2014

Meiofauna community composition at three whale-fall sites in Monterey Bay, California

Gillian Louise Rhett
California State University, Monterey Bay

MEIOFAUNA COMMUNITY COMPOSITION AT THREE WHALE-FALL SITES IN MONTEREY BAY, CALIFORNIA

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A Thesis

Presented to the

Faculty of the

Moss Landing Marine Laboratories

California State University Monterey Bay

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in

Marine Science

_____

by

Gillian Louise Rhett

Summer 2014

CALIFORNIA STATE UNIVERSITY MONTEREY BAY

The Undersigned Faculty Committee Approves the

Thesis of Gillian Louise Rhett:

MEIOFAUNA COMMUNITY COMPOSITION AT THREE WHALE-FALL SITES IN MONTEREY BAY, CALIFORNIA

James R. Harvey

James Harvey, Director
San Jose State University, Moss Landing Marine Laboratories

Kenneth M. Coale

Kenneth Coale, Professor
San Jose State University, Moss Landing Marine Laboratories

Robert H. Vrijenhoek

Robert Vrijenhoek, Senior Scientist
Monterey Bay Aquarium Research Institute

Marsha Moroh

Marsha Moroh, Dean
College of Science, Media Arts, and Technology

July 17, 2014

Approval Date

ABSTRACT

There are localized deep-seafloor habitats where there is a much greater input of nutrients than most of the seafloor. These include hydrothermal vents, cold seeps, and sunken whale skeletons called whale-falls. Meiofauna, a taxonomically diverse group of microscopic invertebrates and single-celled eukaryotes, have been studied in many habitats, though there have been no published studies on meiofauna at whale-falls. The purpose of this study was to test whether the increased energy resources at a whale-fall affected the meiofauna community. To test this hypothesis, I characterized the community of meiofauna living under and around whale-fall at three locations in the Monterey Bay, in terms of biomass (μg carbon per cm^2) and diversity. Nematodes were the most abundant organism, although annelids accounted for the greatest share of biomass in some samples due to their larger body size. More meiofaunal organisms were found near the carcass than far from it and the greatest meiofaunal biomass occurred three to seven meters from the carcass. The greater biomass nearer the bones was probably due to nutrient enrichment from the whale. The lesser numbers under the carcass compared with 3 – 7 m away may be due to toxic chemical gradients in the sediment around the bones, grazing by larger organisms living near the bones, or competitive dominance. These findings are a necessary first step towards a thorough understanding of how the meiofauna at whale-falls differ from meiofauna communities in other habitats.

ACKNOWLEDGEMENTS

I would like to thank my advisor, James Harvey, and my committee members Robert Vrijenhoek and Kenneth Coale for their unwavering help and support throughout this project. Although they are all very busy, they always took the time to help me plan my methods, interpret my results, improve my writing and give me moral support.

Thank you Kurt Buck for all the help you gave me with lab methods and interpreting my data. Thank you Shannon Johnson for giving me the idea for this thesis in the first place and for help and support throughout the process. Thank you Sara Tanner and Ivano Aiello for help with the SEM and Ivano for help with the sediment analysis and the map. Thank you Jonathan Geller and Mike Graham for help with the proposal and planning my sampling design. Thank you Mary McGann and Jeff Baguley for help with identifying the forams and invertebrates respectively. Thank you Julio Harvey for help with the PRIMER software. I would also like to thank the faculty, staff, and students of Moss Landing Marine Laboratories and the Monterey Bay Aquarium Research Institute.

I would like to thank my funding sources: the Molecular Ecology Lab and Benthic Ecology Lab at MBARI for collecting the sediment cores (which was by far the most expensive part) and providing lab supplies and equipment, and the Friends of MLML 2013 James Nybakken Scholarship and the David and Lucile Packard Foundation for monetary grants.

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Introduction

Most of the seafloor has low energy input compared with habitats that rely on photosynthesis, but there are localized seafloor habitats where the concentration of organic carbon is greater, such as hydrothermal vents and cold seeps. These habitats with greater biomass and biodiversity in less productive surroundings resemble oases in a desert (Laubier and Desbruyères 1985). Hydrothermal vents are found in most areas with tectonic activity. Cold seeps occur over a wide range of depths in the ocean, where dissolved reduced chemicals such as methane or hydrogen sulfide are released from the Earth's crust (Paull et al. 1984). The ecosystems immediately surrounding these geological structures are fueled by chemoautrophic bacteria that can synthesize organic carbon using the chemical energy from natural gasses. Hydrothermal vent fields and cold seeps persist for decades, making them a transient habitat (Callender et al. 1990, Coykendall et al. 2011). Hydrothermal vent fields vary in size and can be tens of meters long and host a large biomass of 2 - 8.5 kg/m^2 wet weight (Fustec et al. 1988). The biomass of cold seep communities also is greater than the surrounding environment, and the species composition is more variable than the hydrothermal vent systems (Juniper and Sibuet 1987). The concentration of nutrients in the form of dissolved organic carbon is greater in the immediate area around vents and cold seeps, and rapidly decreases tens of meters away (Barry et al. 1996). Organic carbon and biomass, therefore, are distributed in a halo around the vent and seep systems.

Whale carcasses (and later skeletons) on the seafloor, commonly called whale-falls, are another nutrient-enriched deep sea habitat, hosting their own specialized community. Whale-falls exist on a temporal scale similar to vents and cold seeps, on the order of years to decades, and a similar spatial scale of tens of square meters. They also exhibit a similar distribution of

dissolved organic carbon in the sediment, where the percentage of carbon is greater directly under the bones than far from the bones, similar to the distribution found at vents and seeps (Goffredi et al. 2008). These nutrient-enriched deep-sea habitats are the setting for a variety of intraspecies interactions. The most striking of these is the symbiosis between invertebrates and bacteria (Goffredi et al. 2007), but the abundance of diverse organisms also results in trophic interactions that may be less likely elsewhere. For example, pycnogonid sea spiders prey on attached and drifting sea anemones at cold seeps, whale-falls, and sunken wood, which is another enriched deep-sea habitat (Braby et al. 2009). *Osedax* worms attach to bones via root-like structures that contain their bacterial symbiotes (Vrijenhoek et al. 2009). They break down the bone gradually, and this process makes it possible for *Rubyspira* snails to eat the resulting spongy bone material (Johnson et al. 2010). Thus, organisms that inhabit whale-falls are not only exploiting the nutrients from the carcass directly, they also benefit from and in some cases depend on each other. Macrofauna have been and continue to be well studied at whale-falls and other deep sea habitats, but there are no published studies of how the presence of a whale-fall affects the meiofauna community.

