat flux and intrusion events, creating an ideal time to observe how these environmental anomalies influence zooplankton abundances. The ISIIS is typically towed in long transects (Greer et al., 2014; Briseño-Avena et al., 2020), which provide m to 10-km spatial resolution but are not practical for other sampling strategies where multiple stations are sampled quickly with other shipboard instrumentation (CTD, plankton nets, water samples, etc.). A new smaller shadowgraph imaging system similar to the ISIIS, the Mini Deep-focus Plankton Imager (mDPI), was designed for station-based sampling, making it easier to sample at multiple locations over a broader spatial extent. Image data were collected in a variety of oceanographic conditions in the SAB during August 2021, generally characterized by sharp vertical gradients in temperature and chlorophyll-a fluorescence. By analyzing the vertical changes of certain functional traits new insights into how oceanography structures zooplankton communities and potentially influences their function can be gained.

The mDPI can resolve how changing oceanographic conditions in a continental shelf ecosystem can influences zooplankton distributions in regard to traits and taxonomy. Functional traits chosen to be examined for this study included size, carbon content, motility, and feeding strategy because they are commonly used for understanding environmental influence and interactions in plankton communities (Kiørboe et al., 2018). Size can be used as a proxy for trophic level (Martini et al., 2021), while carbon content provides insight into what kinds of adaptations an animal has and its value as prey to certain predators (Kiørboe, 2013). Swimming speed is a measure of a plankter’s motility in changing conditions and its ability to catch prey (Kiørboe et al., 2018). Feeding strategy can be used as an indicator for what environments might be favorable to a plankter’s hunting behavior or resource acquisition (Kiørboe, 2011). The zooplankton were divided into 23 taxonomic groups. By examining and comparing the trait and taxonomic distributions, we can determine which oceanographic drivers most contribute to mesozooplankton composition changes along the SAB shelf.

Questions

The images and corresponding oceanographic data that were collected with the mDPI were analyzed to answer the following questions:

- What is the fine- scale vertical distribution and abundance of shelf-associated meso and macro-zooplankton with respect to taxonomy and functional traits?
- How does the community respond to changes among stations in the vertical oceanographic structure and environmental factors?

Hypotheses

The primary predictions of this study involve the association of trait abundance and diversity with environmental parameters. A priori expectations of the relationship between functional traits investigated and environmental parameters measured are provided in Table 1 and visualized for two of the traits using a conceptual diagram (Figure 1). The first hypothesis is that the relationship between the environment and size is expected to show that smaller plankton are more influenced by the environment than larger plankton. Larger plankton are likely stronger swimmers and better equipped for navigating a given environment, while also being less likely to be eaten by prey in different areas of the water column. Therefore, larger plankton are expected to be more distributed vertically. The second hypothesis is that zooplankton with high carbon content will be broadly distributed and less influenced by oceanographic properties, while low carbon organisms are expected to be patchier and more correlated with temperature, and chlorophyll-a. These low carbon zooplankton should also have higher abundance in stations that are highly productive (as indicated by total particle abundances). High carbon plankton tend to have mobile feeding strategies and are less likely to aggregate with a particular water environment or oceanographic zone, while low carbon organism can often be less motile and be more dependent on the presence of small plankton. The third hypothesis is that for motility, or swimming speed, the quickest swimmers will be distributed vertically, while the slow or passive swimmers are more likely to aggregate in relation to the pycnocline. The fourth hypothesis regarding feeding or trophic strategy has the most complex predictions because they are related to behavior. Passive ambush and feeding current feeders as well as filter feeders are expected to aggregate with chlorophyll-., This is due to the likely presence of POM and smaller plankton, which can be easily consumed in these areas. Active ambush feeders sit and wait to sense nearby prey that cross their path, they are predicted to aggregate in relation to the pycnocline because of their propensity for sitting still and letting the water carry them. Cruisers and searchers are likely to be vertically distributed but should have higher relative abundance in station with smooth stratification. In addition to the individual trait predictions (Table 1), a fifth hypothesis addresses diversity of the trait categories. Smoothly stratified stations are expected to have lower trait diversity compared to sharply stratified stations, due to the lack of structure, which generates different environments and ecological niches. Diversity is also expected to change spatially with latitude and depth of station.

Table 1: Expected observations organized by trait.

Trait	**Expected Observation**
Size	Larger zooplankton observed to be distributed vertically more consistently than smaller zooplankton which will be observed in higher concentration in density gradients
Carbon Content	High carbon plankton will be distributed throughout water column, more abundant at the pycnocline, and similar abundances among stations. Low carbon: more aggregations in relation to the pycnocline, patchier, and higher abundance in more productive locations
Swimming Ability/Motility	fast swimmers: widely distributed low swimming ability: more aggregated
Trophic or Feeding Strategy	passive ambush/feeding current: aggregate with Chl-a Active ambush feeders: aggregates in relation to pycnocline Filter feeders: aggregate with Chl-a Cruisers/searchers: distributed widely, higher relative abundance in smoothly stratified stations

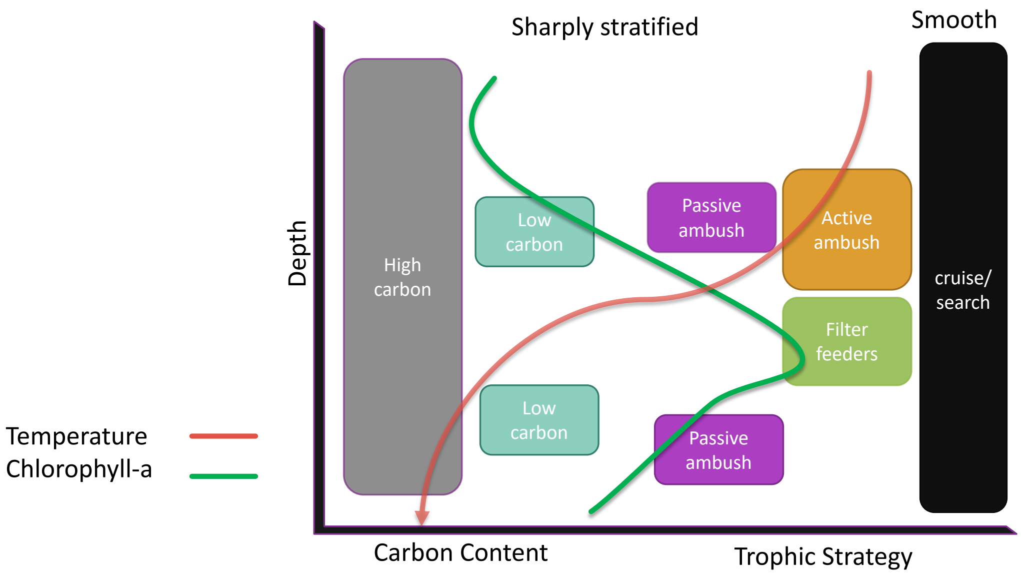

Figure 1: Conceptual diagram of expected observations for carbon content and trophic strategy in sharply and smoothly stratified stations, Boxes display areas of relative abundance in the water column. Lines display vertical profiles of temperature and chlorophyll-a, designated by color.

CHAPTER 2

METHODS

Study area

Data were collected during a research cruise conducted in the SAB aboard the R/V *Savannah* from August 10 – 19, 2021. The goal of this study was to observe the vertical and latitudinal distribution of gelatinous zooplankton during stratified summer conditions. Sampling focused on the middle shelf region where, due to oceanographic conditions, water can reside long enough for zooplankton blooms to fully develop. At each mid-shelf transect, station-based sampling primarily occurred at the inner edge (25 m isobath) and outer shelf edge (45 m isobath) of the mid-shelf region. The 25 m isobath is influenced by tides and river output, while the 45 m isobath is more influenced by bottom water intrusion associate with wind and heat flux, allowing for observation of how these different influences may change the zooplankton community (Pomeroy et al., 1987; Paffenhofer, 1992). Over the course of the cruise, 24 stations were sampled from 31.4°N (Savannah, GA) to 28.2°N (Cape Canaveral, FL). Several stations were sampled multiple times to observe changes in the zooplankton community over time.

