ABUNDANCE AND DIVERSITY OF BENTHIC MEGAFAUNA AT ABYSSAL STATION ALOHA

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

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This thesis is dedicated to my friends and my family, especially to those who have been present with me during the difficult times since the COVID-19 pandemic. Each of you have shaped me into the person I am today. I will continue to reflect on the experiences that we have shared together as I address challenges in the future. Aloha.

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Mahalo.

ABSTRACT

Abyssal seafloor ecosystems, such as at Station Aloha, are poorly understood, although particulate organic carbon (POC) flux is thought to be an important driver of benthic faunal abundance and biodiversity. To better understand these ecosystems, this study aims to address how abyssal benthic megafaunal communities vary with POC flux. Abundance and diversity of megafauna were evaluated at Station Aloha and compared to other stations in the abyssal Pacific with different POC fluxes, to address these variations. Megafaunal abundance and diversity were evaluated at Station Aloha using photographic data collected by the ROV *Lu'ukai*, which consists of high-resolution photos along a transect across the Seafloor at ~4700 m depth. Megafauna in images were identified to the lowest possible taxon assigned to trophic groups and counted using scaled images. Patterns were then compared to other benthic locations at similar depths in the Clarion-Clipperton Zone (CCZ) and off the eutrophic California coast, where megafaunal abundances and seafloor POC flux have also been evaluated. Megafaunal abundance at Station Aloha was low compared to other abyssal benthic sites located under more eutrophic surface waters. Despite low abundance, a diversity of feeding types including suspension feeders, surface deposit feeders, predators, and scavengers occurred at Station Aloha. Ecological patterns at Station Aloha were found to be similar, in terms of metazoan megafauna, to ecosystems with low POC flux in the nearby CCZ. These findings suggest that low food supply at Station Aloha limits megafaunal abundance yet supports a diversity of approaches to utilizing the food reaching the seafloor.

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1.0 INTRODUCTION

The deep sea is the largest biome on the planet due to its incredible volume and sea floor area. The deep sea spans from 200 m below the ocean surface to the sea floor, which in some areas can lie 11 kms below surface waters. Yet, while this biome covers most of the surface of the planet, the structure of ecosystems here remains predominantly unknown. This lack of knowledge becomes more understandable as one realizes the extremity of the living conditions, relative to humans. The deep ocean is a region of the world where humans cannot easily visit due to extreme water pressures, low temperatures, and the absence of light. Even as human technologies have become more advanced and efforts to understand oceans have increased, just a small fraction of the seafloor has been mapped (Mayer, et al. 2018). Many more people have traveled to outer space than have been to the ocean's greatest depths. A better understanding of deep-ocean ecosystems is important because these massive regions are major reservoirs of biodiversity and have the potential to significantly affect the other important biomes on the planet (Lejzerowisc et al. 2021).

1.1 THE DEEP PACIFIC

The Pacific is the largest ocean on earth by a factor of about two (Smith, Demopoulos 2003). This massive body of water can, therefore, support a variety of ecosystems, many of which are poorly understood by humans. Abyssal ecosystems at depths of 4000 – 6000 m can be very different from one another because conditions change significantly with depth, latitude, overlying primary productivity, and bottom topography (Smith 2020). These conditions, along with others, dictate the productivity and biodiversity of Pacific seafloor ecosystems.

Most of the primary production that occurs in the ocean can be attributed to photosynthesis in surface waters, where sunlight is readily available. For photosynthesis to occur, nutrient fluxes are also required, with nutrients generally coming from waters below the photic zone. Nutrient fluxes can often be related to ocean currents and upwelling. Ocean currents in the Pacific are controlled, in part, by two massive oceanic gyres, one in the North Pacific, and the other in the South Pacific. Surface waters within these gyres are known to be generally oligotrophic because they are downwelling and have relatively low fluxes of nutrients from deep waters (Browning, et al. 2017). As a result, the seafloor beneath these gyres tends to be food-poor, biomass deserts because most deep benthic ecosystems rely on particulate organic carbon (POC) that sinks from the surface waters (Smith, Demopoulos 2003; Smith, et al. 2008). Benthic regions that exist under areas of substantial nutrient upwelling, such as along the equator or along continental margins in the eastern Pacific, have greater fluxes of sinking POC, and this typically results in more productive benthic ecosystems (Smith, et al. 2008).

While POC flux can significantly impact the productivity and abundance of organisms in a deep-sea ecosystem, substratum type is also known to influence the variety of organisms that can be found at a given location. The abyssal region, 4-6 km in depth, is relatively flat with low-relief ridges and troughs and occasional seamounts. The abyssal plain is known to have both hard and soft substrata, each of which are known to provide habitat for different kinds of organisms. Hard substrate is known to exist as polymetallic nodules and rocky outcrops, while soft substrate usually exists in the form of muddy red or brown sediments (Smith, Demopoulos 2003). Polymetallic nodules support sessile suspension feeders that can easily anchor themselves to a nodule's hard substrate (Amon, et al. 2016). Soft substrates often contain a greater abundance of mobile megafauna that have the ability to move about the sediments to find food (Smith, Demopoulos 2013).

It is important for humans to gain a better understanding of deep Pacific ecosystems because the roles that the different ecosystems play in both global biodiversity and largescale biogeochemical processes are poorly understood but could be significant. For instance, an overwhelming majority (80%) of species that have been studied on the deep-sea floor have been found to be new to science (Smith, et al. 2008). When these new findings are coupled with the expansive amount of space that the seafloor covers it can be speculated that the deep ocean is a significant reservoir of global biodiversity (Smith, et al. 2008). These deep-water ecosystems can also affect nutrient fluxes and conditions that are integral to human life through a variety of ways, including supporting global fish stocks (Devine, et al. 2012). Lack of understanding also applies to the effects of the deep ocean on the global climate. This region of the planet is known to be one of the most significant carbon sinks on the earth (Jiao, et al. 2011), yet specific carbon flows remain relatively uncharacterized.

1.2 GOALS AND HYPOTHESIS

The goal of this thesis is to evaluate megafaunal abundance and taxonomic richness at oligotrophic Station Aloha and compare them to other abyssal Pacific sites with differing POC fluxes. Specifically, this thesis asks: *How do megafaunal abundance and taxonomic richness compare between abyssal locations with different food availability?* I hypothesize that megafaunal abundance will have a positive relationship with primary production in surface waters and particulate organic carbon (POC) flux to the deep-sea floor, with Station

Aloha having particularly low abundance. Furthermore, this research will provide a baseline for further studies of abyssal benthic communities in oligotrophic and eutrophic regions of the Pacific. Finally, comparing ecosystem parameters between Station Aloha and other sites in the North Pacific Ocean can help characterize global patterns of abyssal ecosystems. These comparisons may help in understanding baseline conditions in the CCZ where deep-sea mining is expected to occur in the near future.

2.0 SITE DESCRIPTIONS

POC flux, megafauna abundance, and megafauna diversity are compared between seven sites in the pacific, including Station Aloha, Station M and six other locations in the CCZ (Fig 1). These comparisons are expected to show how geographic differences in oceanic variables can affect the communities that live on the deep seafloor. Below is a brief description of these sites.

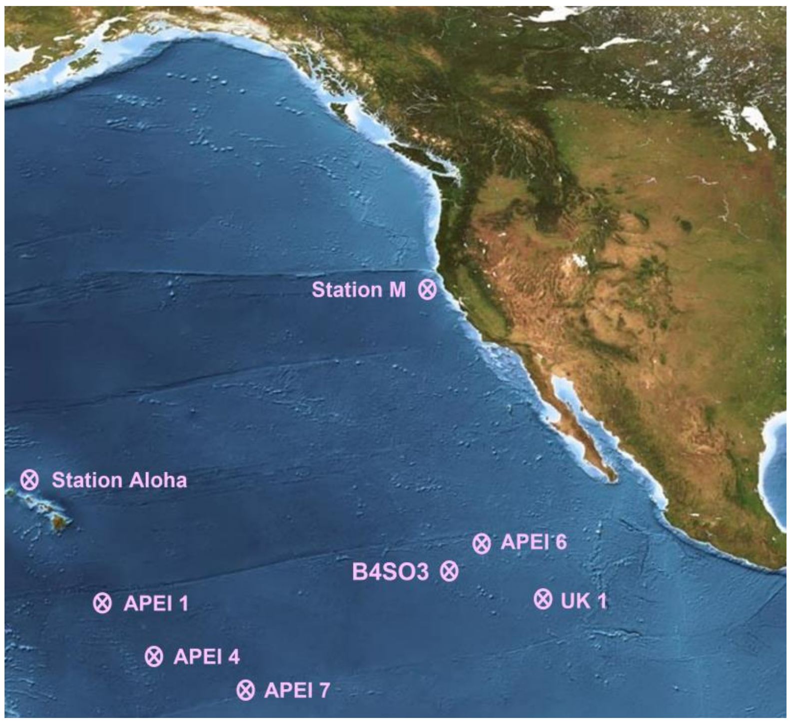

Figure 1. Map of the North Pacific depicting the location of sites that was compared in this study, and their relations to Hawaii and North America.