Smith et al. (1989) hypothesized that whale-falls may provide a stepping-stone for the dispersal of chemosynthetic community organisms. Many of the macroorganisms found on whale-falls are related to organisms in the cold seep and hydrothermal vent communities (Smith et al. 1989). For example, *Riftia* and *Osedax* belong to the same family, Siboglinidae, and both have symbiotic relationships with bacteria (Goffredi et al. 2007), although they are different in that the bacteria in *Osedax* digest collagen and fats (Rouse et al. 2004) and the bacteria in *Riftia* oxidize hydrogen sulfide (Cavanaugh et al. 1981). The density of whale-fall sites on the abyssal plane may hypothetically be as few as one per 935 km^2 although this is an average and their

distribution may be patchy (Jelmert and Oppen-Berntsen 1996). This raises the question: how do the larvae of whale-fall community species find a whale carcass on which to settle? *Osedax* worms can live on large bones from other organisms, such as teleost fish (Rouse et al. 2011). These smaller bones can provide stepping stones between distant whale-fall habitats. Although *Osedax* are typically observed on whale bones, molecular clock data and *Osedax*'s ability to colonize other bones indicate that they existed before whales did (Vrijenhoek et al. 2009).

There has been speculation since the 1930s that the carcasses of large marine animals such as whales would provide an influx of nutrients to deep benthic organisms thereby increasing those organisms' abundance (Krogh 1934). Trawls later in the 20th century brought up whale bones with organisms such as mussels and polychaetes living on them, indicating that these bones provided food or habitat for a variety of organisms (Dell 1987). It was not until the late 20th century when technology allowed direct observation of the deep sea that this phenomenon was studied in detail. Smith et al. (1989) described the community living on whale-fall after the fortuitous discovery of a sunken whale carcass off southern California. MBARI scientists discovered a gray whale carcass on the seafloor of the Monterey Bay in 2002 and later sank four more carcasses at different locations and depths around the Monterey Bay (Fig. 1). Goffredi et al. (2004), thereafter, described unique benthic fauna living on whale bones in the Monterey Bay, including the *Osedax* bone-eating worm, a relative of the hydrothermal vent worms *Riftia*. Much work has been done on the macrofauna and microfauna of whale-falls but there has not yet been a publication on the meiofauna.

Mare (1942) coined the term "meiobenthos" to refer to the assemblage of small metazoans inhabiting the sediment beds of lakes, rivers, oceans, and other bodies of water. Modern-day meiofauna are a taxonomically broad group defined by their size: being able to pass

through a 300 – 600 μm mesh and being retained by a 31 – 44 μm mesh (Giere 2009). They are mobile or haptosessile, and live on the surface of sediment or in the intersitital space among the sediment grains. Some organisms are included in the meiofauna only in their larval stages, with the adult stage being larger and classified as macrofauna. Meiofauna have been studied in a variety of habitats including estuaries, beaches, and the deep seafloor (Giere 2009). Most meiofauna have a broad diet, consuming smaller meiofauna and microorganisms. The most common organisms found in the deep sea meiofauna are nematodes and foraminiferans. Other phyla such as arthropods and annelids are present but less numerous, although their larger bodies mean a relatively small number of them can have a disproportionate effect on total meiofauna biomass.

Meiofauna, being defined only by size, include representatives of most groups of large protists and most invertebrate phyla (Fig. 2). Nematodes are abundant in the meiofauna of many habitats. Nematodes are ecdysozoan organisms belonging to phylum Nematoda. They represent about 90% of organisms (by count) living on the seafloor (Danovaro et al. 2008), and they are usually the most abundant taxon in the meiobenthos (Shimanaga et al. 2000). Their size ranges from 0.2 – 3.0 mm long, and they prey opportunistically on a variety of smaller organisms, including other nematodes. Foraminiferans, or forams, are a phylum of amoeboid protists that form a test of calcium carbonate or agglutinated sediment particles and are typically less than 1 mm but can be as large as 20 cm. Deep-sea forams use their pseudopods for locomotion and to capture and consume bacteria, detritus, and sometimes small animals (Sen Gupta 2002). Ciliata are protistans with distinctive macro- and micro-nuclei. Most ciliates are bacteriovores, some are detritivores and scavengers, and a few are predators. Polychaete annelids are fairly common in the deep meiobenthos. They can be small (< 300 μm), with only a few segments, or large

enough to qualify as macrofauna (> 1 mm). Kinorynchia is a phylum of marine pseudocoelomate invertebrates with eleven segments. They have no limbs or cilia; they locomote using the spines on the head and body. Gastrotricha is another ecdysozoan phylum found in the meiobenthos, named for their cilia that look like hairs. They are small, 60 μm to 100 μm, and they consume bacteria and protozoans. Crustacean arthropods, in particular copepods and ostracods, are common in the deep meiobenthos. Harpacticoids are the most common copepods in the meiofauna. Harpactacoids' tapered bodies range in length from 0.2 mm to 2.5 mm, and they can be identified by the size and shape of their bodies and limbs. Meiofaunal harpactacoids can feed on detritus or more selectively on bacteria, protozoans, or diatoms. Crustaceans' nauplius larvae also are found in the meiofauna. Ostracods have short bodies with few segments and their rounded, hinged carapace resembles a bivalve shell. Like the foraminiferans, ostracods are identified by their carapace shape, although convergent evolution of similar carapaces in distantly related organisms makes their identification more difficult. Ostracod species include scavengers, microphages, and herbivores, and they are preyed on by mites and worms. These are the most common taxa in the meiofauna in general but their density and diversity vary depending on habitat characteristics.

Depth is an important factor influencing the composition of the meiofauna community in a given habitat. In the Gulf of Guinea, density of meiofauna gradually decreases with increasing depth, and varies from about 800 to 1,800 individuals per 10 cm^2 at 1,200 m depth to about 0 to 1,000 individuals per 10 cm^2 at 3,000 to 4,000 m depth (Van Gaever et al., 2009a). The effects of nutrient enrichment also vary at different depths and locations. Meiofauna abundance was greater at a nearshore hydrocarbon seep 18 m deep near Santa Barbara than in the surrounding area (Montagna et al. 1989), a sulfide seep at 906 m depth in Monterey Bay (Buck and Barry

1998), and at 2,150 m depth in the Gulf of Mexico (Robinson et al., 2004). There was no difference, however, at a cold seep and the surrounding area off Japan at 1,170 m depth (Shirayama and Ohta 1990), and meiofauna density was actually less at a shallow methane seep in the North Sea at 150 m depth (Dando et al. 1991). Van Gaever et al. (2009b) compared the metazoan meiofauna at two locations near Norway, a pockmark cold seep at 740 m depth and a mud volcano at 1,280 m depth. Nematodes were the most abundant meiofauna in both Norwegian locations, followed by copepods. Varied meiofauna densities were found at the seep and control sites in both locations, although the pattern of greater density at the seep than non-seep was consistent.