Sampling and data acquisition

Fine-scale distributions of mesozooplankton were resolved with the mini Deep-focus Plankton Imager (mDPI). The mDPI uses shadowgraph imagery to capture consistent sequence of 2330 x 1750-pixel (10.19 cm x 7.66 cm) images, with a depth of field of 20 cm and a volume of 1561 mL at a rate of 5 s^{-1}. These images are collected along with a suite of oceanographic data on the same towed vehicle. The imager is towed for approximately 1 hour at ~2 – 2.5 knots in a "tow-yo" fashion up and down through the water column going from near surface (3-4 m) down to ~5-10m from the seafloor. This motion is achieved by manually paying in and out cable from a fiber-optic winch system. The mDPI is also equipped with four oceanographic sensors including an ECO-FL-RT G4 (125ug/L), 1 Hz, for measuring chlorophyll-a fluorescence, an SBE49 350dbar CTD, 16 Hz, and an Aanderaa oxygen Optode 4330F, 0.5 Hz, for dissolved oxygen. Sensors were calibrated as recommended by the manufacturer prior to use.

Imager data processing and classification

Imagery from the mDPI was processed using the software ImageJ (ImageJ 1.53e, Schneider et al. 2012) and analyzed in R (R Core Team 2022, v4.1.1). Because oceanographic sensors on the mDPI sampled at different rates, R code was developed to use the timestamp from each sensor to merge them based on the closest point in time. The CTD sampled most frequently (~16 Hz), so data from the fluorometer and oxygen optode were repeated for each measurement from the CTD.

Images from the mDPI were separated by station number, and a standard flat fielding routine was applied to remove artifacts and improve contrast between plankton and the background. Flat fielding is done by making an average background of 100 images and subtracting that average background from those 100 images, then performing that operation for the whole set of images. ImageJ macros were used for segmentation and identifying regions of interest (ROIs) by converting full frame images to black and white with a grayscale level of 200 (Figure 2). A size threshold was used to select only ROIs above 2000 pixels (~2.2 mm equivalent spherical diameter) to ensure the inclusion of only mesozooplankton and some larger macroplankton. Particle statistics (i.e., area, height, width, solidity) associated with each ROI were also recorded in the segmentation process and stored in a separate file.

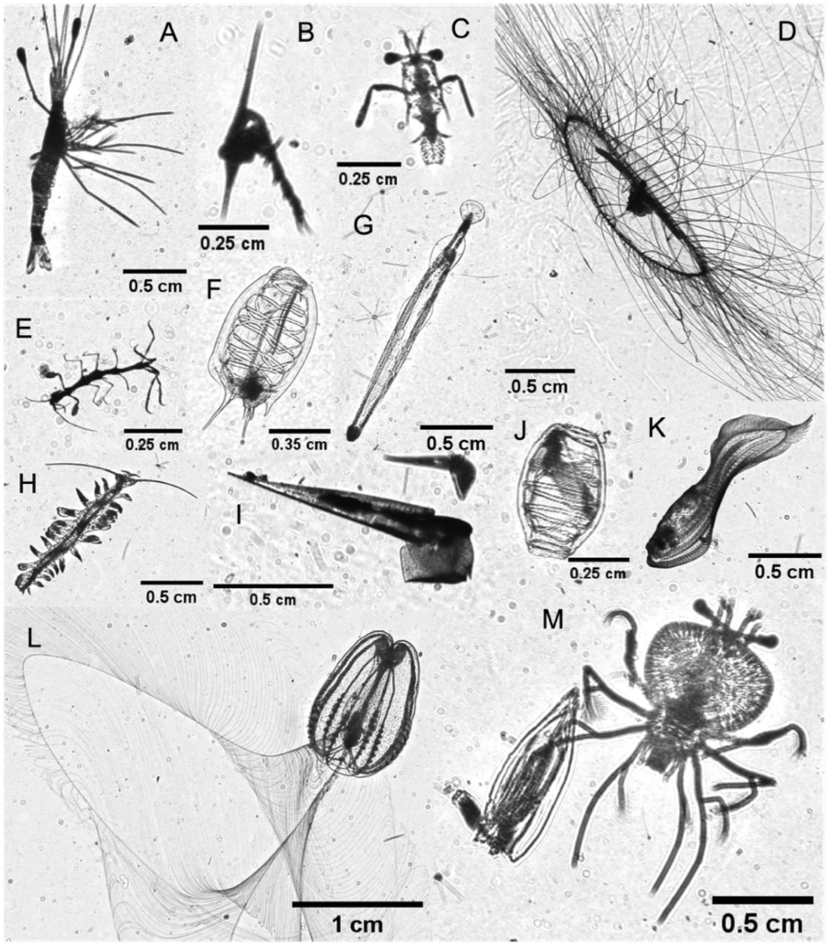

Figure 2: Selected examples of shadowgraph plankton images from the mDPI. A) shrimp, B) crab zooea C) stomatopod larva, D) hydromedusae, E) amphipod, F) salp G) chaetognath, H) polychaete, I) pteropod, J) doliolid, K) larval flatfish, L) ctenophore, M) lobster phyllosoma carrying a siphonophore.

ROIs were individually labeled as one of 23 categories of zooplankton using ImageJ macro customized keyboard shortcuts (Figure 2, Table 2). Tunicates (salps and doliolids) were separated into life stage categories, including gonozooid, phorozooid and nurse for doliolids, and chains and solitary individuals for salps. Taxonomic groups were identified to the lowest practical level for in situ imagery data, typically to general taxonomic groups (e.g., chaetognaths, hydromedusae, etc. – see Table 3). Several stations (4, 7, 14, 20) supported high abundances of protists (*Rhizaria*) that were too numerous to classify individually. Because these protists had consistent properties in terms of particle statistics, a particle solidity threshold of 0.8 was applied to the ROIs to remove them. Solidity is defined as [area]/ [convex area] (Ferreira and Rasband, 2021). Samples of excluded images demonstrated that ~96% of ROIs excluded using this method were protists (Table 3). ROIs of zooplankton were selected from the resulting file of non-excluded images and assigned a taxonomic label. Labeled ROI particle statistics were merged with physical data as previously described (Greer et al., 2014; Takahashi et al., 2015) by associating the closest oceanographic data point to the moment the image was taken based on a calculated Julian time.

Trait categories and assignment

Each ROI was grouped into four different functional trait categories based on taxonomic identity as previously described (Kiørboe, 2011; Kiørboe, 2013; Kiørboe et al., 2018; Martini et al., 2021) (Table 2). The size trait was assigned to each ROI as one of three size classes, with each size class being a larger range to obtain similar numbers of ROIs in each size class. The size ranges for class 1, class 2, and class 3 were 2000 – 3000 pixels (2.2-2.7 mm equivalent spherical diameter, ESD), 3000 – 5000 pixels (2.7 – 3.5 mm ESD), and >5000 pixels (>3.5mm ESD). Other traits values were assigned to each ROI based on its taxonomic identity. Carbon content was assigned as either low, medium, or high percent carbon, following the general patterns of % wet weight of carbon (Kiørboe, 2013).

Table 2: Taxonomic categories with associated label and assigned traits.

ID	***label***	***trophic***	***motility***	***carbon***
Shrimp	shrimp	cruise/search	quick	high carbon
fish	fish	cruise/search	quick	high carbon
amphipods	amphi	cruise/search	slow	high carbon
zooea (crabs & lobsters)	zooea	cruise/search	slow	high carbon
stomatopods	stoma	cruise/search	slow	high carbon
copepods	copepod	active ambush	sufficient	high carbon
isopods	isopod	cruise/search	sufficient	high carbon
pteropods	ptero	passive ambush	sufficient	intermediate carbon
polychaetes	poly	cruise/search	sufficient	intermediate carbon
chaetognaths	chaeto	active ambush	sufficient	intermediate carbon
salp chain	salpchain	filter feeders	passive	low carbon
salp ring	salpring	filter feeders	passive	low carbon
appendicularians	app	filter feeders	slow	low carbon
doliolid	dolio	filter feeders	slow	low carbon
doliolid phorozooid	dolphor	filter feeders	slow	low carbon
doliolid nurse	dolnurse	filter feeders	slow	low carbon
salp	salp	filter feeders	slow	low carbon
Hydromedusae	hydro	passive ambush	passive	low carbon
scyphomedusae & ephyra	scypho	passive ambush	passive	low carbon
anemone	anemo	passive ambush	passive	low carbon
ctenophores	cteno	passive ambush	slow	low carbon
siphonophores	siphon	passive ambush	sufficient	low carbon
Heteropods	hetero	cruise/search	sufficient	Intermediate carbon

Table 3: Data from protist exclusion procedure performed on four stations. Percentages were calculated from random samples of 1000 taken from excluded ROIs.