2.1 OLIGOTROPHIC STATION ALOHA

Station Aloha is approximately 120 km north of Oahu, at 22°45' N, 158° W as seen in Figure 1, putting it in the middle of the oligotrophic North Pacific gyre (Chiswell 1996). The seafloor at this site lies approximately 4,720 m below the surface ocean, placing it within the depth range of the abyssal plain. This station sits in a basin that essentially runs parallel to the Hawaiian Ridge feature (Smith, Sandwell 1997). According to one study conducted over several weeks, deep waters at Station Aloha consistently flowed westward through the basin, though flow direction deviated by tens of degrees (Alford, et al. 2014). Therefore, while current direction and intensity can vary here due to abyssal forcing (Alford, et al. 2014), the bottom-water flows of Station Aloha may follow trends that are directionally similar to the larger scale water flow of the North Pacific gyre. This gyre also makes the site is oligotrophic, and POC flux to the seafloor, as measured by sediment traps, is low (Karl, et al. 2012).

Figure 2. The location of Station Aloha (marked by the red circle) in relation to the Hawaiian Islands, while illustrating the cable route (marked by yellow line) of the cabled observatory (Karl, Church 2019).

Station Aloha is an excellent location to use for ecosystem comparisons because it has been the site of the Hawaiian Ocean Time-series (HOT) since 1989. The HOT program has accumulated many forms of data from a variety of depths, and some of these data span large temporal scales. One significant finding of the HOT is that primary productivity in the surface waters fluctuates with El Nino - Southern Oscillation (ENSO; Karl, et al. 1996).

These changes in surface ocean conditions can also have an effect on deep-sea ecosystems. It was found that while primary production increased during an ENSO event, POC flux to the abyssal Station Aloha simultaneously decreased (Karl, et al. 1996), which can lead to changes in abyssal food webs. The HOT program continues to the present day and can be used to better understand Station Aloha in a variety of ways even at abyssal benthic depths. This continuous long-term study measured POC flux to the deep sea from sediment-trap deployments (Karl, et al. 2012). This provides a valuable opportunity to characterize relationships between vertical POC flux and abyssal ecosystems.

The POC flux at Station Aloha is the main source of available food to the abyssal zones in this region of the ocean (Smith, et al. 2008). The annual seafloor POC flux can also be estimated using a flux model within Lutz, et al. (2007). POC flux estimations require several variables such as net primary production (NPP) rates, NPP variability, and sea surface temperature (SST; Lutz et al. 2007). Based on these values and using the Lutz, et al. (2007) model, deep seafloor POC flux at Station Aloha was estimated to be 1.35 $gC/m^2/yr$. Meanwhile, the POC flux at Station Aloha was measured to be 0.60 $gC/m^2/yr$ when using sediment traps from 1992 – 2004 in the region (Karl, et al. 2012). Both values tend to be lower than many other areas of the deep sea due to the oligotrophic nature of the subtropical gyre. It has been found that POC flux to the deep seafloor between 1200 and 5000 m depth can vary from 0.36 $gC/m^2/yr$ to 6 $gC/m^2/yr$ with the lowest values existing within the oligotrophic gyres (Smith, Rabouille 2002). Therefore, due to this relatively low POC flux, the abundance of megafauna at the seafloor of Station Aloha is expected to be relatively low (Smith, et al. 2008).

While the HOT program has provided a thorough understanding of different environmental parameters at Station Aloha, many biological aspects of the abyssal ecosystems remain poorly characterized here. However, there has been some research previously done on the epibenthic megafauna of this region. One study at Station Aloha found that the dominant epibenthic metazoan megafauna in the region were made up of burrowing urchins, holothurians (sea cucumbers), and sponges (Smith, et al. 1997). The existing megafaunal data do not, however, characterize the abundance of individual taxa or taxonomic richness (Smith, et al. 1997). Thus, there is a gap in understanding in the benthic ecosystem of Station Aloha, which provides relevance for the research performed in this thesis.

2.2 EUTROPHIC STATION M

The data collected and analyzed at the oligotrophic Station Aloha will be compared to the more eutrophic Station M. Stations M and Aloha are similar in several ways, for instance, the seafloor at Station M is about 4100 m below the ocean surface (Ruhl, Smith 2004), which similarly to Station Aloha, exists within the range of abyssal plain. While there are similarities, these two stations also have significant contrasts in environmental conditions. Station M is located off the continental rise of the California coast at 34°50’ N, 123° W (Ruhl, Smith 2004) and is more eutrophic due to the upwelling that occurs off the California coast (Ruhl 2007).

Station M, like Station Aloha, has also been studied over long time scales to assess seasonal and interannual variability in ocean conditions, making it an excellent location for comparison. Station M has been continuously studied since 1989 to obtain a better understanding of benthic processes and ecology (Ruhl, Smith 2004). It has been found that the food supply at Station M is also affected by seasonal variations in overlying surface waters (Ruhl, et al. 2008). From 1989 to 2002, Station M recorded a range of POC fluxes from about 0.37 - 6.2 $gC/m^2/yr$ (Ruhl, Smith 2004). Notably, a correlation has been found between these fluctuations in POC flux and changes in the benthic megafaunal community. These correlations were especially perceptible during the strong El Nino/La Nina event that took place from 1997-1999 (Ruhl, Smith 2004).

One study of epibenthic megafauna at Station M found densities ranging from about 2.7 to 4.0 individuals/m^2 across nine ~1 km long transects (Lauerman, et al. 1996). The polychaete tube worm *Paradiopatra* sp. was found to be the most abundant organism at Station M, making up 26% to 48% of the megafauna, followed closely by echinoderms making up 24-41% (Lauerman, et al. 1996). While *Paradiopatra* sp. may dominate the transects in terms of abundance, echinoderms dominated this region in terms of biomass (Ruhl 2008). The dominance of echinoderms here follows the trend that mobile creatures tend to dominate regions of soft substrate, since the seafloor at this northeast Pacific abyssal site is primarily composed of silty-clay (Smith, et al. 1994). It should also be noted that xenophyophores were found to be the second most dominant organisms at five of the nine transects at Station M.

2.3 CLARION-CLIPPERTON FRACTURE ZONE (CCZ)

The Clarion-Clipperton Fracture Zone, known as the CCZ, is an expansive region of the abyssal Pacific that lies between Hawaii and Mexico, spanning several thousands of kilometers longitudinally. The seafloor here typically lies between 4-6 km depths, similar to the depths of Station M and Station Aloha. The CCZ is bounded to the north and south by the Clarion and Clipperton transform faults on the seafloor, respectively. Environmental conditions on the abyssal seafloor vary over the massive area of the CCZ. Some examples include environmental variation in bathymetry, sedimentation rates, POC flux, and manganese nodule abundance. Gradients in POC flux can be observed across the CCZ, with POC flux increasing from west to east and north to south (Washburn, et al. 2021).

One of the most significant differences between the CCZ and Stations M and Aloha is that manganese nodules are abundant in some areas of the CCZ. Environmental conditions at the abyssal seafloor in the CCZ can lead to the formation of polymetallic nodules, also called manganese nodules, which are an important characteristic of the region. These nodules are primarily made up of manganese, iron, copper, nickel, and cobalt, along with several rare earth metals (Miller, et al. 2018). Some of these metals are important in the production of electronics like smartphones, computers, and electric vehicle batteries, and as these devices are produced more widely across the globe, some look to nodules as a potential source of minerals for future device production. Interests in seafloor mining for these nodules has led to significant scientific studies across this region to characterize environmental baseline conditions and how deep-sea ecosystems could be affected by mining practices (Drazen, et al. 2020; Smith, et al. 2020).

These ecosystem studies have increased the understanding of the benthic megafaunal communities at several locations in the CCZ (Amon, et al. 2016; Durden, et al. 2021; De Smet, et al. 2021; Simon-Lledó, et al 2019). However, megafaunal studies of the CCZ have been conducted over much larger spatial scales, but over much shorter temporal scales, than megafaunal studies at Station M. Therefore, conclusions made about communities of the CCZ address spatial, but not temporal patterns (Durden, et al. 2021).

The range of environmental conditions in the CCZ inevitably leads to variations in ecosystem structure. Changes in ecosystems tend to correlate with the natural gradients that occur across a region. One example of this can be seen as nodule densities vary from high to low. Areas of the Eastern CCZ with higher nodule density tend to have larger populations of suspension feeders, since 80% of suspension feeders were found to use nodules as anchor points of hard substrate (Simon-Lledó, et al 2019). Furthermore, the diversity of substrata (both hard and soft) in nodule fields can lead to a complexity within ecosystems in which both sessile and mobile megafauna can have greater standing stocks (Amon, et al. 2016; Simon-Lledó, et al 2019). Therefore, areas of nodule fields can lead to the existence of different ecosystems due to the habitat complexity induced by nodules.

Several regions within the CCZ have been shown to differ in terms of ecosystem structure. In the western CCZ, benthic megafauna can occur at low abundance, but with high species diversity (Durden, et al. 2021). This lower abundance of megafauna correlates with low food supply in the region, based on the Lutz et al. (2007) model (Durden, et al. 2021). The invertebrate communities of the western CCZ were also shown to have little overlap with communities from the eastern CCZ, or from seamounts in the region (Durden, et al. 2021). The variety of benthic environments that are prevalent within the CCZ along with the low biological overlap between sites could lead to a greater vulnerability to genetic loss if ecosystems here are disturbed.

One Megafaunal taxonomic group found to be common across the CCZ is the xenophyophores. Xenophyophores are unicellular, multi-nucleate foraminifera that belong to the kingdom Rhizaria. These creatures are worth noting because they can be found in significant numbers even when metazoan megafaunal populations are low. For instance, studies have found that in some areas where metazoan megafaunal abundance is less than 0.5 individuals/m^2, xenophyophore abundances range from 2.1 – 3.7 individuals/m^2 (Durden, et al. 2021; Simon-Lledó, et al. 2019). This shows that in several areas in the CCZ xenophyophores dominate the megafauna in terms of abundance.