In addition to depth, the age of a whale-fall site is likely to be a factor influencing meiofauna community composition. Smith et al. (1989) described a succession of communities colonizing a whale carcass as it decomposed, although the number and duration of distinct stages is different at other locations from what Smith found and may depend on the oxygen concentration of surrounding water and the rate of decomposition (Lundsten et al. 2010). In anoxic conditions at a southern California site, three stages were observed and a fourth was predicted (Bennett et al. 1994). The first was the mobile scavenger stage, lasting less than a year, when large scavengers consumed soft tissues of the carcass. The second was the enrichment-opportunist stage, lasting from months to a few years, in which the carcass was colonized by a diverse assemblage of macrofauna that live on the bones and nutrient-enriched sediment. The third stage was the sulfophilic stage, hypothesized by Smith et al. (1989) to last about 2 to 50 years, when the bones were colonized by chemosynthetic community organisms. It is now known the bones also are colonized by bone-specialized organisms such as *Osedax* worms (Johnson et al. 2010). The fourth and final stage, which has been predicted but not

observed, was the reef stage, when nutrients would be depleted and the remaining mineral structures from the bones would provide a hard substrate for attachment and potentially providing flow enhancement that would benefit suspension feeders (Smith and Baco 2003). These studies of succession are all based on macrofauna; as there are no published studies on meiofauna communities at whale-fall, it is not yet known whether they undergo similar ecological succession.

In contrast, whale-falls in the Monterey Bay, where oxygen concentration varied between 0 mL oxygen per liter of seawater (mL/L) and 4.17 mL/L with a mean of 1.368 mL/L, the stages were shorter and the enrichment-opportunist stage overlapped completely with the sulfophilic stage, calling into question the validity of describing whale-falls over time in terms of discrete stages. The oldest whale-fall in this study, at 2,893 m depth with a mean dissolved O_2 of 2.0 mL/L, was first observed in February 2002 with the skeleton intact. The bones had completely disappeared by 2009, leaving behind only a dark patch on the seafloor. *Osedax* worms on whale-falls of Monterey Bay colonized and degraded the bones, accelerating the progression of stages (Braby et al. 2007).

Although the ages of the three whale-fall sites in my study were all within a five-year range of each other, there may have been changes in the meiofaunal community on a shorter time scale, that were not captured by this study. In addition to having different ages, the carcasses were located at different depths, ranging from 633 m to 2,893 m depth. Depth has an effect on communities at cold seeps (Levin 2005), therefore, it also was reasonable to expect depth to affect the meiofauna at these whale-fall sites. Depth can affect community composition of macrofauna: of the four known species of *Osedax*, *O. rubiplumus* and *O. frankpressi* were found at the two deepest whale-fall sites, below the oxygen minimum zone, and *O. japonicus* and *O.*

mucofloris were found more recently at shallower, more oxygen-poor whale-fall sites (Braby et al. 2007). Therefore, depth and age of carcass (time elapsed between discovery or deployment of the carcass and the collection of samples) were taken into account in this project.

The size of the whale carcass, which is proportional with the amount of carbon it brings to the seafloor, is another factor to consider. The whales at 633 m and 2,893 m depth were gray whales (*Eschrictius robustus*) approximately 10 m long, and the 1,019 m deep whale was a blue whale (*Balenoptera musculus*) approximately 17 m long. Extrapolating from the average mass and carbon biomass values for whales given by Smith and Baco (2003), the gray whales contained approximately 1.20×10^6 g carbon each, and the blue whale approximately 2.85×10^6 g carbon.

The hypotheses tested were (1) total density of organisms (in terms of biomass per sediment area, following the convention for reporting meiofauna biomass) would be greatest under the bones and decrease at greater distance from the bones, following approximately the same pattern as the distribution of organic carbon in the sediment and (2) there would be greater diversity in the meiofaunal community at an intermediate distance from the bones. I expected a peak in diversity at the transition zone between the whale-fall-affected sediment and the surrounding "background" sediment due to the ecotone effect, and because this pattern has been observed specifically for meiofauna in other nutrient-enriched habitats (Boucher and Gourbault 1990).

Methods

MBARI's Molecular Ecology Lab collected comparable sets of sediment core samples from three of their five whale-fall sites (Fig. 1) at three different water depths (Table 1). The 0 m cores (0 m from the whale carcass) were taken by moving a bone aside and sampling the

sediment that had been directly under the bone. Sediment cores were collected using the ROV Doc Ricketts in June 2011. The corers used were polycarbonate, 7 cm in diameter and 25 cm long. They are optimized to avoid sediment/water interface disturbance. For each core, a 23.5 mL meiofaunal subsample of the top 3 cm of sediment was preserved in gluteraldehyde for visual identification of the organisms.

Water depth was assumed to be constant among cores within each site, based on previous examination of sites by MBARI. I assumed a greater number of samples at about 0 – 7 m distance would be necessary to characterize unique but rare species that would only occur under or near the whale carcass, therefore, distance from the carcass was sampled using a stratified method (Table 1), with proportionally more samples taken near the carcass. This pattern of rare organisms only occurring on or close to the carcass has been observed in the macrofauna at whalefall sites (Goffredi et al. 2004, Johnson et al. 2010). The 633 m and 1,019 m depth sites had intact bones when the cores were collected, but the bones at the 2,893 m depth site had degraded and there was only a dark patch on the seafloor to indicate where the skeleton had been.

Samples were prepared for microscopy using the procedure described by Burgess (2001), in which sediment is sieved then suspended in colloidal silica (Ludox™) to separate by density, and as much sediment as possible was removed to make viewing and identifying the organisms easier. The sample was passed through a 355 μm mesh before the silica procedure to remove any organisms too large to qualify as meiofauna and then rinsed onto a 32μm sieve to wash away silt and bacteria. Three steps of centrifugation and resuspension in Ludox™ were performed to remove as much sediment as possible. The material remaining after processing (organisms and

some remaining sediment) was funneled onto an 8μm filter that was placed on a glass microscope slide.