Station	***#animals/sample size***	***Percent protist***
2	87/1000 = 8.7%	91.3%
7	22/1000 = 2.2%	97.8%
14	46/1000 = 4.6%	95.4%
20	18/1000 = 1.8%	98.2%

Zooplankton taxa tend to be either gelatinous and low carbon or non-gelatinous and high in carbon, with a small group of taxa containing intermediate levels of carbon (e.g., polychaetes, chaetognaths, and pteropods, Kiørboe, 2013). Swimming speed categories, while not quantified for most zooplankton taxa, were determined based on field observations from live net tow samples that were filmed using a lab shadowgraph imaging device with similar optics to the mDPI. The assignment of four swimming speed categories, including passive, slow, sufficient, and quick, was based off the cruising speeds of zooplankton, rather than jumping or escape speeds (e.g., copepods and shrimps have extremely fast hopping capabilities for escape responses), because cruising is most relevant to feeding. Passive swimmers are the least efficient and include those with body plans not well adapted for quick movements or changes in direction. Passive swimmers include medusae, anemones, salp chains, and salp rings. Slow swimmers are slightly quicker and more maneuverable and include doliolids (all life stages), salps, ctenophores, appendicularians, stomatopods, amphipods, and zooea. Sufficient swimmers are highly maneuverable and capable of sustained velocity for use in capturing prey. Pteropods, copepods, polychaetes, chaetognaths, isopods, heteropods, and siphonophores all fall into this category. While one might presume that because siphonophores are gelatinous, they must be slower, in the SAB they tend to have aerodynamic body plans that allow them to swim more efficiently than other gelatinous animals. Quick animals include fishes and shrimp, which have the strongest swimming capabilities of the zooplankton examined in this study.

For trophic or feeding strategy, one of four categories was assigned: filter feeder, cruise/search, active ambush, and passive ambush feeders (Kiørboe, 2011). While these categories are general and not every species in a taxon fits neatly into them, it is assumed that the majority of species in these groups fit with the assigned trait. Filter feeders included zooplankton known for filtering large amounts of water over a filter or mesh to catch prey (e.g., doliolids, salps, and appendicularians). While current producing feeders that do not filter over a mesh, such as hydromedusae, may be considered filter feeders, this strategy was grouped with passive ambush feeders here. Kiørboe (2011) argued that hydromedusae display both current producing and passive ambush behaviors. Passive ambush feeders swim freely and capture prey utilizing specialized anatomical adaptations and include medusae, anemones, siphonophores, ctenophores, and pteropods. Active ambush feeders sense prey in their vicinity and strike quickly when detected. Chaetognaths are the most common members of this group, but copepods were also included. While copepods are very well studied and highly adaptive to different feeding strategies, active ambush feeding is common amongst larger copepods (which were segmented by the image processing routine) and is one of their more specialized feeding methods (Kiørboe, 2011). Cruisers/searchers are those that swim and search for prey using sight as a primary means for sensing. Fish, stomatopods, shrimp, zooea, polychaetes, isopods, heteropods, and amphipods are examples of this group. While some species in a particular taxon might differ in the expression of a trait, it was assumed that the general categories and assigned traits presented in Table 2 applied to the vast majority of observed species.

Water column abundance, oceanographic zones, and diversity metrics

In order to address the questions regarding the structure of zooplankton communities, the locations of organisms in the water column and their abundances, as well as the associated environmental data, were analyzed using several methods. A Spearman correlation analysis was performed on the oceanographic data to assess their relationships using the Hmisc package and corrplot packages in R (Harrell, 2022; Taiyun, 2022). Vertical profiles of environmental data from each station were created to assess the major oceanographic changes in the water column and to help determine smooth or sharply stratified stations. Environmental data was averaged across the length of a tow to get a representative vertical profile for each station. This was acceptable because tows were not conducted over large transects and likely never exceeded a length of greater than two nautical miles.

Both environmental and ROI data were binned into 1 m depth bin for the creation of vertical profiles and to analyze the influence of the environment on abundances. Vertical 1-m bins were chosen because they allowed for analyses to be fine-scale and, at the same time, allowed each bin to have an adequate volume of water and number of ROIs of each taxon to calculate accurate abundances. Additionally, 1-meter bins eliminated noise from the calculation of stratification ($N^2 = -(-g/\rho)\, d\rho/dz$) derived from the buoyancy frequency ($N = [(-1/\rho)\, d\rho/dz]^{1/2}$)) as a measure of vertical density stratification.

To assess community differences between oceanographic zones, binned ROI data was used to assign a stratification value (N^2) to each ROI, calculated for each 1 m bin using the GSW package in R (Kelley and Richards, 2022). For stations with sharp stratification, ROIs with a stratification value higher than 0.0025 s^{-2} were labeled as being in the pycnocline, while ROIs above and below were labeled respectively. These oceanographic zones make up the water column of stations defined as sharply stratified or "sharp." ROIs in stations with no pycnocline or smooth stratification were classified as being in a "smooth," which was considered its own oceanographic zone.

Counts of the total numbers of ROIs in each station, including those not labeled, were used as a proxy for productivity and divided by the total volume sampled at the station for a raw abundance value for the whole station (Figure 3). Classified ROI abundances were calculated for each taxon at each station by dividing counts of classified ROIs in a given bin by the volume sampled in that bin. Visualizations were generated in RStudio (R Core Team 2022, v4.1.1) using the ggplot2 package (Wickham, 2016).

Taxonomic diversity and evenness of traits was assessed using Shannon diversity (Hill, 1973) and Pielou's evenness, respectively, with the vegan package in R (Oksanen et al., 2022). Shannon diversity considers richness (the number of taxa present) and evenness (how evenly distributed the abundances of taxa are). Shannon diversity is calculated as:

$$Shannon\ Index\ (H') = -\sum_{i=1}^{S} p_i ln(p_i)$$

where p is the counts or abundance of a specific taxa or trait, S is the number of traits or taxa present at a location.

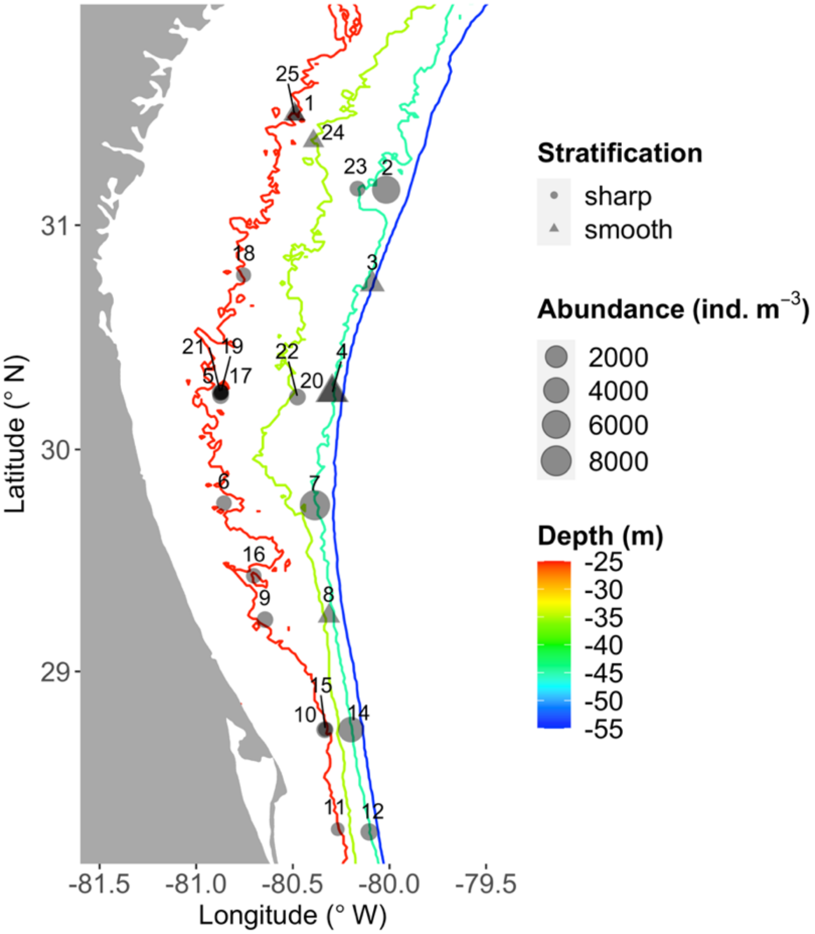

Figure 3: Locations of the stations where mDPI tows were conducted between Savannah, GA and Cape Canaveral, FL numbered by order sampled. Several locations were sampled more than once at different times creating darker points that are stacked. Image data were not collected at station 13 so it is not displayed. The color of the contour lines depicts isobaths starting at 25 m and increasing by increments of 10 m. Abundance of segmented images was calculated by dividing the total number of ROIs at each station by the volume sampled and is shown by size of point. Shape depicts if a station was categorized as smooth or sharply stratified.