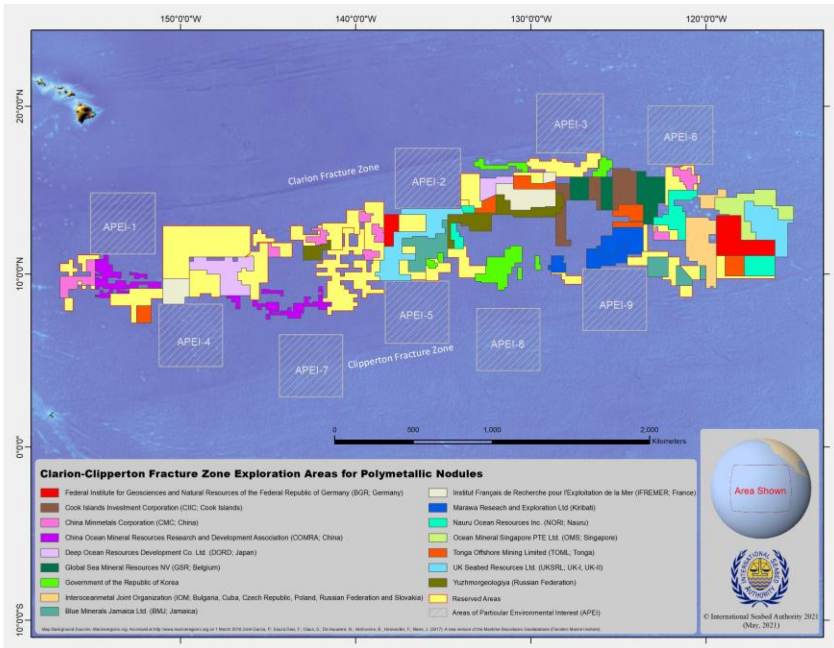

Figure 3. Map of mining exploration contract areas (colored blocks not including yellow), and areas reserved for mining (yellow blocks) within the CCZ. Each color (besides yellow) represents a different exploration contractor as depicted in the legend below the map (ISA, 2021).

It is useful to compare the results at Station Aloha to megafaunal community structure in the CCZ because when large scale nodule mining begins in the region, megafaunal communities could be affected drastically. Serious effects from mining could reach many ecosystems within the CCZ since exploration contract areas, and areas reserved for mining, spread over vast spaces of the CCZ (Fig. 2). Studies have shown that the vehicles designed for nodule collection in these areas are highly destructive to the ecosystems of the deep CCZ because they will destroy, smother, and bury habitats and biota living on and around the polymetallic nodules (Jones, et al. 2017). Furthermore, nodule extraction methods are expected to produce large deep-sea sediment plumes that will have many adverse effects on deep-sea ecosystems (Amon, et al. 2016; Drazen, et al. 2020; Gillard, et al. 2019; Miller, et al. 2018; Smith et al. 2020). Therefore, mining in the CCZ could lead to significant ecosystem destruction, and these ecosystems are known to recover very slowly (Jones, et al. 2017; Smith, et al. 2020). If there is little overlap found between megafaunal communities of the CCZ and of Station Aloha, then biodiversity loss may be expected to be greater from polymetallic nodule mining.

3.0 METHODS

3.1 PHOTO COLLECTION

Photographic data at the seafloor of Station Aloha was acquired to characterize the megafaunal community for this study. To perform this characterization, organisms were identified and counted within the collected photographic images. Photographic images were obtained during *RV Kilo Moana* cruise KM2002 in January 2020 to Station Aloha. This research cruise deployed the University of Hawaiʻi's *ROV Luʻukai* to the seafloor to collect a variety of samples and conduct photo transects across the seafloor. Multiple seafloor transects were scheduled to be taken at Station Aloha, but only one photo transect was successfully completed due to complications with the ROV and the mounted downward facing camera.

The methods used for the photo transect at Station Aloha were similar to those of Durden et al. (2021). The Station Aloha transect was taken on July 19th of 2019, on ROV *Luʻukai*'s 118th dive. The ROV moved in one continuous direction on a randomly selected heading (270° in this instance), within a 45° range constrained by currents. The photo transect was approximately 1150 m long, and the speed of the vessel was ~0.5 knots. A downward-facing Ocean Imaging Systems DSC 24000 digital camera system (Nikon 7100 camera set to aperture F8, shutter speed of 1/60, focal distance 8.5 ft, images 4,000_6,000 pixels) was mounted to the front of *ROV Lu'ukai* and collected images at 10-second intervals. The camera was meant to remain at a constant altitude above the seafloor, with photos including two laser scale marks spaced 35 cm apart. However, it should be noted that ROV operations were not precise, and therefore variation existed in both ROV speed and elevation of the camera above the seafloor. Thus, some of the photos were not usable due to insufficient lighting (Fig. 4) and overlapping images.

Figure 4. Depiction of the variations in light intensity on the seafloor in photos collected by *ROV Lu'ukai* resulting from variation in ROV elevation above the seafloor.

3.2 PHOTO ANNOTATION

The transect produced 575 photos, of which 525 images were used for analysis. To obtain the information needed to characterize the region, multiple measurements were taken for each photo using the VARS annotation software (Barr 2019). The adequately lit area in which megafauna could be counted was measured with the help of the parallel laser scale markers. Visible megafauna within the lit area were identified and counted. Both of these measurements were needed to perform statistical analysis on the megabenthic community of Station Aloha.

Measurements and several calculations were applied to each image to calculate its area. All images used had an equal pixel area of $1.0036*10^7$ pixels^2, but this value needed to be converted to meters to obtain the area of each photo. To do this the x and y coordinates were estimated at each laser point. The Pythagorean theorem was then applied to calculate the distance between laser points in pixels. Once the ratios of pixels to meters was obtained for each image through the known laser spacing these ratios were applied to the x and y dimensions in pixels to obtain the dimensions in meters. Dimensions in meters were then multiplied together to calculate the total area for each image in square meters.

A small percentage of the area of each image could not be annotated due to a shadow that was cast on the sediment from an appendage on the ROV. Further measurements and calculations were necessary to correct for this uncountable shadowed area in each image. Because shadows varied in size across the dataset due to the altitude, pitch, and roll of the ROV, shadows were characterized into three shadow types. Ten images were randomly selected for each shadow type, so that the shadowed area could be estimated in pixels and then converted to area in m^2 using each image's pixel to meter ratio, which was previously obtained to calculate the total area. Once the shadowed area was calculated for all ten images, the ratio of shadowed area to image area was calculated and then converted into a percentage. The ten percentages were then averaged for each shadow type, and the average ratios were applied to all images within their respective shadow type. Shadow Type 1 covered the least space in images, averaging about 1.06% of the total image area; shadow Type 2 covered an average 2.27% of image space; and shadow Type 3 covered an average of 4.6% of the image area. These percentages were subtracted from the total area of each image depending on the image's shadow type.

Images were annotated in a random order, and identifiable megafauna was counted and tabulated within the visible area for each usable image. Organisms were identified to the lowest possible taxonomic level, which ranged from phylum to genus, depending on the organism. Due to photo resolution and small size of organisms, some annotations were marked as *unidentified organism.* Other objects were also counted and tabulated, such as trash and other objects that were difficult to identify.

3.3 PHOTO DATA ANALYSIS

To characterize the megafaunal population on the seafloor of Station Aloha several types of analyses were performed. These analyses included tabulation of relative abundance of taxa, and calculation of overall megafaunal abundances (individuals/m^2) within the transect. Furthermore, some statistical analyses were executed to estimate taxonomic richness and the completeness of taxonomic sampling at Station Aloha.

To perform these analyses, the data collected with the VARS photo annotation software was converted into tables in Excel. These data consisted of the laser measurement in pixels, the id number that specified the order in which each photo was snapped, and the organisms identified in each photo, along with each organism's phylum. In Excel, the data from unusable photos was sorted out, and removed from the dataset. Usable data was then tabulated to illustrate the prevalence of each taxon across the transect.

The total number of organisms identified was divided by the corrected area in meters to obtain the abundance of metazoan megafauna for each photo in individuals/m^2. These abundances were calculated for the entire data set and then averaged to determine the mean metazoan megafaunal abundance for the entire transect. Similar calculations were then repeated to obtain the mean abundance of xenophyophores in the transect.

The Chao 1 analysis was used to estimate taxonomic richness (the total number of morphotypes that occur) at Station Aloha, and to assess how many megafaunal morphotypes remain to be sampled. This calculation estimates the taxonomic richness of a region based on the idea that rare species contribute significantly when assessing community diversity. A separate bootstrap analysis was performed, which, similarly to the Chao 1 analysis, estimates the number of megafaunal morphotypes that might exist in the region. The Bootstrap curve estimated the total number of taxa in the region based on statistical assumptions (Primer 7). These statistical analyses help to illustrate how well the region was sampled.

Finally, to better understand the community structure of Station Aloha it was necessary to compare community structure parameters to other sites in the North Pacific. POC flux, mean megafaunal abundances, and taxonomic richness data were gathered from the northeast Pacific and from the western CCZ (Lauerman, et al. 1996; Durden et al. 2021). Similar data was also collected from the eastern CCZ (Amon, et al. 2016; Simon-Lledó, et al. 2019; De Smet, et al. 2021), however, De Smet et al. (2021) did not incorporate xenophyophores into megafaunal abundances.