Individual organisms were identified to the most specific taxon possible, enumerated, and photographed using the epifluorescence microscope at MBARI. All slides were examined at 100x initially for counts and identification under the epifluorescence microscope using excitation wavelengths for DAPI (358 nm), and fluorescein (494 nm) because some of the organisms such as nematodes had more clearly visible outlines under the longer-wavelength light, which made measuring them on the photographs easier. Some organisms such as foraminiferans have hard parts that could remain intact long after death, and those organisms were not analyzed; the DAPI stain ensured that only organisms with intact nucleic acids at the time of collection would be counted. Using the method from Baguley et al. (2004) for size and biomass calculations, organisms were photographed at 100x or 200x depending on their size. I used ImageJ to measure the organisms and the biomass conversion factors from Nozais et al. (2004), Hillebrand et al. (1999), and Menden-Deuer and Lessard (2000) to estimate their biomass from the photographs.

I measured the length and width of a subsample of organisms (chosen by where they happened to fall on the filter) from each core to save time rather than measuring every individual, I calculated the biomass of each organism, then took the average biomass for that type of organism in that core and multiplied it by the count per volume of that organism in that core to obtain the total biomass (μg carbon) per volume (mL) for each type of organism in each core. I converted biomass per volume to biomass per cm^2 of 3 cm sediment depth as this is the standard way of reporting meiofauna biomass. I assigned organisms to nineteen broad taxonomic categories, based on their typical abundance in the meiofauna (such as nematodes)

and my ability to correctly identify them. These categories were: Amphipoda, Isopoda, Copepoda, Cumacea, Tanaidacia, nauplius larva, other Arthropoda, Gastrotrichia, Gnathostomulida, Kinoryncha, Nematoda, Nemertea, Oligochaeteae, Polychaeteae, Priapulidae, Turbellaria, other invertebrate, Foraminifera, and other Protista (Fig. 2).

Sediment characteristics, especially grain size, influence the composition of meiofaunal communities (Giere 2009) therefore, it was necessary to analyze them, to ensure they are not a confounding factor. I took an approximately 1 mL subsample from randomly selected cores at three distances from each whale (0 m, 3 – 7 m, and greater than 7 m) and obtained a distribution of grain sizes for each core using the laser particle size analyzer at MLML. I calculated the percent of grains in each size category rather than using the count, so that comparisons could be made among the cores although the volumes of subsamples may not have been equal. I assumed cores at the same site and the same distance would have the same particle size distributions as other cores at that site and distance.

Results

No significant difference was found in particle sizes among sites or among cores within sites (Fig. 3). This supports the assumption that differences in meiofauna were affected by presence of the whale, or other factors such as depth and oxygen, and not by sediment characteristics.

Total meiofauna biomass was greatest at an intermediate distance from the carcass at two of the three sites. The meiofauna biomass density for the two shallower sites had a general negative trend with greater distance from the carcass, with a maximum at 3 – 7 m distance; the relationship between biomass and distance from the carcass at the deepest site was weaker and had the greatest value at the 0 m distance (Fig. 4). Biomass was greatest 3 – 7 m from the bones

at the two newer, shallower sites. The strength of the relationship between biomass and distance from whale carcass correlated well with age and depth of the carcass, with the 633-m site having the greatest difference between its least and greatest biomass values. The 2,893-m site had only a weak, positive relationship between meiofauna biomass and proximity to the carcass. At the two shallower, newer sites, the meiofauna biomass was less in samples near the carcass than at an intermediate distance, which is counterintuitive if one subscribes to the halo-like distribution of biomass in response to local enrichment. It appears that another factor caused the biomass to be less at 0 – 3 m distance than it would be otherwise. The 2,893-m site did not have this 0 – 3 m biomass suppression. The biomass versus distance relationship for the 1,019-m site was intermediate between the other two sites, meaning depth and age of the carcass may have had an effect on the distribution of meiofauna biomass.

Nematodes and forams were the most numerous meiofauna in all cores, but annelids were the largest group in terms of biomass in most of the cores from the 633-m site due to their large bodies, and other protists (besides forams) accounted for a small share of total biomass due in part to their smaller size (Fig. 5). At the 633-m site, biomass of all taxa at 0 m was minimal compared with further distances. More annelid biomass was found at 3 – 7 m distance than at the 0-m or >7-m distances. There was little crustacean biomass at < 7 m. No clear trends among sites existed for biomasses of nematodes or forams but fewer annelids and more arthropods occurred at the 1,019-m site than the 633-m site. The biomass of nematodes at 1,019 m was similar in the 3 – 18-m cores and minimal in the 0-m cores. Foram biomass did not follow the general trend of lesser biomass at 0 m distances.

The distribution of meiofauna at the 2,893-m site was more similar to the 1,019-m site than the 633 m site. Total meiofauna biomass at all distances from the carcass at 2,893-m was

less than the other two sites. The 2,893-m site had the weakest relationship between distance from the carcass and biomass. Whereas the 1,019-m and 633-m sites had a large difference between the 0-m and 3-m samples, there was only a small difference between the biomasses at 0 m and 3 m at the 2,893-m site, although meiofauna biomass was greatest at 3 m from the carcass, which is consistent with the other two sites. This was largely driven by nematodes, crustaceans, and other arthropods. Crustaceans in particular were more abundant in the 0 – 3 m distance cores at the 2,893-m site in comparison with the other sites. The biomass of forams versus distance had no consistent trend among sites.

To quantify diversity, I made a table of the count per sediment area for each distance at each site (Table 2). "Diversity" is a broad concept and can be analyzed using many different methods (Whittaker 1972, Legendre 2005). I have chosen to examine alpha diversity, beta diversity, and evenness to get a reasonably comprehensive view of how distance from the carcass affected different aspects of meiofauna diversity, at least at a broad taxonomic level.

Alpha diversity is the variance in how many and which species of organisms are present within a site and beta diversity is this variance or species turnover along a habitat gradient or among sites (Whittaker 1972). I used the Shannon-Weiner index for alpha diversity because it does not give too much weight to dominant species as does Simpson's index (Whittaker 1972). I calculated the Shannon-Weiner Diversity Function using the formula from Krebs (1999): $H' = \sum(p_i \log_2 p_i)$ where p_i = percent number of each taxonomic group for each distance within each site (Fig. 6). The relationship between distance from the carcass and diversity was similar to the relationship with biomass: lesser values at 0 m distance and greater values at 3 m. Unlike biomass, the strength of the relationship was about the same at all three sites, and was weak. The relationships between diversity and distance were similar among all three sites, unlike biomass.

This makes sense given the taxonomic categories I was able to identify were broad, and most meiofauna habitats could be expected to have representatives of most of these broad groups. I may have found differences in diversity among the sites if I had been able to identify the organisms at the genus or species level.