The abundances of different life stages of doliolids and salps were pooled together for the calculation of Shannon diversity. Because the number of trait categories present in each environment is consistent, Pielou’s evenness index was suitable for assessing trait diversity and provided a more interpretable scale understanding trait composition (0-1). Pielou’s evenness is calculated as:

$$Pielou's\ Eveness\ (J') = \frac{H'}{ln(S)}$$

where H' is the Shannon diversity of the traits, and S is the number of trait categories. While other measure of functional diversity have been used (Pomerleau et al., 2015), Pielou’s evenness is a simple calculation that can be used for assessing the evenness of trait categories if the number of categories in a trait is taken as the number of species or richness value. The calculations were performed on count data for each station and oceanographic zone. Evenness was also paired with oceanographic zone to determine the influence environmental factor might have on trait diversity.

Vertical distributions and trait percentages within oceanographic zones

A bootstrap resampling method was implemented to use ROIs from the different defined oceanographic zones at all stations to investigate trait composition changes among different water masses. ROIs from all stations were pooled into their respective oceanographic zone categories for resampling. The bootstrapping was done by taking 1000 random samples with replacement of 20% of each oceanographic zone. Totals for each trait value in each sample was used to calculate a proportion of each trait. A mean and 95% confidence interval were calculated using the sets of 1000 samples to determine the average percentages of trait values in each oceanographic zone. Corresponding in situ abundances for each oceanographic zone were also calculated by taking the total number of each trait value from a specific oceanographic zone pooled across stations and dividing by the volume sampled in that bin.

Influence of environmental factors on trait abundance

Generalized Linear Modeling (GLM) using a quasi-Poisson distribution helped determine which environmental variables significantly influenced the distributions and abundance of each trait. The GLM technique can model a non-normal distribution of a response variable through the use of a link function. Because our response variable is taken to be abundance of a trait, the quasi-Poisson distribution family with a log link function was chosen because of the use of count data in the form of abundances. In addition, the quasi-Poissson distribution includes a parameter to account for overdispersion, allowing for quantification of the aggregation tendencies of the different traits. The Poisson distribution assumes the variance and the mean are equal, while quasi-Poisson accounts for the case in which the data are overdispersed, (variance is greater than the mean) by using an additional parameter that represents the ratio of the variance to the mean. Abundances of each trait from 1-m depth bins for every station were calculated by dividing the number of ROIs of a certain trait by the volume sampled at a specific depth and station. The explanatory variable data were average environmental parameter values at each depth bin for each station. Salinity did not change substantially across the study area (36.08 – 36.37) therefore, it was excluded from the model. The generalized linear models were performed using the stats package (R Core Team 2022, v4.1.1).

CHAPTER 3

RESULTS

Twenty-four stations (labeled 1 - 25 with no station 13) were sampled during this study. A total of 681,980 ROIs (many of which were excluded as *Rhizaria* colonies) included 114,438 labeled ROIs. The zooplankton used in this analysis represented 64,821 individual ROIs. With the exception of oxygen, oceanographic variables were highly correlated. (Figure 4). The environmental factors with the most observed variability were temperature and chlorophyll-a (i.e., Figure 5 and 6). Of the stations sampled, there were 17 sharply stratified stations and 7 stations with smooth stratification. Density and stratification were highly dependent on vertical changes in temperature.

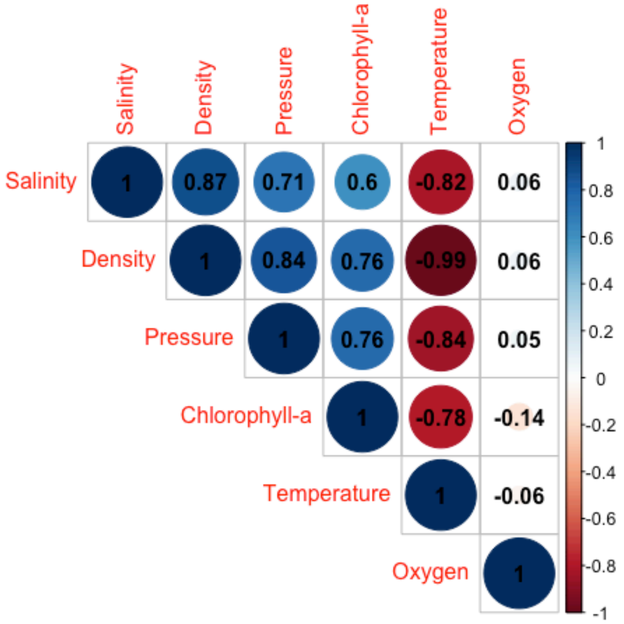

Figure 4: Spearman correlation plot of environmental variables with correlation coefficients. Blue indicates an increasingly positive correlation, while red indicates a negative relationship.

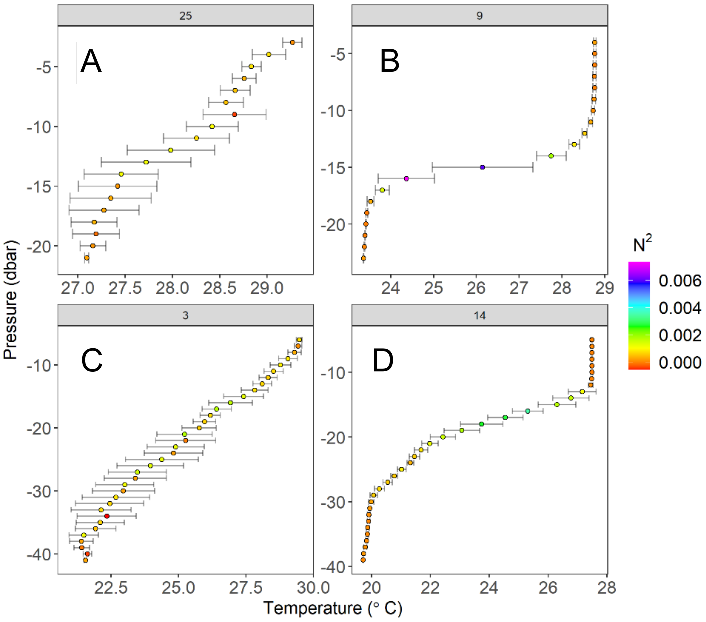

Figure 5: Vertical temperature gradients at several sampling stations based on 1-m binned averages of temperature, A) 25m smooth station 25, B) 25m sharp station 9, C) 45m smooth station 3, D) 45m sharp station 14. Error bars depict standard deviation of temperature values. Stratification (N^2) derived from buoyancy frequency is shown as the color of points.

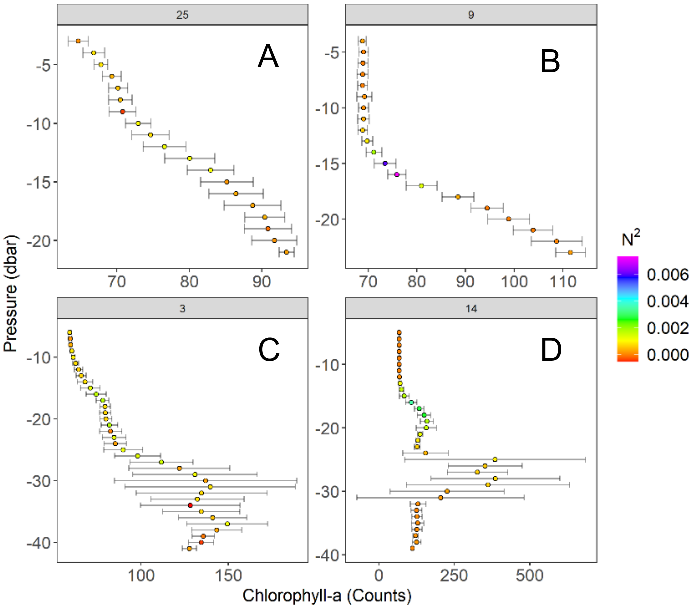

Figure 6: Chlorophyll-a gradients at several sampling stations based on 1-m binned averages of chlorophyll-a, A) 25m smooth station 25, B) 25m sharp station 9, C) 45m smooth station 3, D) 45m sharp station 14. Error bars depict the standard deviation of chlorophyll-a values. Stratification (N^2) derived from buoyancy frequency is shown as the color of points.