3.4 STATISTICAL ANALYSIS

The correlation between POC flux and both metazoan megafaunal and xenophyophore abundance across sites was tested with a product-moment correlation coefficient (*R)* calculated in Excel. The significance of each value was tested by using the table for critical values for correlation coefficients (Carr 2021). A one tailed test was applied to the correlation coefficient of the metazoan megafauna, because this relationship was expected to be positive. However, a two tailed test was applied to the xenophyophores, because their relationship with POC flux had the potential to be either positive or negative.

4.0 RESULTS AND DISCUSSION

Table 1. The total abundances and taxonomic identity of the megafauna observed at Station Aloha, sorted by phyla. Note that groups identify organisms at a variety of taxonomic levels from class to genus, and that the morphotype column is used if there are unidentified organisms within lower taxonomic levels. Likely feeding type for each morphotype is also included in this table.

Phylum	Lower Taxonomic Level (rank)	Morphotypes (rank)	Likely Feeding Type	Number of Individuals
Annelid				
	Polychaeta (class)	Sabellida (order)	Deposit feeder	1
		Unidentified		1
Arthropod				
	Amphipoda (order)	/	Many	3
	Aristeidae (family)	/	Predator	3
	Munnopsidae (family)	/	Predator	1
	Unidentified	/		5
Chordate				
	Ophidiidae (family)	/	Scavenger/omnivore	3
	Ipnopdae (family)	/	Unknown	1
	Unidentified	/		1
Cnidaria				
	Umbellula (genus)	/	Suspension feeder	59
Echinoderm				
	Asteroidea (class)	/	Unknown	9
	Echinoidea (class)	/	Deposit feeder	21
	Holothuria (class)	*Paelopatides* (genus)	Deposit feeder	2
		Penniagone (genus)	Deoosit feeder	3
		Unidentified		5
Foraminifera				
	Xenophyophorea (clade)	/	Deposit/suspension feeder	89
Porifera				
	Chondrocladia (genus)	/	Suspension feeder	4
	Cladorhiza (genus)	/	Suspension feeder	24
	Euplectellidae (family)	/	Suspension feeder	1
	Hyalonema (genus)	/	Suspension feeder	2
Unidentified				
	Unidentified	/		21
Grand Total				260

4.1 MEGAFAUNAL ABUNDANCES AT STATION ALOHA

Within the 525 photos that were annotated, 260 individual organisms were identified, including both xenophyophores and metazoan megafauna (Table 1), along with each morphotypes likely feeding types (Levin 1994; Kapiris, Thessalou-Legaki 2011; Craig R. Smith). Megafauna were identified in less than half of the photos in the dataset. The average megafaunal abundance, using images as replicates for the entire transect, was 0.15 ± 0.012 individuals/m^2 (mean $\pm$ std. error). A variety of the megafauna that were found at Station Aloha can be viewed in Figure 5.

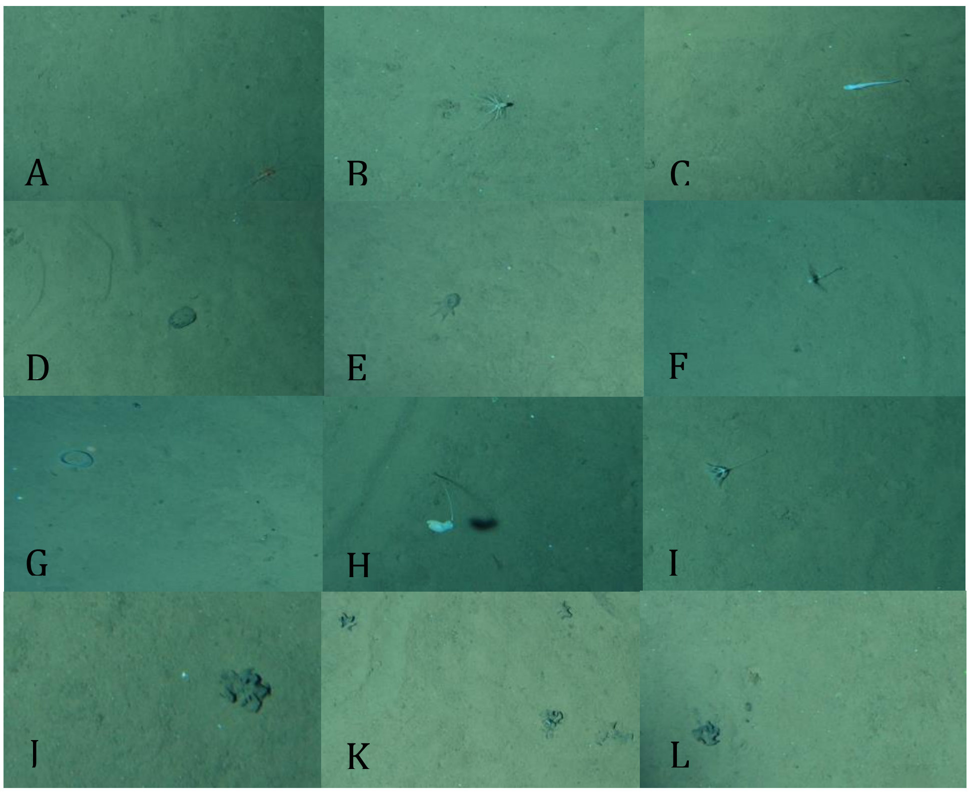

Figure 5. Examples of different metazoan megafauna observed at Station Aloha areas in cropped images from the transect collected at Station Aloha. (A) Bottom right: shrimp in the family Aristeidae. (B) Crab in the family Munnopsidae. (C) Fish in the family Ophidiidae. (D) Sea urchin, Echinoidea. (E) Holothuroid in the genus *Peniagone*. (F) Sponge in the genus *Cladorhiza*. (G) Top left: Sponge in the family Euplectellidae. (H) Sponge in the genus *Hyalonema*. (I) Sea pen in the genus *Umbellulla*. (J) Xenophyophore. (K) Three Xenophyophores. (L) Xenophyophore.

The megafaunal community consisted of a variety of organisms within seven phyla, which ranged from Porifera to Chordata (Figures 5 and 6). It is clear that three phyla dominate the megafauna at Station Aloha. Foraminifera, Cnidaria and Echinoderms alone made up 73% of the total megafaunal abundances, while the four remaining phyla and unidentified organisms only made up 27%.

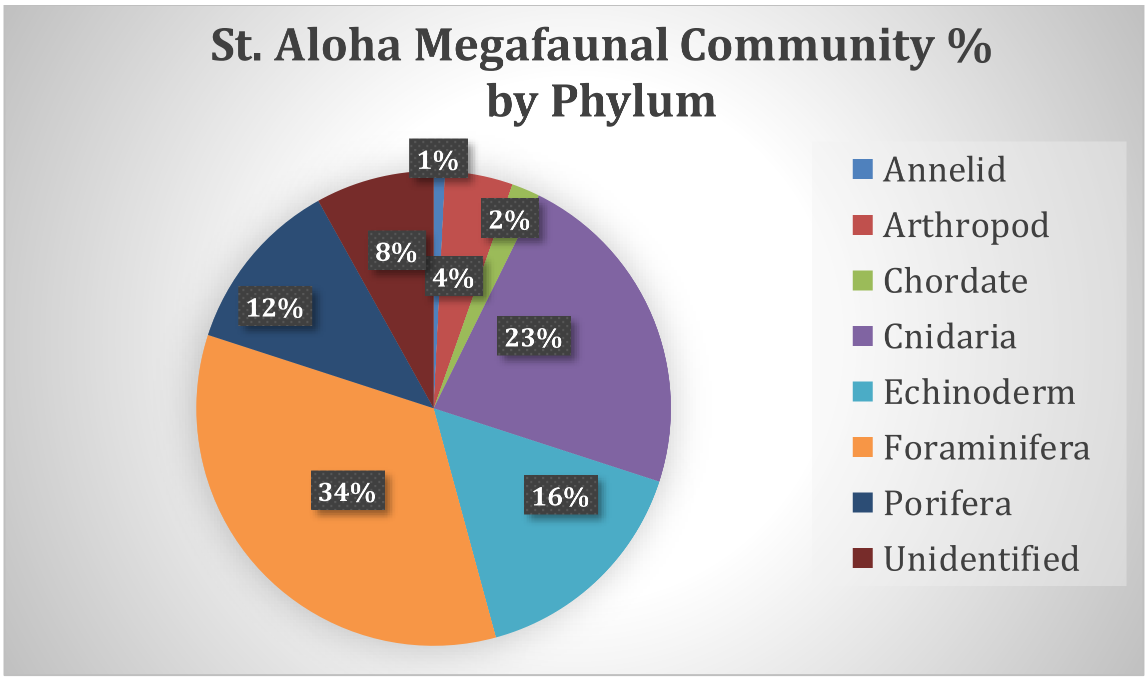

Figure 6. Proportional contribute of megafaunal phyla to the community at Station Aloha.

The dominant phylum was the foraminifera (34%), within which, The Class Xenophyophoroidea (xenophyophores) was the only taxon that was identified. The exclusivity within this phylum is likely due to the large size of the xenophyophores, allowing the class to be easily recognized. It is likely that other species of foraminifers exist at the site, but they are too small to be identified with the method of sampling used in this study. Yet, even with just one identified taxon, the foraminifera appeared to dominate megafauna of this area of seafloor. Their dominance is made clear when looking at the relative abundance of each taxon (Figure 6) at Station Aloha.