I performed principal components analysis (PCA) using the PRIMER software application to determine beta diversity. I chose PCA over other multivariate analyses because it clearly and easily displays the contributions that each group made to the differences among the samples. I made plots based on square root transformed biomass (Fig. 7a) and square root transformed counts (abundance) (Fig. 7b). The data were transformed by square root to prevent the most abundant species from overwhelming the signal of rare species (Legendre and Gallagher 2001). Neither the sites nor the distances formed distinct clusters. The values for 3 – 20 m distances at the 2,893-m site were the most tightly clustered group for both biomass and abundance, but the 0-m data point was far from the rest, mainly due to it being more dominated by nematodes than the others. The clusters for the 1,019-m and 633-m sites overlapped, although there were some differences. The 633-m site had greater foram abundance, especially for the 13-m and 18-m distances, and in terms of biomass the 1,019-m site was somewhat more nematode-dominated, and the sediments at 3 m, 13 m and 18 m distances from the 633-m site were most heavily dominated by polychaetes and oligochaetes.

Evenness is another characteristic of biological communities that can be quantified. Whereas diversity represents which taxa are present, evenness indicates the degree to which one or a few taxa are dominant (Whittaker 1972). Two samples could have the same number of species present but one could have 90% of its organisms belong to a single species and the other have equal numbers from each taxon; the evenness of the first sample would be less than the

evenness of the second. The greatest possible value of evenness occurs when there is an equal number of individuals from every taxon. I used Simpson's Measure of Evenness from Krebs (1999): E = (1/D)/s, where D = Simpson's Diversity Index ($D = \sum p_i^2$) and s = the total number of taxa in the sample (Fig. 8). As with alpha diversity, there was not a clear difference among the sites or a strong trend versus distance. The 633-m site had the greatest variation in evenness. The 1,019-m and 2,893-m sites had a general trend of increasing evenness with increasing distance from the carcass.

Some taxa were present at every site and most distances and others were only found in a few of the sediment cores (Table 2). Nematodes, forams, and other protists were present in every sediment core, and annelids were present in most. Some taxa were not found at every site or every distance: there were no tanaids in the cores from the 633-m site. Nemerteans were only found at 7 m distance or greater. Tanaids were only found at distances 3 – 7.5 m and were only found at the 1,019-m and 2,893-m sites. The probability of finding large-bodied, less ubiquitous organisms in a given sediment core is less than that of finding a more common organism, such as nematodes, therefore it was more problematic to get a reasonable estimate of their total biomass and abundance. If it had been feasible to sample a greater volume of sediment and to identify all of the organisms to a finer taxonomic level, I may have been able to estimate diversity more accurately and a clearer pattern may have emerged.

Discussion

Nutrient enriched marine habitats often exhibit greater biomass and diversity overall than non-enriched sites, with lesser biomass and diversity at the center of the site (under the bone or at the fissure leaking gas) and greater diversity a few meters from the center of the site (Montagna et al. 1989; Buck and Barry 1998; Van Gaever et al. 2009a). One possible reason for the reduced

diversity is a few taxa may colonize a whale-fall early and establish competitive dominance, then competition may decrease the diversity of organisms under and near the carcass although the total abundance of organisms could be great. In addition to the effect of competition, it is possible that chemicals that are at greater concentration under the carcass may be harmful to meiofaunal organisms. Whale-falls, like cold seeps, have greater concentrations of sulfide than the surrounding sediment and are inhabited by mats of bacteria, such as the Beggiatoaceae, that require hydrogen sulfide (Bernhard et al. 2004). Sulfide is toxic to most eukaryotes and an environment with a high sulfide concentration is expected have less meiofauna diversity because only the organisms adapted to tolerate sulfide would survive. Pesticides are another potential factor and may be found at whale-falls, but not seeps or vents. Pesticides bioaccumulate in large mammals such as whales (O'Shea and Tanabe, 2003) and these pesticides might be found at greater concentration near the carcass, whereas at a short distance from the carcass there would still be some nutrient enrichment but a lesser concentration of toxins such as organochlorines. Different species of meiofauna likely have varying resistance to pesticides and if this is the case it would further decrease diversity near the carcass. I did not obtain data on organochloride or other pesticide concentrations in the sediment, so the effect of pesticide on whale-fall meiofauna remains open to speculation.

Some organisms survive in a sulfide-rich environment despite its toxicity. One possible mechanism for this survival is bacterial symbiotes that oxidize sulfide and mitigate the harm to their host. Nematodes are the dominant group in meiofauna communities in many habitats (Montagna et al., 1989; Buck and Barry, 1998; Robinson et al., 2004; Van Gaever et al., 2009a) and they also were numerically the most abundant group in most of my samples. Some nematodes have thiophilic, sulfide-oxidizing bacteria as ecto- or endosymbionts (Nussbaumer et

al. 2004, Musat et al. 2007). I did not investigate whether the nematodes in my samples had these symbiotes but if those in the 0-m cores did, where sulfide concentration is expected to be greatest, that would explain why the nematodes were able to survive in that sediment where most meiofauna did not.

I did not collect data on the habitat characteristics that caused the differences in the meiofauna communities along the distance gradient and among the three sites, apart from the age and depth of each site, but others have collected data that are useful, particularly Goffredi et al. 2008 and Treude et al. 2009, whose figures I will refer to below. Nutrient enrichment is likely to be a major factor, supporting the greater meiofauna biomass I found 3 – 7 m from the carcass (although this alone does not explain why biomass was less at 0 m). Enrichment is greatest at 0 m from a whale carcass (Goffredi et al. 2008: Fig. 3) and declines roughly linearly with distance from the carcass, therefore, something may be suppressing the organisms immediately under the bones or driving the abundance and biomass greater at intermediate distances, or both. Bacteria are prey for many meiofauna, so their distribution around the whale likely had an effect on meiofauna distribution. Goffredi and Orphan (2010) reported bacteria, as measured by protease activity, at the 2,893-m site were most abundant under the whale and steadily decreased with increasing distance from the whale. They also showed that bacteria at 0 m distance were less diverse although there were more of them (greater abundance). Particulate organic carbon (POC) is another indicator of overall food availability for meiofauna. Goffredi et al. (2008) reported POC, similar to the pattern of bacteria activity, was greatest at 0 m distance and decreased linearly. This linear decrease the food for many meiofauna would explain the pattern from 3 m and further, but food limitation does not explain the lesser biomass at 0 m as there is plenty of food at 0 m distance.