Diversity and abundance

The stations sampled showed a notable difference in taxonomic abundance with some consistency throughout (Figure 7). Hydromedusae and chaetognaths were the most abundant zooplankton at across the sampling area. Several of the stations had notable blooms or patches of less common zooplankton. Station 9 had high abundances of pteropods. Stations 2 and 7 had a relatively high concentration of shrimp. Stations 3 and 5 had the highest abundances of doliolids of all life stages, while salps were common at stations 6 and 21. Stations 4 and 10 had the highest abundance of appendicularians. Stations 7 had the highest abundance of all the stations at 268 ind. m^{-3}, the majority of which were hydromedusae. Total zooplankton were least abundant at Station 23 ~70 ind. m^{-3}.

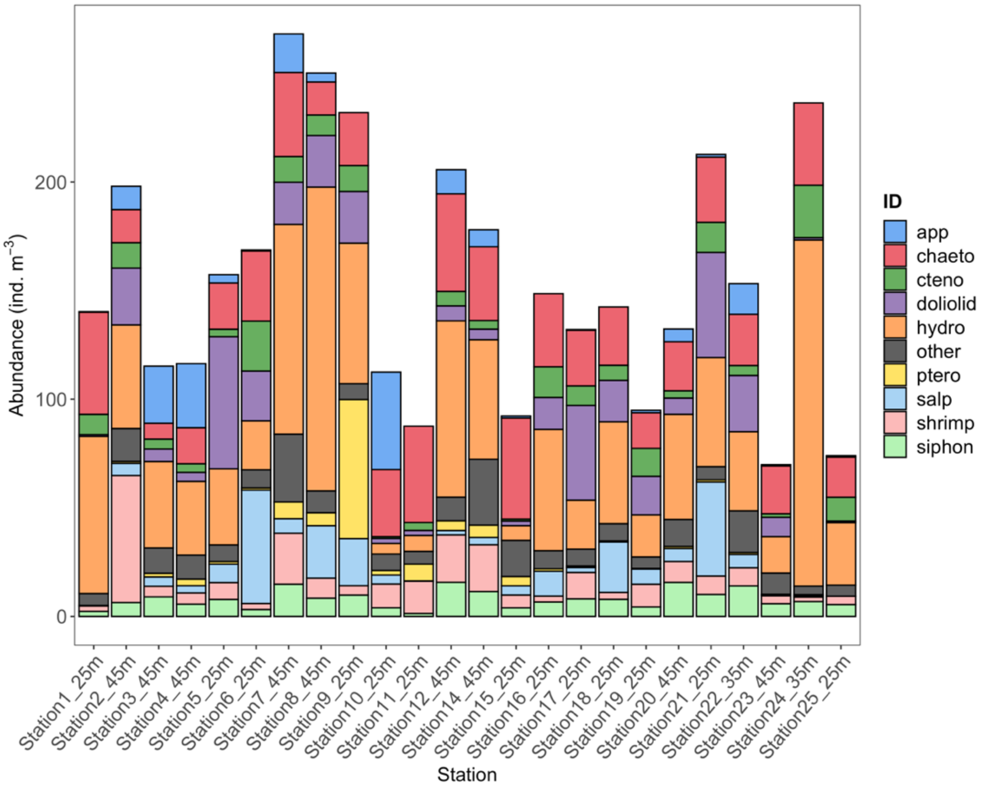

Figure 7: taxonomic abundances of zooplankton depicted across stations

While the same diversity metric was not used for both taxa and traits, observations can be made between diversity and evenness across the sampling area. Taxonomic diversity generally increased from south to north over the sampling area, reaching as high as ~2.5, with some very low diversity values below ~1.25 found at the northernmost latitudes of the sampling area (Figure 8). Shallow 25m stations were generally less diverse than 45m stations and smoothly stratified stations were less diverse than sharply stratified stations at the same latitudes. Trait evenness of different water types was consistent with notably lower diversity in smoothly stratified stations (Figure 9). Carbon had the lowest evenness overall with values consistently below ~0.8 and as low as 0.6 in smoothly stratified stations. This is likely due to the almost universal dominance of low carbon organisms observed in the abundance analysis. Across latitude, motility and trophic strategy had a fair amount of variability (Figure 9). Carbon and size showed more consistent trait diversity among stations.

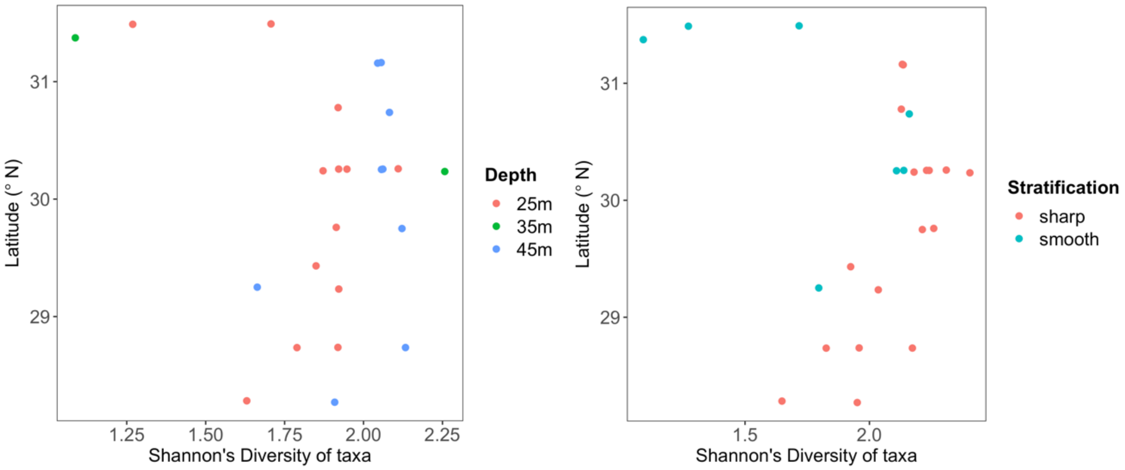

Figure 8: Shannon diversity of taxonomic categories across the sampling area with, A) depth as color, B) stratification category as color.

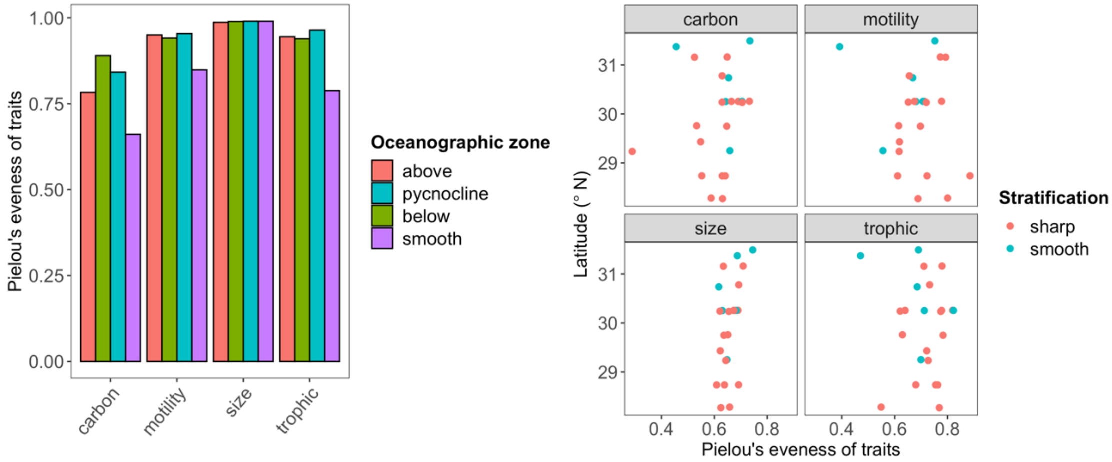

Figure 9: Pielou's evenness of each trait, A) in each oceanographic zone represented as color, B) across latitude with stratification category depicted as color.

Zooplankton traits and oceanographic zone

Zooplankton traits differed among the oceanographic zones with respect to proportions (Figure 10) and abundances (Figure 11). The abundance within each oceanographic zone was observed to be highly variable for all trait categories, with the exception of size. Carbon percentages were skewed towards low carbon having the highest percentage of each oceanographic zone, with its lowest found below the pycnocline (~52%), this corresponded to higher proportions of high and intermediate carbon zooplankton below the pycnocline (~18% and ~25% respectively). The abundances of the carbon content trait were dominated by low carbon across all water types as well (>90 ind. m^{-3}).