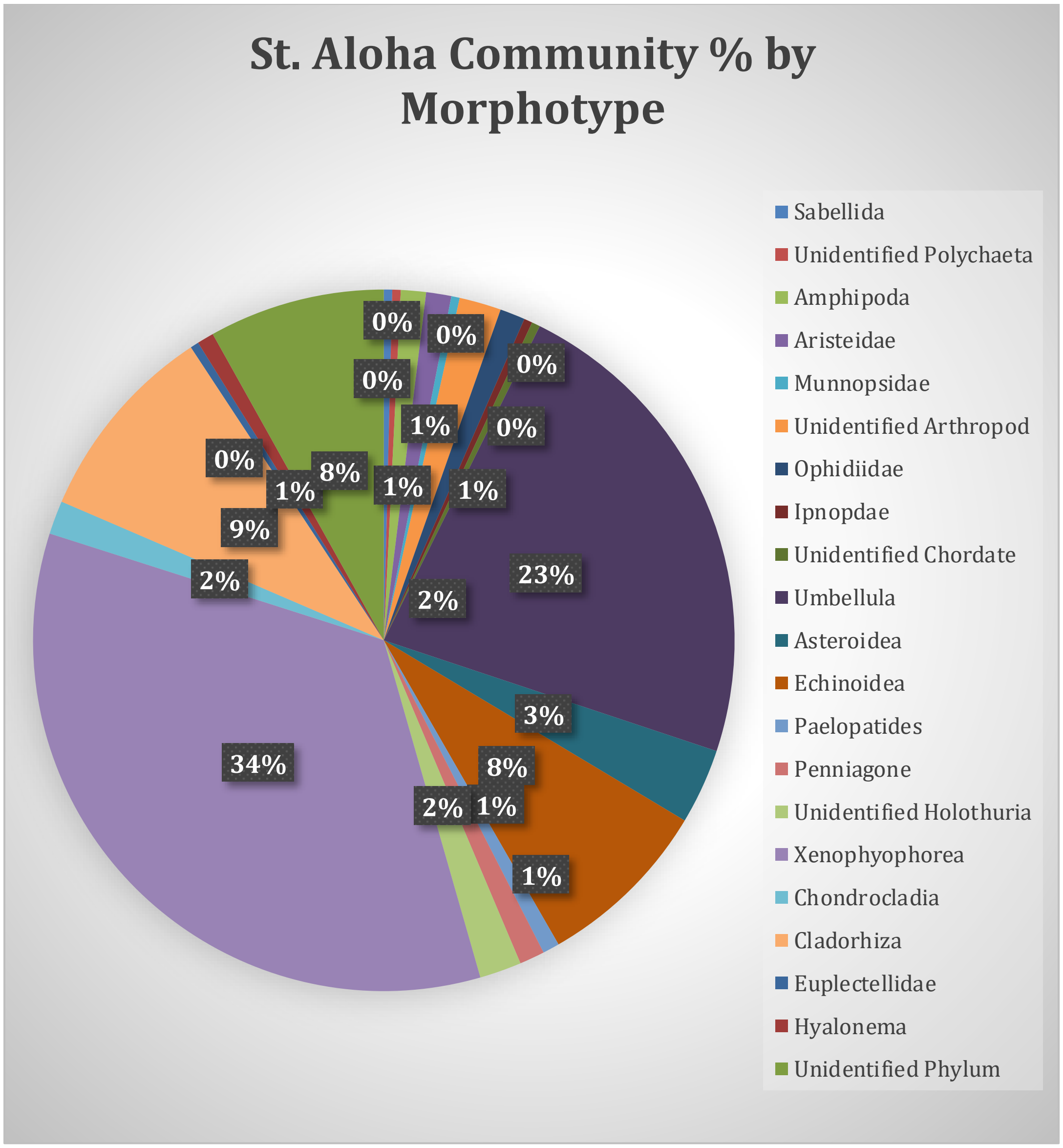

Figure 7. Proportional contribute of megafaunal morphotype to the community at Station Aloha.

Within the metazoan megafauna, a subset of taxa dominated abundance. Two of the three dominant metazoan phyla were Cnidaria and Porifera. Within these phyla, only suspension (or filter) feeders were identified within the transect. Most of these suspension feeders (59 individuals) consisted of cnidaria in the genus *Umbellula*, although there was also a significant number of porifera in the genus *Cladorhiza* with 24 individuals. Furthermore, there were several other types of known suspension feeders within the

Porifera phylum including the genus *Chondrocladia*, the family Euplectellidae, and the genus *Hyalonema*. However, these taxa were much less abundant than *Umbellula* and *Cladorhiza*. Suspension feeders dominated metazoan abundance at Station Aloha.

The phylum that made up the third largest proportion of identified organisms was Echinodermata. Thus, echinoderms are also significant to this ecosystem by constituting 16% of the overall megafaunal abundance, with numerous morphotypes identified within the phylum.

4.2 MEGAFAUNAL DIVERSITY AT STATION ALOHA

As previously stated, there were seven phyla observed within the transect. Within several phyla, there were a number of different taxa observed. In total, 20 morphotypes were identified across the dataset, disregarding the 21 organisms that were not identifiable at the phylum level (Table 1). Organisms were identified to a wide range of taxonomic ranks varying from species to class. The variation of taxa that were identified shows that the benthic megafaunal community has both a variety of taxa and feeding types including suspension feeders, scavengers, deposit feeders, and predators.

Of the seven phyla identified in this study, five had multiple morphotypes (Table 1). Echinoderms made up the most diverse group, with five morphotypes. Sponges and arthropods were the next most diverse groups, each with four observed morphotypes. While arthropods had low abundance compared to other phyla, the high abundance of sponges was primarily concentrated within the *Cladorhiza* morphotype (Table 1). The lowest observed diversity existed within cnidarians and forams at one morphotype each. This is almost certainly an artifact of limited ability to identify these organisms to lower taxonomic levels due to image resolution, since foraminifers and cnidarians make up the two highest abundances across the site. Furthermore, these two phyla are known to harbor high diversity in abyssal habitats (Gooday, et al. 2020; Durden et al. 2021).

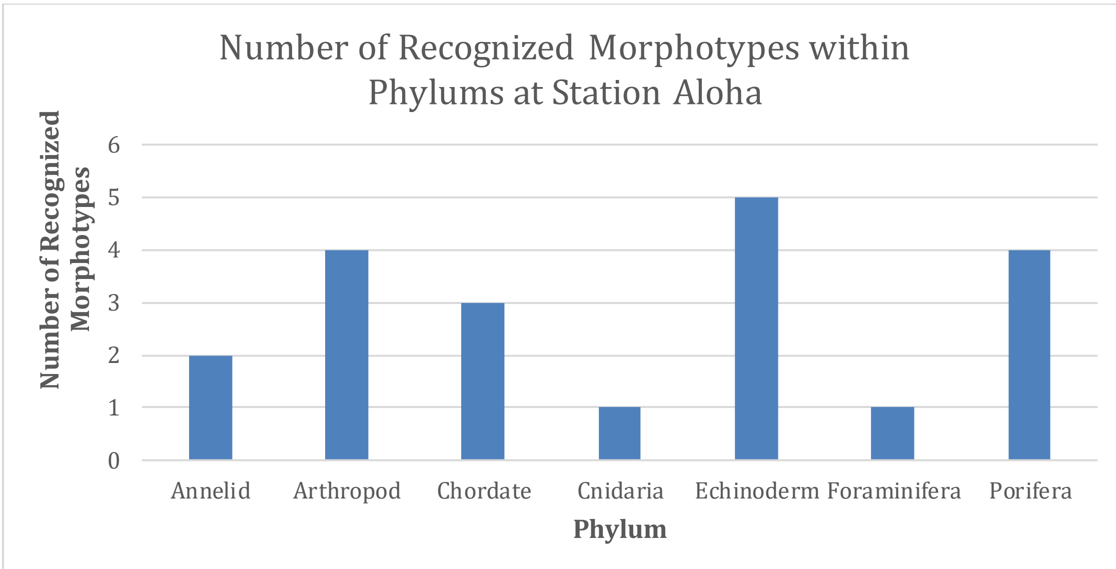

Figure 8. Bar graph showing the number of megafaunal morphotypes found within each identified phylum at the seafloor of Station Aloha.

Figure 8 indicates that there were 20 morphotypes across the dataset, however, this chart disregards those 21 organisms that were not identified to the phylum level, which can be seen in the unidentified phylum group of Table 1. The number of morphotypes that were seen within this dataset would be greater if any of these individuals belong to a previously unidentified morphotype.

One way of quantifying diversity in an ecosystem is assessing species richness. To do this it is necessary to estimate how many taxa, or in this case morphotypes, exist within the region. The analysis of this dataset identified 20 different morphotypes; however, it should be noted that it is likely that the region was significantly under sampled due to ROV issues limiting data to a single photo transect. To understand how well taxonomic richness was sampled, statistical taxa accumulation curves and taxa richness estimators were applied to the dataset (Figure 9).