Chemical gradients in the sediment under a whale carcass, such as sulfide and low pH, limit meiofauna's ability to live directly under the carcass but do not spread as far as the halo of nutrients. Treude et al. (2009) reported gradients of several chemicals that may affect meiofauna abundance, at a whale-fall at 1,675 m depth in the Santa Cruz basin. pH changes with depth of sediment over a range of 7.4 to 7.6 within the top 3 cm; pH is greater at the sediment surface and decreases to a minimum of 7.4 to 7.5 at 3 cm depth (Treude et al. 2009: Fig. 3). There is not a clear trend in pH with distance from the whale but the lowest pH of 7.4 was found at 0 m distance. Oxygen concentration decreases to almost zero within the top 3 cm of sediment and the decrease is sharper near the whale and more gradual far from the whale (Treude et al. 2009: Fig. 3). Sulfide accumulates around the whale carcass as a result of increased bacteria metabolism in the sediment and suppresses the growth of meiofauna (Fenchel & Reidell 1970, Powell et al. 1980). Treude et al. (2009) reported the amount of sulfate reduction, and concentrations of methane, sulfate, and sulfide, the percent dry weight of sulfur, nitrogen, and total organic carbon, and acridine orange direct counts, a measure of bacteria abundance; all of these are shown in Treude et al. 2009: Fig. 4. Sulfate reduction was greatest under a carcass, and sharply decreased about 1 m away. Methane was greatest under a carcass and less at distances of 1 – 9 m. Sulfate was similar at all distances. Sulfide was greater at 0 m than 1 m, but there were no data for further distances. Sulfur was greatest under the carcass then decreased. Nitrogen was greatest 0 – 0.5 m then decreased but the difference was small, 0.5 to 1.1% dry weight. Total organic carbon was greatest for 0 – 0.5 m distance then decreased, which was the same pattern as Goffredi et al.'s (2008) results for particulate organic carbon. Acridine orange direct count was greatest at 0 to 1 m distance (there are no data for 0.5 m) then decreased. The lesser meiofauna biomass in the 0-m cores is similar to the pattern observed at cold seeps, where

sulfide toxicity prevents most organisms from surviving at the seep but there are abundant organisms near the seep where the deleterious sulfide concentration is less (Buck et al. 2004, Buck et al. 2013. The data from Goffredi et al. (2008) and Treude et al. (2009) support my assumption that the halo of carbon in the sediment is more widely spread around the carcass than that of sulfide, which is found at approximately 100-fold greater concentration less 0 – 0.5 m from the carcass than at further distances (Treude et al. 2009: Fig. 4). Sulfide toxicity was likely to be the most important chemical factor decreasing meiofauna biomass under the whale carcass.

Predation, by larger organisms that are attracted to the food and shelter provided by the whale-fall habitat, is another potential suppressor of biomass near the carcass (Smith et al. 1989). Even in the later stages when there was no flesh remaining, the bones may provide shelter and attract prey organisms to the site: the bones' physical structure affects the flow of seawater and acts as a drift fence, concentrating objects and particles that would otherwise be swept away (Smith et al. 1989).

The effects of depth, age, and size of the carcasses also should be considered when examining differences among the three sites. Oxygen concentration and depth influence which meiofauna species will be present (Giere 2009), and the three sites in my study span a depth range of 2,260 m, with the two shallower sites in the oxygen minimum zone. Low oxygen conditions are favorable for anaerobic, sulfate-reducing bacteria that increase the concentration of hydrogen sulfide in the sediment at the center of a whale-fall or cold seep (Treude et al. 2009, Buck et al. 2013); this may explain why the difference between the 0-m and 3-m cores was greatest at the 633-m site, which was in the oxygen minimum zone and had the least concentration of oxygen in the seawater among the three sites (Table 1).

The striking difference between the 2,893-m site and the two shallower, newer sites was the absence of intact large bones, which may be another important factor. The relationship between biomass and distance from the carcass was weakest at this oldest and deepest site, which was to be expected because the bones had degraded and the site is gradually returning to the state it was in before the carcass was present. Few bone pieces remained at this site but a darkly stained spot on the seafloor was still visible (Lundsten et al. 2010). The relationships of biomass versus distance from carcass for the two shallower, newer sites were similar to each other. The 633-m site had the greatest maximum biomass values and the strongest effect of distance on biomass, though it is unknown whether that is the result of depth, age of the carcass, or another trait that differed among the sites but could not be teased apart. If the trends in biomass and diversity are driven primarily by chemical gradients in the sediment then it is likely age of the carcass that would be a significant factor because the gradients would take time to accumulate and then diminish after the carcass has completely decayed away; time series data would be necessary to test that hypothesis.

A possible confounding factor for one or more of my sites is sediment transport in the canyon. The forams at the 2,898-m site included some forams that live at shallower depths, which demonstrated that sediment had been transported from shallower parts of the canyon into the site (Mary McGann, personal communication). Therefore, the results from this site may have been influenced by input of sediment from higher in the canyon. Landslides in submarine canyons bring sediment from the canyon wall to deeper sites (Lee 2005). This process introduced noise that could obscure differences among cores at that site, although the cores should all have been affected equally by introduced sediment. If sediment transport happens often and meiofauna are buried under deeper, hypoxic sediment, this could be the cause of the

overall lesser meiofauna biomass at the 2,893-m site, although the age of the carcass also was likely a factor.

The sample sizes for this project were small, as is unfortunately the case for many projects involving deep-sea sediments, due to the expense and complexity of collecting samples from the deep seafloor. Obtaining a broad overview of the meiofauna communities at whale-falls is a necessary first step but there is still a lot of work to be done before we have a thorough understanding of this system. I am confident that I have a good estimate of the abundance and biomass of the more common taxa, nematodes and forams, but I may not have sampled enough sediment to provide accurate information about the less common taxa, whose distribution may be so sparse that they could be present but missed unless a larger area of sediment is collected. I found no cumaceans at the 2,893-m site, and I found kinorynchs only at the 633-m site. I cannot tell whether these are accurate representations of the actual abundance of these taxa; it is possible they are present at the other sites but their abundance was so minimal that my sediment cores did not happen to catch one. The value of this project was determining the effect of the whale carcass on total biomass. It is less clear what effect the carcass had on diversity and more work needs to be done, both with more sediment cores and more specific taxonomic identification. It also would be useful to examine the meiofauna at more whale-fall sites, ideally with multiple whales at the same depth and multiple carcasses of the same age, species and approximate size at different depths, in addition to measurements of chemical parameters that may confer habitat quality such as sulfides, pH, oxygen, and pesticides.