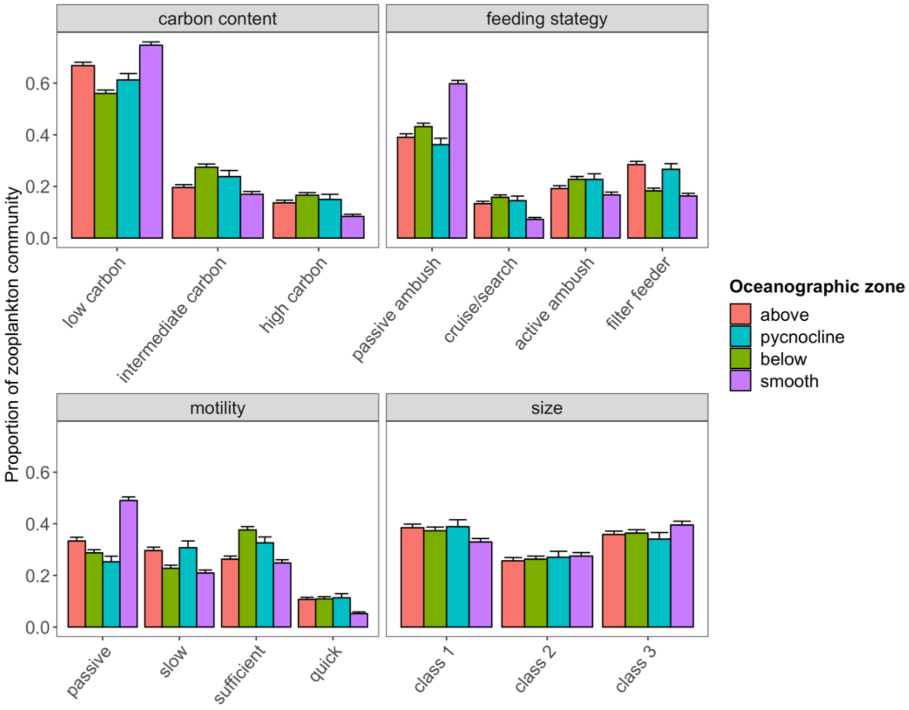

Figure 10: Proportion of the zooplankton community corresponding to each trait and oceanographic zone.

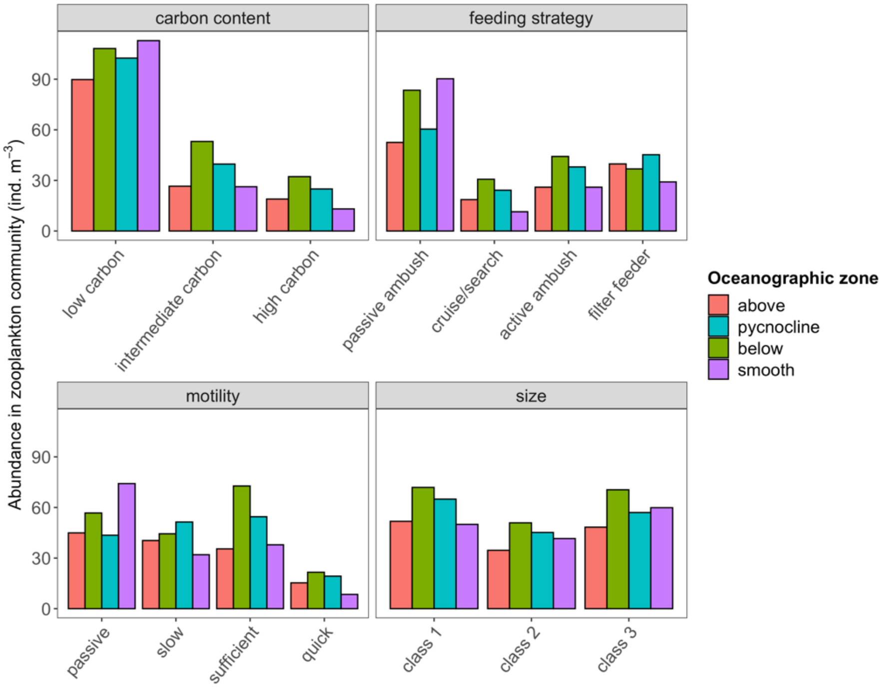

Figure 11: Abundance of trait values (ind. m^{-3}) for each trait and oceanographic zone.

Intermediate and high carbon had higher abundances below and within the pycnocline compared to above and at smoothly stratified stations. Passive ambush feeders were consistently the predominant feeding trait in most water columns, especially in smooth water where they accounted for nearly 60% of the zooplankton. Active ambush feeders had higher proportions below the pycnocline (~22%), while filter feeders had higher proportions above and within the pycnocline (~30%). Passive ambush feeders had their highest abundances in smooth water (~90 ind. m^{-3}). Cruisers and active ambush feeders were in highest abundance below or in the pycnocline at ~30 ind. m^{-3} for cruisers and ~40 ind. m^{-3} for active ambush. Filter feeders had similar abundances throughout sharply stratified waters (<30 ind. m^{-3}) but lowest in smooth waters (>30 ind. m^{-3}). Motility percentages were approximately evenly distributed amongst passive, slow, and sufficient swimmers, with quick zooplankton having significantly lower percentages. Passive swimmers made up 50% of the community in smoothly stratified samples while sufficient swimmers made up ~40% of the below pycnocline community. The general trend observed was that passive and slow zooplankton were common above the pycnocline or in smooth waters, while sufficient swimmers were more likely to be located below the pycnocline in sharply stratified waters. Motility trait abundances were variable with peaks of passive swimmers in smooth stations (~75 ind. m^{-3}) and high abundances of sufficient swimmers below the pycnocline (50 ind. m^{-3}). The quickest swimmers had generally low abundances at ~20 ind. m^{-3} in sharply stratified waters and 10 ind. m^{-3} in smoothly stratified waters. Size proportions had very little variability between oceanographic zones, but every size class tended to be most abundant below the pycnocline.

Modeling the influence of environmental factors on traits

The GLMs fit to the fine-scale abundances of traits demonstrated that chlorophyll-a and temperature influenced most traits and typically had more significant correlations (Table 4). Chlorophyll-a always has a positive correlation with traits, while both positive and negative correlations with temperature were observed. Oxygen was negatively correlated with some traits while pressure (location in the water column) was positively correlated with some traits. Three of the trait values (passive ambush, low carbon content, and passive swimming) were patchy compared to other traits (dispersion coefficient > 40).

Table 4: Coefficients and associated significance for each trait value with each environmental factor in the GLM. Model: Trait value~ temp + chla + oxy + pressure. Family: quasi-Poisson using a log link function. Dispersion coefficient provides a relative measurement of patchiness, with values >40 in Bold. Significance levels: '.', p ~ 0.05, '*', P < 0.05 level, '**', p< 0.01 level, '***', p<0.001.

Trait	***Chlorophyll-a***	***Temperature***	***Oxygen***	***Pressure***	***Dispersion coefficient***
active ambush	0.0015***	-0.0668***	-0.0441	-0.002	12.3973
passive ambush	0.0012**	0.02868	-0.0769	0.0288***	**51.8169**
filter feeders	0.0027***	-0.0107	-0.1295	-0.0142.	30.0255
cruise/search	0.0003	-0.1384***	-0.1099	-0.0020	32.4408
size 1	0.0010**	-0.0236	-0.0517	0.0120*	24.3879
size 2	0.0013***	-0.0146	0.0069	0.0167***	15.5397
size 3	0.0021***	-0.0269	-0.1549*	0.0029	27.1546
low carbon	0.0021***	0.0415*	-0.1348*	0.0195***	**45.8919**
intermediate carbon	0.0000	-0.1424***	-0.0507	-0.0097	29.4288
high carbon	0.0011.	-0.1348***	0.1506	-0.0008	28.5759
passive	0.0018***	0.0854***	-0.2800***	0.0339***	**45.3136**
slow	0.0028***	-0.0196	-0.0175	-0.0105	26.4207
sufficient	0.0004	-0.1399***	0.0573	-0.0047	24.5847
quick	0.0009	-0.0812.	0.0681	0.0075	34.3383

Both low carbon content and passive swimmer trait groups were significantly influenced by all four selected environmental parameters in the model, suggesting that these zooplankton taxa are the most sensitive to environmental variability. Passive ambush feeders were positively correlated with chlorophyll-a and pressure. Cruise/search feeders, intermediate carbon, high carbon, and sufficient swimmers all had a highly significant negative correlation with temperature and no other influences. Active ambush feeders were positively correlated with chlorophyll-a and negatively correlated with temperature. Filter feeders and slow swimmers were positively correlated with chlorophyll-a and had no other significant influences. Quick zooplankton showed almost no correlation with environmental parameters except for a small amount of significance attributed to a negative correlation with temperature. All size classes had a positive relationship with chlorophyll-a, while the first two size classes were deeper, and the third size class was negatively correlated with oxygen.