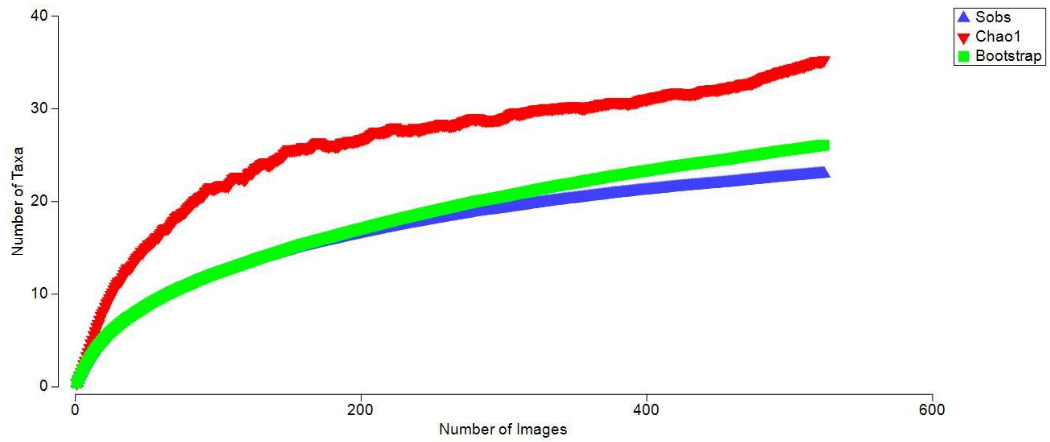

Figure 9. Graph showing species accumulation curve (Sobs) within the dataset, and two curves representing species richness estimators Bootstrap and Chao 1, all generated with Primer 7. Blue - Number of total morphotypes that accumulated using our analytical methods. Green - Number of species estimated in the region using the Bootstrap statistical estimation. Red - Number of species accumulated in the region using the Chao 1 statistical estimation test.

The taxon accumulation and richness estimator curves indicate that this study under sampled the region (Figure 9). The actual number of morphotypes that were identified within the study (blue curve in Figure 8), is below both the Bootstrap and the Chao 1 estimated richness curves. This suggests statistically that there are more taxa at Station Aloha than were observed within the dataset. The Bootstrap test estimates that this study under sampled the region by approximately three morphotypes. The Chao 1 curve estimates that this region was under sampled by ~12 morphotypes. Because Sobs, Chao 1 and Bootstrap curves are all still rising at the x-axis limit, they likely underestimate morphotype richness.

4.3 POC FLUX VERSUS MEGAFAUNAL ABUNDANCE ACROSS SITES

Across the sites compared in this study, all but one have fairly similar POC fluxes. The POC flux, which is an estimate of food availability, for Station Aloha was estimated to be 1.35 $gC/m^2/yr$ based on the Lutz et al. (2007) model. This value is much lower than the POC flux of Station M, which make sense because Station M lies under highly eutrophic waters off of the California coast (Fig. 9). However, the Station Aloha value for POC flux sits within the range of POC fluxes at the six CCZ sites, as they range from 1.1 $gC/m^2/yr$ to 1.8 $gC/m^2/yr$ (Fig. 10).

The seafloor POC flux patterns follow known trends related to primary productivity in surface waters of the North Pacific. For instance, within the CCZ, POC flux was observed to be greater at locations closer to the equator, which follows the trend of more primary production near equatorial upwelling (Smith, et al. 1997). Furthermore, seafloor POC flux at the CCZ sites increases when moving longitudinally from west to east, which is a trend that has been previously documented in the CCZ (Washburn, et al. 2021).

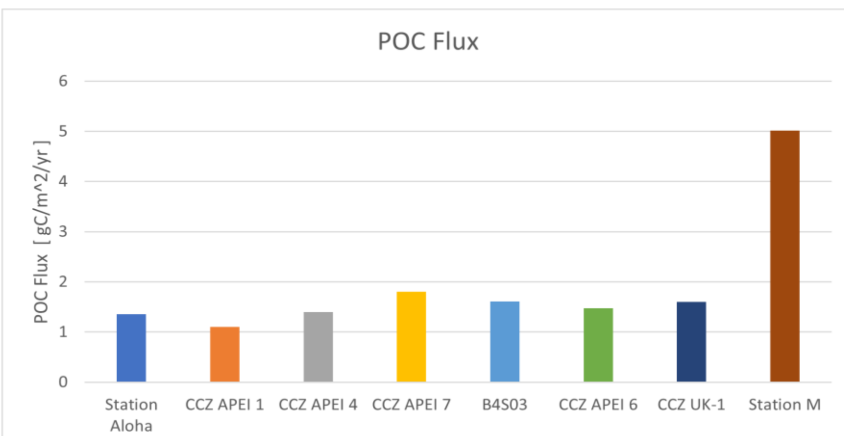

Figure 10. Bar graph comparing POC flux across North Pacific sites, with sites being organized from left to right with respect to proximity to Station Aloha, with Station M last due to the difference in surface conditions.

It was originally hypothesized that increased food availability would lead to increased abundance of megafauna across these deep-sea sites. Variations in megafaunal abundance across sites are shown in Figure 11. Xenophyophores were analyzed separately because they are not metazoan organisms, but rather unicellular foraminifers and their response to nutrient limitation could be different from the metazoan (Gooday, et al. 2020).

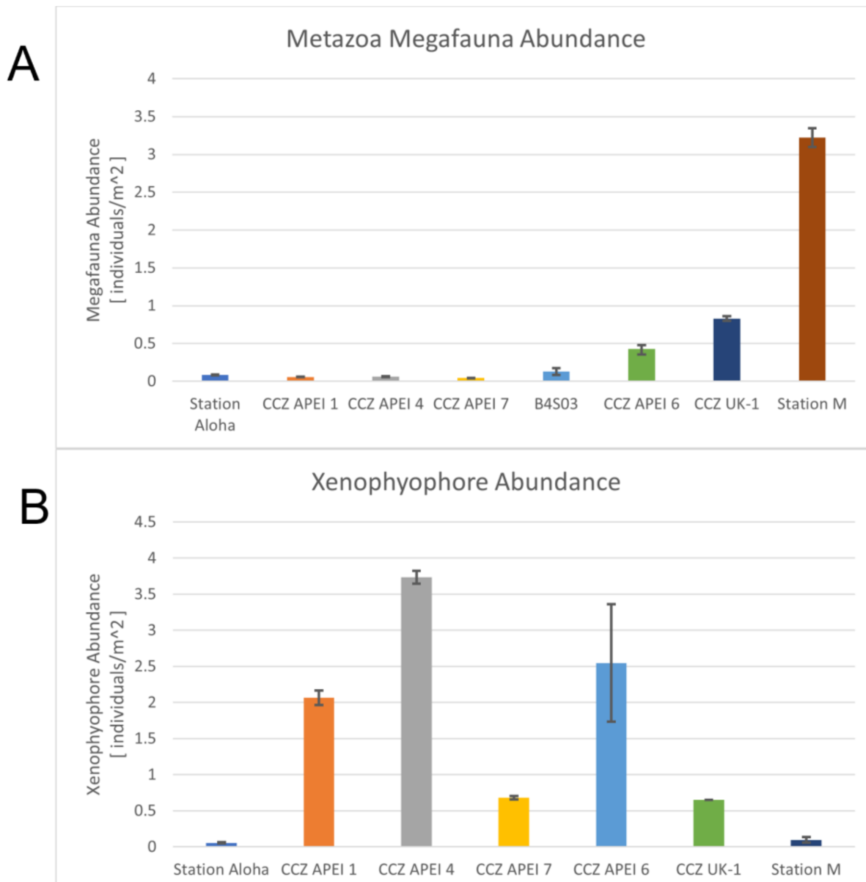

Figure 11. Bar graph that compares the abundance of (A) megafaunal metazoans and (B) megafaunal xenophyophores across North Pacific sites. Sites are organized from left to right with respect to proximity to Station Aloha, with Station M last due to the difference in surface conditions. Note that site B4S03 does not contain data for xenophyophore abundance.

Station Aloha was similar to most of the abyssal sites compared in this study, with low metazoan megafaunal abundance relative to Station M. None of the benthic locations that lie at significant distances from continental land masses reach a megafaunal abundance greater than 1 individual/m^2, although an increasing trend can be observed at the eastern locations within the CCZ. Both APEI 6 and UK-1 have abundances greater than 0.25 individuals/m^2, while the other sites have abundance values closer to zero. It should also be noted, the metazoan megafaunal abundance at Station M, similar to POC flux, is higher than the rest of the sites.

The patterns of xenophyophore abundance (Figure 11) were not the same as those seen with the metazoan megafauna or POC flux graphs. This could be for a variety of reasons including seasonal variation in infauna foram abundance (Drazen, et al. 1998), and presence of nodules at some locations, as this could affect xenophyophore populations (Durden, et al. 2021). APEIs 4 and 6 in the CCZ had the highest abundances of xenophyophores. One thing that these two sites have in common is that they have a similar POC flux. However, Station M and Station Aloha have the two lowest abundances of all the sites. Station M and Aloha have significantly different POC fluxes providing no evidence of a monotonic relationship between xenophyophore abundance and POC flux (Fig. 12).