Conclusion

Meiofauna are a substantial component of the ocean's ecosystems, and they have only recently been studied extensively. Meiofauna are a critical and easily overlooked part of the

carbon and nitrogen cycles, and they are part of the larger food web including bacteria and microalgae, on which they graze, and larger organisms that prey on them.

The study of organisms inhabiting whale-falls highlights another aspect of whales' importance to the ocean ecosystem that is not widely known. No other organism has such robust, oily bones that form the whale-fall habitat for most of its duration. Whale-fall macrofauna may not depend on whales, for example *Osedax* can live on other types of bones (Rouse et al. 2011), but no other organism besides whales has such a large skeleton that would remain intact on the seafloor for as long or provide a sudden pulse of so much carbon to the seafloor. In the absence of large marine vertebrate bones, whale-fall community organisms may not go extinct but their distribution would be affected. The depopulation or extinction of whales also might impact other chemosynthetic communities, if whale-falls provide stepping stones for dispersal of organisms among seepage sites. It is possible there is a threshold density of whale-falls below which they can no longer adequately serve this function. The first publication on organisms living on whale-fall was in 1989, after whaling had severely depleted the numbers of large whales. Collecting more data over time as whale populations recover or decline would make it possible to determine the effects of whale population size on whale-fall communities. Deep-sea chemosynthetic communities are so different from the familiar communities of terrestrial ecosystems that humans inhabit that they appear almost alien to us. It is important both to avoid damaging these communities by human activity and to improve our understanding of them to better understand the diversity of life.

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Table 1: The three sites where samples were collected for this project.

depth	location	species	carcass length	implanted or discovered	start date	date sampled	age of carcass (months)	average seawater $[O_2]$	push cores collected and their distances from the carcass
633m	36.802°N -121.994°W	gray whale (*Eschrichtius robustus*)	10m	implanted	11 April 2007	5 June 2011	50	0.4129	2 (0m), 2 (3m), 2 (7m), 2 (13m), 1 (18m)
1,019m	36.772°N -122.083°W	blue whale (*Balaenoptera musculus*)	17m	implanted	5 October 2005	2 June 2011	68	1.4532	2 (0m), 3 (3m), 2 (7.5m), 1 (15m), 1 (18m)
2,893m	36.613°N -122.434°W	gray whale (*Eschrichtius robustus*)	10m	discovered	6 February 2002	3 June 2011	112	2.2381	2 (0m), 2 (3m), 2(6m), 2 (15m), 1 (20m)

Table 2: Count of individual organisms per sediment area (cm^2), divided into taxonomic groups. Blank indicate no individuals of that group were found in the core(s) at that site and distance. This does not necessarily mean these taxa are absent entirely from these locations, only that my sampling did not collect any.

distance:	0m			3m			6m	7m	7.5m	13m	15m		18m		20m
depth:	633m	1,019m	2,893m	633m	1,019m	2,893m	2,893m	633m	1,019m	633m	1,019m	2,893m	633m	1,019m	2,893m
amphipod	2.28		5.55			2.04		4.15		2.63		2.63	11.82		2.07
copepod		2.63	7.35		6.57	1.44			5.25	3.94			7.88		2.07
cumacean		1.31						4.15	2.63						
foram	276.41	290.19	111.46	154.77	157.57	181.33	205.49	133.81	209.43	472.70	66.97	236.35	301.35	100.45	184.64
gastrotrich		3.94	2.39	0.93		1.44	1.31					7.88			
gnathostomulid							3.94		6.57						
isopod		1.31				1.76		3.11		2.63					
kinorinch				0.93											
nauplius									1.31						
nematode	68.53	28.89	571.06	218.17	270.93	192.87	127.37	139.00	185.80	168.07	207.46	116.86	265.89	240.95	130.70
nemertean								1.04				3.94			2.07
oligochaete	14.85	5.25	10.45	51.28	15.76	1.44	11.82		1.31	15.76		10.50	9.85	1.97	
other arthropod			1.31		1.31	1.76	1.31		1.31	1.31	7.88	5.25		1.31	
other invertebrate			1.31												
polychaete			7.35	35.43	20.35	13.00	4.60		3.28	9.19	1.31	15.76	13.79		7.26
priapulid						0.72									
protist	50.26	73.53	188.88	93.23	182.51	65.41	22.32	45.64	140.50	90.60	34.14	15.76	43.33	30.20	32.16
tanaid					1.31	0.72			2.63						
turbellaria			4.78		1.31	1.44	1.31					1.31	0.98		

Figure 1: Locations of the three sites sampled for this project in the Monterey canyon and Soquel canyon, labeled by their depths.