CHAPTER 4

DISCUSSION

Oceanographic factors favoring the abundance, composition, and diversity of different zooplankton groups classified by their functional traits were investigated in the mid-shelf region of the southern portion of the SAB during the summer. The diversity calculation showed that both trait and taxonomic diversity were lower in smooth oceanographic zones. The resampling analysis demonstrated a changing trait community based on water type, and GLMs suggested that chlorophyll-a and temperature were the primary oceanographic influences on zooplankton distributions in the SAB during the sampling period. The relatively long residence times and stratified summer conditions of the SAB (Savidge and Savidge, 2014), provided ideal conditions to answer questions about how water column structure influences ecological properties of the mesozooplankton community. The results of the two most consequential traits, carbon content and feeding strategy, are summarized in a conceptual diagram (Figure 12). In addition, the data showed that the overwhelming majority of every water type was dominated by low carbon organisms that would be destroyed in net samples that are traditionally used for zooplankton studies (Remsen et al., 2004). The observations discussed here likely have consequences for trophic transfer, productivity, and overall ecosystem functioning in shelf seas (Hébert et al., 2017).

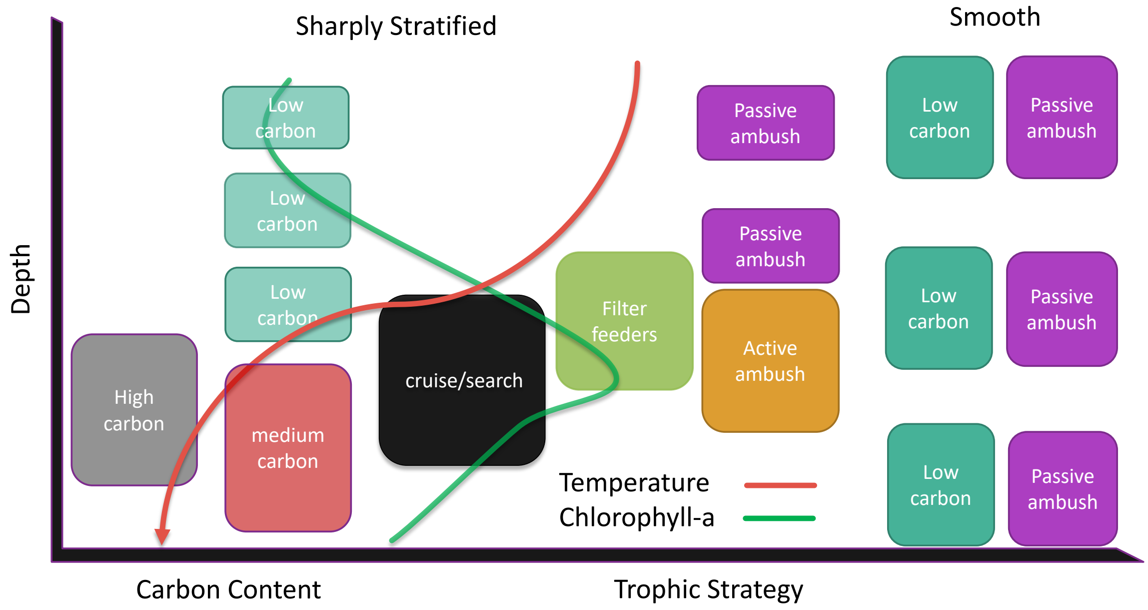

Figure 12: Conceptual diagram of observations for carbon content and trophic strategy in sharply and smoothly stratified stations, boxes display areas of relative abundance in the water column. Lines display vertical profiles of temperature and chlorophyll-a, designated by color.

The value of fine-scale analysis for trait-based approaches

Trait-based studies conducted in other regions have demonstrated that trait groups can change depending on the oceanographic environment. However, these studies have not typically measured the water column on a fine spatial scale, or been capable of detecting population responses of gelatinous taxa that are difficult to sample. Ge et al. (2022) utilized trait-based methods to analyze diel vertical migrations (DVM) in the North Pacific Gyre using 50 m bin sizes and found distinct DVM patterns for different functional groups. Venello et al. (2021) applied the use of traits to a ~35-year time series to determine the impact of environmental drivers on zooplankton abundances off the coast of Vancouver, Canada and found that the biomass of different functional groups varies with environmental variable fluctuations. Though these studies addressed scientific questions using innovative methods, neither study was able to look at fine-scale vertical distributions, causing them to potentially miss valuable insight into how fine-scale vertical structure influences the zooplankton communities. These studies also likely under sampled gelatinous taxa because of the use of conventional net sampling. Ge et al. (2022) sampled a much deeper region and used 50-m bins, but they may have missed responses of the community to changing water column structure. Because time series often deploy consistent and conventional sampling techniques, Venello et al. (2021) did not include a vertical component and simply looked at total abundances. Both studies were likely not able to detect how oceanographic changes influence zooplankton traits on fine spatial scales. This study is the first to use a towed imaging system in the SAB that can resolve fine-scale water column structure to answer questions about how it influences zooplankton traits.

Abundance and diversity of traits in the SAB

Taxonomic abundances and diversity were variable among stations, which allowed for sampling of a wide range of communities. Chaetognaths and hydromedusae were consistently dominant taxa, as both are common in the SAB year-round (Baier and Purcell, 1997; Lopez-Figueroa, 2017). Several productive stations (based on the abundance of total ROIs, Figure 3) had relatively high abundances of other taxa (e.g., pteropods, shrimp, appendicularians). The majority of stations where zooplankton were most abundant were at the 45 m isobath, with only a couple abundant stations at the 25 m isobath. Taxonomic diversity was lower in smoothly stratified stations and trait evenness was lower in smoothly stratified waters compared to oceanographic zones from sharply stratified stations. Because diversity appears to be influenced by water column structure, seasonal changes in structure could influence zooplankton community diversity (Yoder et al., 1983; Castelao, 2011). It is possible that trait diversity may decrease in the SAB during mixed or smoothly stratified conditions, with higher diversity under sharply stratified conditions.

This study focused on mesozooplankton, whereas many previous studies conducted in the SAB quantified a wider size range than addressed here. Paffenhöfer et al. (1984) looked at how different taxa respond to bottom water intrusions and showed that some zooplankton gather in nutrient rich bottom layers and in the pycnocline caused by intrusions rather than in the upper mixed layer. They also found that larger mature animals favored near-bottom water during intrusion events compared to larva or nauplii, which were more abundant in near surface water. Net tows were conducted in the upper mixed layer, within the pycnocline, and below. Abundances were in the 1000-10000 ind. m^{-3} range for copepod species and 100 – 1000 ind. m^{-3} range for most other taxa compared to a max total abundance of ~250 ind. m^{-3} and average around ~100 ind.$^{-3}$ recorded in this study. This is likely due to the size range for this study being restricted to animals >2.2 mm ESD whereas Paffenhöfer et al. (1984) a different size class (<150 µm). addressed a much greater size range of zooplankton. The raw large particle abundances (Figure 2) were much closer to what was observed by Paffenhöfer et al. (1984), with a range of 2000 – 8000 ind. m^{-3}. However, Paffenhöfer et al. (1984) primarily examined various copepod species, nauplii, and doliolids on the SAB shelf and was not able to account for a wide variety of taxa or larger macroplankton, especially gelatinous animals, which were larger than the size range addressed and were likely destroyed in the conventional nets used for sampling.