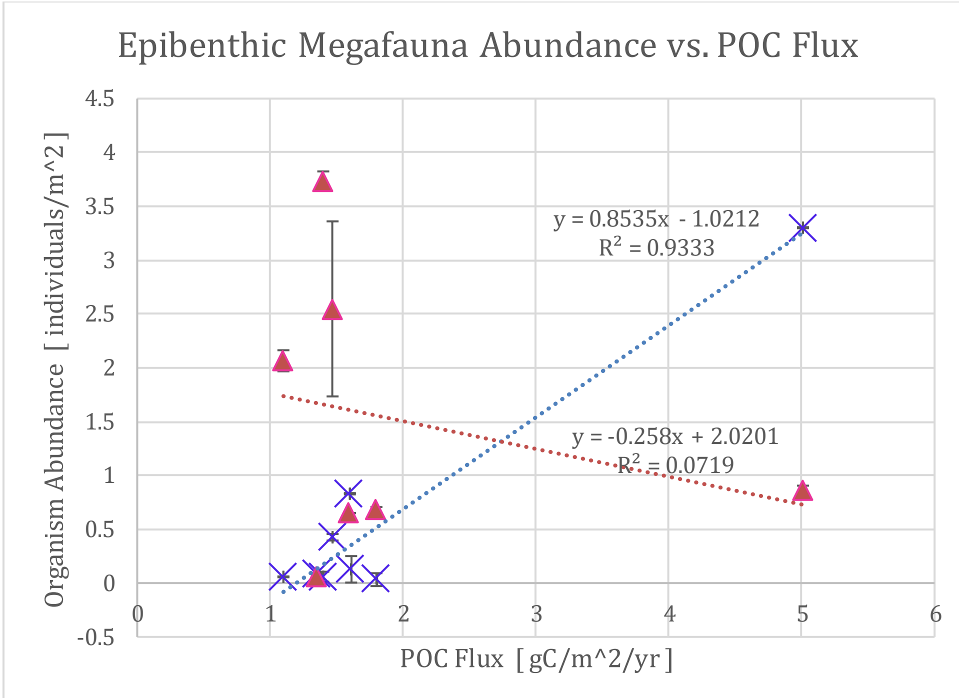

Figure 12. The relationships between organism abundances and POC flux to these deep-sea sites. Blue Xs represent metazoan megafauna abundances while the orange triangles represent xenophyophore abundances. The blue and orange dotted trend lines represent metazoan megafauna and xenophyophores respectively, and equations for each line are given.

In contrast to the results of xenophyophores, the metazoan megafauna density is positively correlated with food supply (seafloor POC flux; Figure 12). Furthermore, this correlation between POC flux and metazoan abundance was found to be statistically significant ($p < 0.01$, one tailed test). The correlation between POC flux xenophyophore abundance, however, was not statistically significant ($p > 0.05$). A two tailed test was applied to the xenophyophore abundances because this relationship had the potential to be either positive or negative.

It must also be noted that the POC flux at Station M was nearly four times greater than any other location. This heavily influenced the strength and slope of the relationship between metazoan megafaunal abundance and food supply. Thus, more data regarding megafauna, xenophyophores, and POC flux should be collected at other sites in the abyssal North Pacific, especially at sites with intermediate POC fluxes between the CCZ and Station M.

4.4 COMPARISON OF MORPHOTYPE RICHNESS

To analyze the diversity of Station Aloha, this study looked at the morphotype richness of Station Aloha. 20 morphotypes were detected within the single transect that was collected in this study, although abyssal plain ecosystems are known to have a high species richness (Smith, et al. 2006). The Chao 1 taxonomic richness curve suggested that the region was under sampled due to the continuing rise of the curve (Figure 8). Therefore, it is likely that the single transect that was analyzed was insufficient to draw complete conclusions about the taxonomic richness of Station Aloha. There are likely more morphotypes in this region than were identified in this study. This must be kept in mind when comparing taxonomic richness to the other benthic sites in the North Pacific.

Station M is the site with the greatest POC flux and abundance by a significant margin. At this location, 102 morphotypes were accumulated within transect areas (Kuhnz, et al. 2014). Furthermore, an additional 19 taxa were identified at this location outside of the transect area (Kuhnz, et al. 2014). This taxonomic richness is significantly higher than that of Station Aloha. However, it is important to keep in mind that many of the Station M morphotypes were identified to the species level, whereas at Station Aloha no morphotypes were identified to the species level. It is for this reason that it is necessary to compare the number of phyla that accumulated at each site. In this study, seven phyla were identified at Station Aloha. At Station M, 15 phyla were identified (Kuhnz, et al. 2014). This suggests that Station M does have a higher morphotype richness compared to Station Aloha, although this conclusion must be tempered by the limited sampling at Station Aloha.

The eastern CCZ is the region where species richness was found to be the greatest. This region consists of the Belgian contract areas (abbreviated for simplicity's sake to B4S03; the region located in the middle of the contract area), the UK-1 contract area, and APEI 6. B4S03 had the lowest species richness of the region at 41 identified morphotypes. While this had lowest species richness in the region, it is almost two times the number of morphotypes identified at Station Aloha. The site with the next greatest species richness was APEI 6, with 129 morphotypes identified, but only ten phyla were identified (Simon-Lledo, et al. 2019). Similar to APEI 6, UK-1 had ten identified phyla, however, it also had the highest species richness (Amon, et al. 2016). The UK-1 site contained 170 total morphospecies observed and 196 morphospecies estimated from Chao 1 (Amon, et al. 2016). The eastern most CCZ has been estimated to have a high megafaunal diversity, yet when comparing to Station Aloha these sites are more similar in terms of phylum richness.

The western CCZ is most similar to Station Aloha in terms of morphotype richness levels, but still remains significantly higher than Station Aloha (Durden, et al. 2021). However, because of difficulties identifying most individuals to lower taxonomic rankings at Station Aloha, it is more relevant to compare phyla diversity. These two regions become much more similar when looking at phylum accumulation. The western CCZ had a total of eleven phyla compared to Station Aloha's seven. But because the Station Aloha taxonomic richness estimations continue to rise (Fig. 8), it is safe to assume that Station Aloha has a more similar phylum richness compared to the western CCZ. The fact that Station Aloha and the western CCZ have the most similar richness levels makes sense because these two regions have similar POC fluxes (Durden, et al. 2021).

Station Aloha was found to have a low megafaunal morphotype richness relative to those other sites where significant megafaunal studies have taken place. There are a variety of possibilities explaining why Station Aloha was observed to have a lower morphotype richness. The first is that the region is home to fewer taxa due to environmental conditions. It is more likely, however, that Station Aloha does have more taxa living in the region, but the single transect that was taken did not fully sample the species richness. Furthermore, it is possible that some organisms may have remained unidentified due to image resolution and lighting, and the shadow of the ROV. Further studies should be made at this site to gain a more accurate understanding of the community's diversity, and why there may be fewer identified taxa.

5.0 CONCLUSIONS

Station Aloha has a low overall benthic megafaunal abundance and morphotype richness compared to other sites in the North Pacific. The hypothesis that abyssal community structure has a positive correlation with POC flux was not entirely supported. This study supported the known positive relationship between POC flux and metazoan megafaunal abundances; however, xenophyophore abundances varied nonmonotonically with POC flux. This makes it difficult to draw any conclusions about the relationship between xenophyophores and POC flux in the North Pacific. It is likely that the low abundance of Station Aloha is in part due to the low POC flux of the region. Furthermore, when combining all of the variables of this study, the community structure of Station Aloha was found to be different from each of the other north Pacific sites that were compared, suggesting that benthic ecosystems of the North Pacific are not homogenous.

More benthic megafauna studies should be performed at Station Aloha and in other regions of the North Pacific to obtain a better understanding of the relationship between these megafaunal communities and POC flux. This could help to better characterize the megafaunal community structure of Station Aloha, while also helping to draw broader conclusions about the deep ocean in general. A greater understanding of how megafaunal communities are related across abyssal Pacific sites is desirable before deep-sea mining is permitted to commence over expansive regions of the seafloor.

LITERATURE CITED

Alford, M. H., Lukas, R., Howe, B., Pickering, A., & Santiago-Mandujano, F. (2011). Moored observations of episodic abyssal flow and mixing at station ALOHA. *Geophysical research letters*, *38*(15).

Amon, D. J., Ziegler, A. F., Dahlgren, T. G., Glover, A. G., Goineau, A., Gooday, A. J., ... & Smith, C. R. (2016). Insights into the abundance and diversity of abyssal megafauna in a polymetallic-nodule region in the eastern Clarion-Clipperton Zone. *Scientific Reports*, *6*(1), 1-12.

Barr, N. (2019, December 17). *Video annotation and reference system*. MBARI. Retrieved December 11, 2021, from https://www.mbari.org/products/research-software/video-annotation-and-reference-system-vars/.

Browning, T. J., Achterberg, E. P., Rapp, I., Engel, A., Bertrand, E. M., Tagliabue, A., & Moore, C. M. (2017). Nutrient co-limitation at the boundary of an oceanic gyre. *Nature*, *551*(7679), 242-246.

Carr, D. S. M. (2021). Critical values for correlation coefficients. Retrieved December 12, 2021, from https://www.mun.ca/biology/scarr/Critical_Values_of_r.htm.

Chiswell, S. M. (1996). Intra-annual oscillations at Station ALOHA, north of Oahu, Hawaii. *Deep Sea Research Part II: Topical Studies in Oceanography*, *43*(2-3), 305-319.

De Smet, B., Simon-Lledó, E., Mevenkamp, L., Pape, E., Pasotti, F., Jones, D. O., & Vanreusel, A. (2021). The megafauna community from an abyssal area of interest for mining of polymetallic nodules. *Deep Sea Research Part I: Oceanographic Research Papers*, *172*, 103530.

Devine, J. A., Watling, L., Cailliet, G., Drazen, J., Muñoz, P. D., Orlov, A. M., & Bezaury, J. (2012). Evaluation of potential sustainability of deep-sea fisheries for grenadiers (Macrouridae). *Journal of Ichthyology*, *52*(10), 709-721.

Drazen, J. C., Baldwin, R. J., & Smith Jr, K. L. (1998). Sediment community response to a temporally varying food supply at an abyssal station in the NE Pacific. *Deep Sea Research Part II: Topical Studies in Oceanography*, *45*(4-5), 893-913.

Drazen, J. C., Smith, C. R., Gjerde, K. M., Haddock, S. H., Carter, G. S., Choy, C. A., ... & Yamamoto, H. (2020). Opinion: Midwater ecosystems must be considered when evaluating environmental risks of deep-sea mining. *Proceedings of the National Academy of Sciences*, *117*(30), 17455-17460.

Durden, J. M., Putts, M., Bingo, S. R. D., Leitner, A. B., Drazen, J. C., Gooday, A. J., ... & Smith, C. R. (2021). Megafaunal ecology of the western Clarion Clipperton Zone. *Frontiers in Marine Science*, *8*, 722.

Gillard, B., Purkiani, K., Chatzievangelou, D., Vink, A., Iversen, M. H., Thomsen, L., & Ackley, S. F. (2019). Physical and hydrodynamic properties of deep sea mining-generated, abyssal sediment plumes in the Clarion Clipperton Fracture Zone (eastern-central Pacific). *Elementa: Science of the Anthropocene*, *7*.

Glover, A. G., Smith, C. R., Paterson, G. L. J., Wilson, G. D. F., Hawkins, L., & Sheader, M. (2002). Polychaete species diversity in the central Pacific abyss: local and regional patterns, and relationships with productivity. *Marine Ecology Progress Series*, *240*, 157-170.

Gooday, A. J., Durden, J. M., Holzmann, M., Pawlowski, J., & Smith, C. R. (2020). Xenophyophores (Rhizaria, Foraminifera), including four new species and two new genera, from the western Clarion-Clipperton Zone (abyssal equatorial Pacific). *European Journal of Protistology*, *75*, 125715.

ISA. (2021). *Clarion Clipperton Zone*. International Seabed Authority. https://www.isa.org.jm/index.php/map/clarion-clipperton-fracture-zone.

Jiao, N., Tang, K., Cai, H., & Mao, Y. (2011). Increasing the microbial carbon sink in the sea by reducing chemical fertilization on the land. *Nature Reviews Microbiology*, *9*(1), 75-75.

Jones, D. O., Kaiser, S., Sweetman, A. K., Smith, C. R., Menot, L., Vink, A., ... & Radziejewska, T. (2017). Biological responses to disturbance from simulated deep-sea polymetallic nodule mining. *PLoS One*, *12*(2), e0171750.

Kapiris, K., & Thessalou-Legaki, M. (2011). Feeding ecology of the deep-water blue–red shrimp Aristeus antennatus (Decapoda: Aristeidae) in the Greek Ionian Sea (E. Mediterranean). *Journal of Sea Research*, *65*(1), 151-160.

Karl, D. M., & Church, M. J. (2019). Station ALOHA: A Gathering Place for Discovery, Education, and Scientific Collaboration. *Limnology and Oceanography Bulletin*, *28*(1).

Karl, D. M., Christian, J. R., Dore, J. E., Hebel, D. V., Letelier, R. M., Tupas, L. M., & Winn, C. D. (1996). Seasonal and interannual variability in primary production and particle flux at Station ALOHA. *Deep Sea Research Part II: Topical Studies in Oceanography*, *43*(2-3), 539-568.

Karl, D. M., Church, M. J., Dore, J. E., Letelier, R. M., & Mahaffey, C. (2012). Predictable and efficient carbon sequestration in the North Pacific Ocean supported by symbiotic nitrogen fixation. *Proceedings of the National Academy of Sciences*, *109*(6), 1842-1849.

Kuhnz, L. A., Ruhl, H. A., Huffard, C. L., & Smith Jr, K. L. (2014). Rapid changes and long-term cycles in the benthic megafaunal community observed over 24 years in the abyssal northeast Pacific. *Progress in Oceanography*, *124*, 1-11.

Lauerman, L. M. L., Kaufmann, R. S., & Smith Jr, K. L. (1996). Distribution and abundance of epibenthic megafauna at a long time-series station in the abyssal northeast Pacific. *Deep Sea Research Part I: Oceanographic Research Papers*, *43*(7), 1075-1103.

Lejzerowicz, F., Gooday, A. J., Barrenechea Angeles, I., Cordier, T., Morard, R., Apothéloz-Perret-Gentil, L., ... & Pawlowski, J. (2021). Eukaryotic biodiversity and spatial patterns in the Clarion-Clipperton Zone and other abyssal regions: insights from sediment DNA and RNA metabarcoding. *Frontiers in Marine Science*, *8*, 536.

Levin, L. A. (1994). Paleoecology and ecology of xenophyophores. *Palaios*, 32-41.

Lutz, M. J., Caldeira, K., Dunbar, R. B., & Behrenfeld, M. J. (2007). Seasonal rhythms of net primary production and particulate organic carbon flux to depth describe the efficiency of biological pump in the global ocean. *Journal of Geophysical Research: Oceans*, *112*(C10).

Mayer, L., Jakobsson, M., Allen, G., Dorschel, B., Falconer, R., Ferrini, V., ... & Weatherall, P. (2018). The Nippon Foundation—GEBCO seabed 2030 project: The quest to see the world’s oceans completely mapped by 2030. *Geosciences*, *8*(2), 63.

Miller, K. A., Thompson, K. F., Johnston, P., & Santillo, D. (2018). An overview of seabed mining including the current state of development, environmental impacts, and knowledge gaps. *Frontiers in Marine Science*, *4*, 418.

Ruhl, H. A. (2007). Abundance and size distribution dynamics of abyssal epibenthic megafauna in the northeast Pacific. *Ecology*, *88*(5), 1250-1262.

Ruhl, H. A., Ellena, J. A., & Smith, K. L. (2008). Connections between climate, food limitation, and carbon cycling in abyssal sediment communities. *Proceedings of the National Academy of Sciences*, *105*(44), 17006-17011.

Ruhl, H. A., & Smith, K. L. (2004). Shifts in deep-sea community structure linked to climate and food supply. *Science*, *305*(5683), 513-515.

Scharek, R., Tupas, L. M., & Karl, D. M. (1999). Diatom fluxes to the deep sea in the oligotrophic North Pacific gyre at Station ALOHA. *Marine Ecology Progress Series*, *182*, 55-67.

Simon-Lledó, E., Bett, B. J., Huvenne, V. A., Schoening, T., Benoist, N. M., Jeffreys, R. M., ... & Jones, D. O. (2019). Megafaunal variation in the abyssal landscape of the Clarion Clipperton Zone. *Progress in oceanography*, *170*, 119-133.

Simon-Lledó, E., Bett, B. J., Huvenne, V. A., Schoening, T., Benoist, N. M., & Jones, D. O. (2019). Ecology of a polymetallic nodule occurrence gradient: Implications for deep-sea mining. *Limnology and Oceanography*, *64*(5), 1883-1894.

Craig R. Smith, personal communication

Smith, C. R. (2020). The heterogeneous abyss. *Proceedings of the National Academy of Sciences*, *117*(29), 16729-16731.

Smith, C. R., Berelson, W., Demaster, D. J., Dobbs, F. C., Hammond, D., Hoover, D. J., ... & Stephens, M. (1997). Latitudinal variations in benthic processes in the abyssal equatorial Pacific: control by biogenic particle flux. *Deep Sea Research Part II: Topical Studies in Oceanography*, *44*(9-10), 2295-2317.

Smith, C. R., De Leo, F. C., Bernardino, A. F., Sweetman, A. K., & Arbizu, P. M. (2008). Abyssal food limitation, ecosystem structure and climate change. *Trends in Ecology & Evolution*, *23*(9), 518-528.

Smith, C. R., & Demopoulos, A. W. (2003). The deep Pacific ocean floor. *Ecosystems of the World*, 179-218.

Smith, C. R., Drazen, J., & Mincks, S. L. (2006, March). Deep-sea biodiversity and biogeography: perspectives from the abyss. In *International Seabed Authority Seamount Biodiversity Symposium* (pp. 1-13).

Smith, C. R., & Rabouille, C. (2002). What controls the mixed-layer depth in deep-sea sediments? The importance of POC flux. *Limnology and Oceanography*, *47*(2), 418-426.

Smith, C. R., Tunnicliffe, V., Colaço, A., Drazen, J. C., Gollner, S., Levin, L. A., ... & Amon, D. J. (2020). Deep-sea misconceptions cause underestimation of seabed-mining impacts. *Trends in Ecology & Evolution*, *35*(10), 853-857.

Smith Jr, K. L., Kaufmann, R. S., & Baldwin, R. J. (1994). Coupling of near-bottom pelagic and benthic processes at abyssal depths in the eastern North Pacific Ocean. *Limnology and Oceanography*, *39*(5), 1101-1118.

Smith, W. H., & Sandwell, D. T. (1997). Global sea floor topography from satellite altimetry and ship depth soundings. *Science*, *277*(5334), 1956-1962.

Washburn, T. W., Jones, D. O., Wei, C. L., & Smith, C. R. (2021). Environmental Heterogeneity Throughout the Clarion-Clipperton Zone and the Potential Representativity of the APEI Network. *Frontiers in Marine Science*, *8*, 319.