Water column structuring zooplankton communities

There were observable differences in the percentage of the community occupied by trait values in different oceanographic zones. Low carbon zooplankton and passive swimmers dominated every station, especially ones that were categorized as smoothly stratified. Sharp stratification creates more niche space, which may be favorable to zooplankton that are able to sense or search for prey where they are most abundant. Passive and slow swimmers, along with filter feeders, tend to aggregate above or in the pycnocline in sharply stratified stations, which could be due to vertical pushing of phytoplankton to the upper portion of the water column caused by bottom water intrusion (Yoder and Ishimaru, 1989). These animals may also get caught in the upper mixed layer during intrusion events (Graham et al., 2001; Treible et al., 2022). Intermediate carbon, sufficient swimmers, active ambush feeders, and cruise/search feeders, occupy larger portions of the community in water that is below or within the pycnocline, which is likely due to high nutrient subsurface intrusions on the shelf that stimulation primary productivity and an abundance of prey (Paffenhöfer et al., 1984).

Carbon content likely holds significance for estimating the value of zooplankton as prey. High and intermediate carbon plankton are more nutrient dense making them more valuable prey than low carbon zooplankton for most predators (Kiørboe, 2013; Heneghan et al., 2020), which could explain why these animals are found in higher abundances at depth. Intermediate and high carbon organisms may favor waters below or in the pycnocline to avoid prey detection due to less light. Stratification can delay settling of marine snow aggregates (Prairie et al., 2015), which could also provide shelter and potential prey items. Sufficient swimmers, active ambush feeders, and cruise/searchers may favor bottom water habitat because of a greater availability of prey (Paffenhöfer et al., 1984). Cruisers/searchers and quick swimmers proved to have opposite responses compared to initial predictions by taking up the least percentage of the community in every oceanographic zone, and the least of all in smooth water (Figure 10). Oceanographic zones did not demonstrate significant differences in the size classes. Though size class is considered a master trait that transcends taxonomic distinctions and has far reaching ecological relevance, especially to trophic level (Andersen et al., 2016; Kiørboe et al., 2018; Martini et al., 2021), it is possible that stratification does not affect the size trait at this scale. Size class abundance did show that abundances across all size classes was highest below the pycnocline in sharply stratified condition. Splitting the data into more size classes could be used to capture the potential effect of this trait.

Diel Vertical Migration (DVM) plays a role in the aggregation of zooplankton in bottom water during the daytime (Ashjian et al., 1998; Ge et al., 2022), but the role cannot be assessed here, as almost all sampling was done at during the day. DVM is the daily migration some zooplankton taxa make during the night to day transition from near surface waters to deep waters in order to avoid being easily detected by prey. High carbon animals or sufficient swimmers are more likely to perform DVMs because they are more commonly preyed upon and are capable of swimming between oceanographic zones, unlike low carbon or passive swimming animals, which tend to be physiologically limited and osmoconformers (Graham et al., 2001). Motile animals may also migrate to areas of high nutrients near the bottom or in the pycnocline, while passive swimmers may experience vertical advection by bottom intrusions pushing them into the upper mixed layer (Graham et al., 2001; Treible et al., 2022).

Environmental factors influence on traits

The distribution of zooplankton vertically in the water column and spatially is at least in part affected by the physical parameters of the environment. Many of the observations made from examining at oceanographic zone abundances are informed and contextualized by the correlations observed between traits and the environmental factors. All four traits analyzed were correlated with oceanographic variables measured for this study. Low carbon, passive swimmers, and passive ambush feeders all had significantly higher dispersion coefficients, meaning these traits tended to be patchier than most. Low carbon zooplankton was positively correlated with temperature while intermediate and high carbon zooplankton were negatively correlated with temperature. Low carbon zooplankton and passive swimmers tend to aggregate at density gradients compared to active, highly motile zooplankton that can more easily migrate with changing oceanographic conditions (Graham et al., 2001), which explains why they have a positive correlation with temperature and are very patchy. Within trophic strategy, passive ambush and filter feeders displayed a high correlation with chlorophyll-a, as predicted. Passive ambush and filter feeders may benefit from aggregating with phytoplankton prey sources or areas with a high abundance of grazers because they have limited sensory capabilities and must physically intercept prey. Active ambush and cruise/search feeders also had a significant negative correlation with temperature and were aggregated within the pycnocline. High nutrients within bottom water intrusions, which increase primary productivity and the concentration of prey in the pycnocline, could play a role in these correlations (Paffenhöfer et al., 1984; Prairie et al., 2015). Correlation factors with size classes were only slightly different and was likely affected by the dominance of low carbon zooplankton (particularly hydromedusae) across the sampling area. With respect to motility or swimming speed trait, passive swimmers correlated significantly with all four environmental factors, with the only negative correlation with oxygen concentration. Slow swimmers positively correlated with chlorophyll-a, while sufficient swimmers had a negative correlation with temperature. Slow swimmers likely hunt non-motile prey and may aggregate with chlorophyll for feeding, while sufficient swimmers are better adapted to hunt motile prey but may aggregate in areas of lower temperature because of an abundance of small prey (Kiørboe, 2011).

Implications for ecosystem functioning

These findings can help to predict which communities may emerge in different conditions and the zooplankton community response to stressors. It is well understood that trait-based analysis can lead to results that have implications for ecosystem functioning, such as how traits can predict DVM behavior or describe relationships between biogeochemistry and the zooplankton community (Hébert et al., 2017; Ge et al., 2022. The correlation of certain traits with environmental factors, namely temperature and chlorophyll-a, along with the change in communities in smooth or sharply stratified waters demonstrates that changes in these environmental factors can have impact on the amount of carbon being transferred to higher trophic levels (Hébert et al., 2016; Venello et al., 2021). Sharply stratified conditions are much more common in the summer months due to bottom intrusion and heat flux, while mixed or smoothly stratified conditions are more common in the winter, meaning potentially less diversity of traits and more low carbon and passive swimmer dominance of the ecosystem. In extensively mixed times of year, we might expect to see increased carbon flux to the sea floor and less to higher trophic levels as the high carbon, nutrient rich meso- and macrozooplankton that fulfill this intermediate trophic level have less favorable habitat (Chi et al., 2021; Tinta et al., 2021). Wind mixing may favor of gelatinous dominated communities while deep-water intrusions appear to favor high carbon animals that vertically migrate and actively transport carbon (Steinberg and Landry, 2017; Lebrato et al., 2019).

Future directions

The extent of environmental influence on the zooplankton community still leaves important knowledge gaps that could be further addressed in future studies. While some physiological, morphological, and behavioral traits have been addressed here, including other variables and traits could lead to a more holistic understanding of the key ecological functions at play. It would also be valuable to address time-scales of these trait responses to changing oceanographic conditions. The use of a shadowgraph imaging system has proved to be invaluable for gathering fine-scale data needed for this kind of study, particularly for fragile and understudied gelatinous zooplankton. Studies looking at traits of zooplankton in the SAB should compare results across seasons to see how the influence of environmental factors on zooplankton might change. The use of other traits (e.g., reproductive method, diet) and the addition of more sensors to the mDPI for the measurement of other environmental factors (e.g., PAR, CDOM, POM) should be considered as well (Kiørboe et al., 2018; Ge et al., 2022). Additionally, comparing results across regions and ecosystems could allow us to determine the consistently influential environmental variables influencing trait composition (Hemingson and Bellwood, 2018;

Greer et al., 2022). Future trait-based studies may also incorporate the analysis of functional groups, which is a method of grouping taxa by environmental role that share the same trait categories. In functional groups analysis, abundance of functional groups is examined rather than abundances of each trait category. The trait-based approach taken in this study determines the relationship between traits and environmental factors, while functional groups help deduce the environmental role of certain taxa (Litchman et al., 2013). A temporal study could also be performed in which a changing water column at a given location is tracked through time to understand the evolution of the zooplankton community through time.

Conclusions

Analyzing traits allows us to address different scientific questions that can lead to a more functional understanding of zooplankton communities compared to when looking at taxonomic groups alone. Trait-based approaches can offer key insight into ecosystem functioning of mesozooplankton communities, and how they are influenced by water column structure. Water column stratification and mixing, as well as other environmental factors, affect the composition and distribution of zooplankton traits and structure the water column into distinct environments, which may allow us to forecast changes in the zooplankton community. These findings and potential quantifying zooplankton ecological function has implications for carbon flux and trophic transfer that includes valuable fisheries. Intensified water column structure associated with deep-water intrusion may favor zooplankton that are more valuable as prey to higher trophic levels, potentially leading to increased trophic transfer, while wind mixing may favor the development of a gelatinous dominated community. Future studies pertaining to zooplankton ecology will benefit greatly from utilizing trait-based analysis methods paired with the fine-scale capabilities of a towed imaging system, such as the mDPI, and should implement the analysis of cross-seasonal effects, additional traits and environmental factors, as well as temporal analyses of water column structure to better capture the time-scales of zooplankton community responses.

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