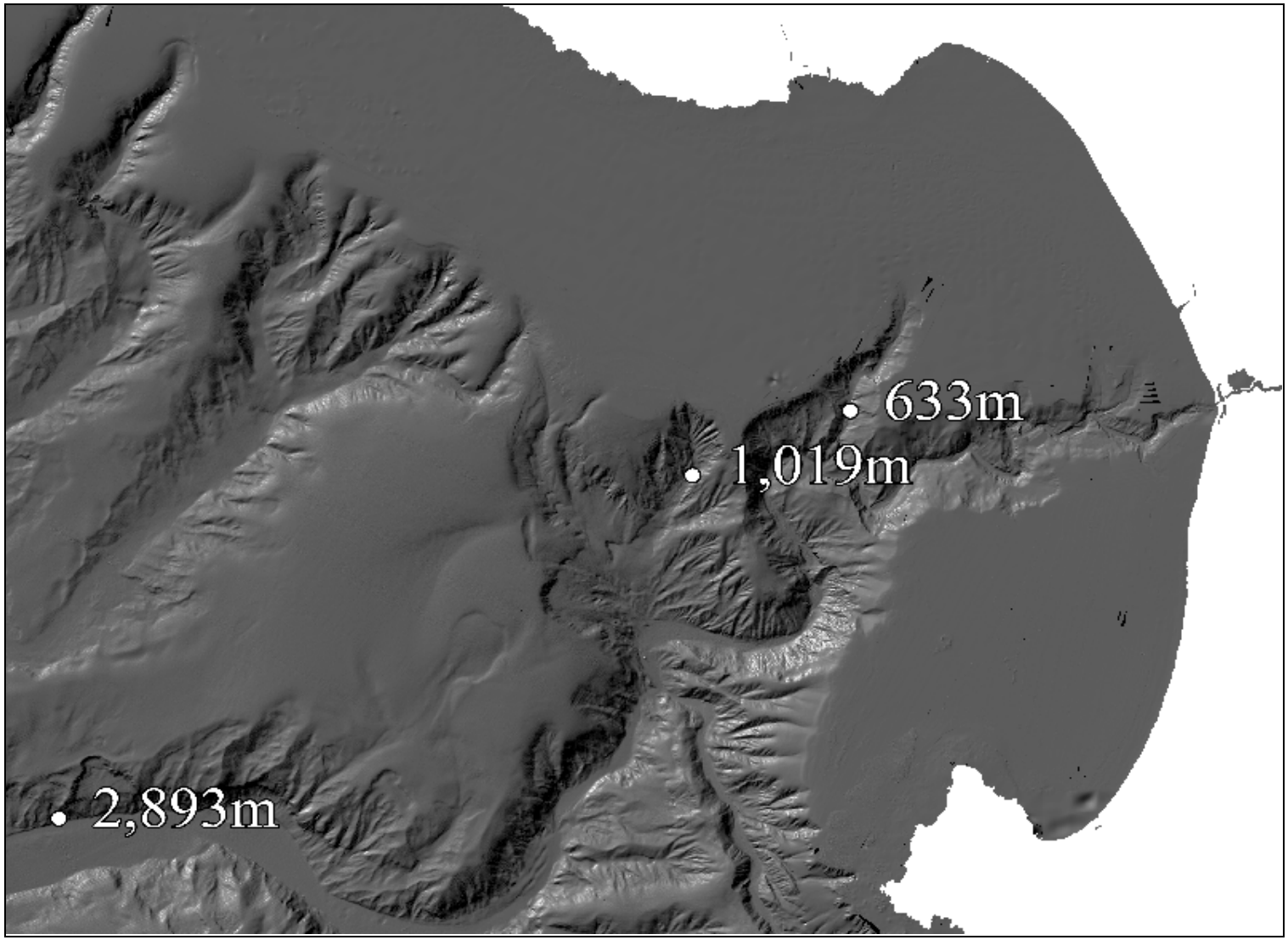


Figure 2: Examples of 16 of the 19 taxonomic groups the meiofauna were sorted into. (a) amphipods, (b) copepods, (c) cumaceans, (d) forams, (e) gastrotrichs, (f) gnathostomulids, (g) isopods, (h) kinorynchs, (i) nauplii, (j) nemerteans, (k) nematodes, (l) oligochaetes, (m) polychaetes, (n) priapulids, (o) tanaids, (p) turbellarians. Not pictured are "other arthropods," "other invertebrates," and "other protists," as they were more variable in appearance.

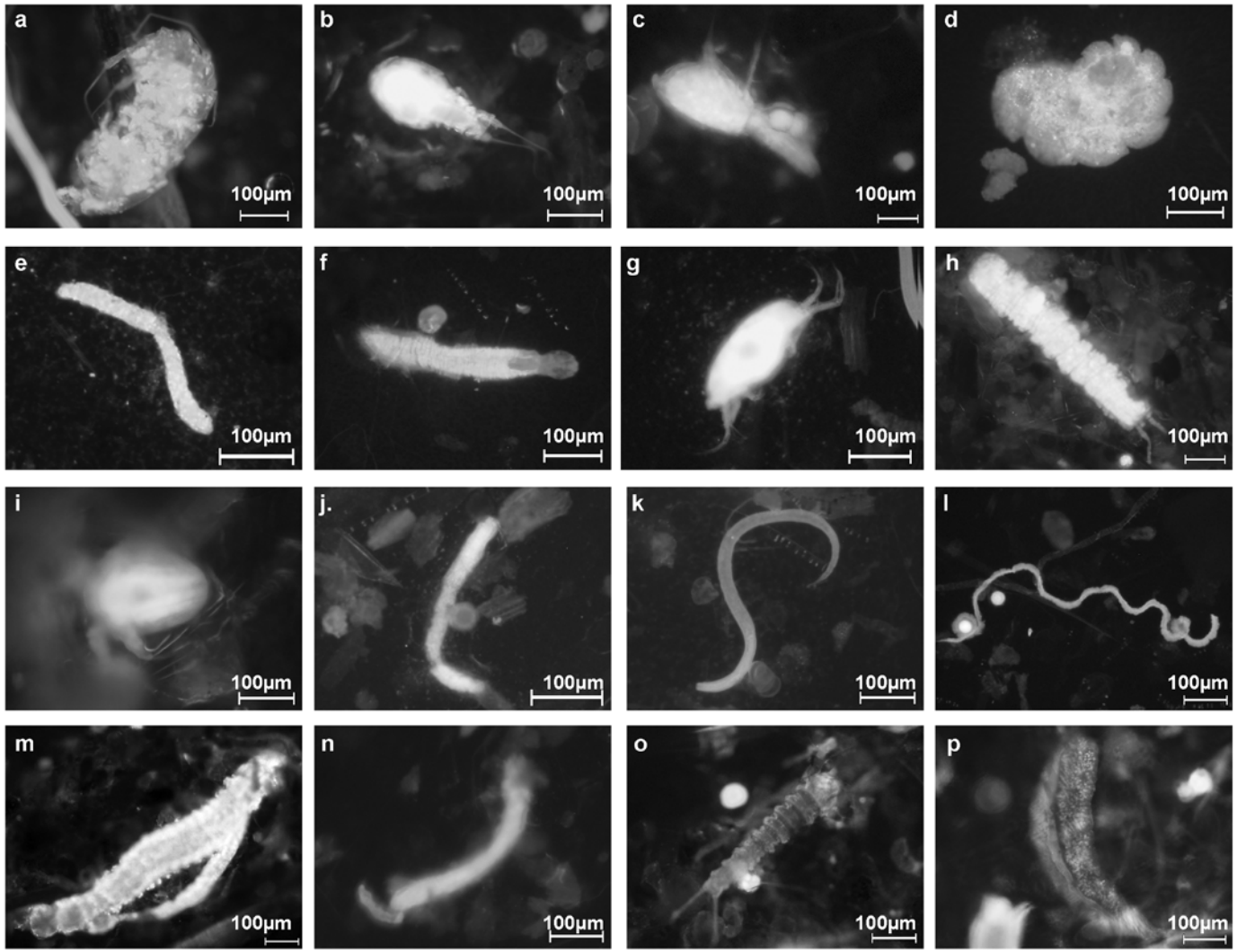


Figure 3 : Distributions of average sediment grain sizes for the three sites, labeled by their depths. The size distributions are similar enough that it is reasonable to assume any difference in the meiofauna communities among the sites is not due to differences in sediment grain sizes.

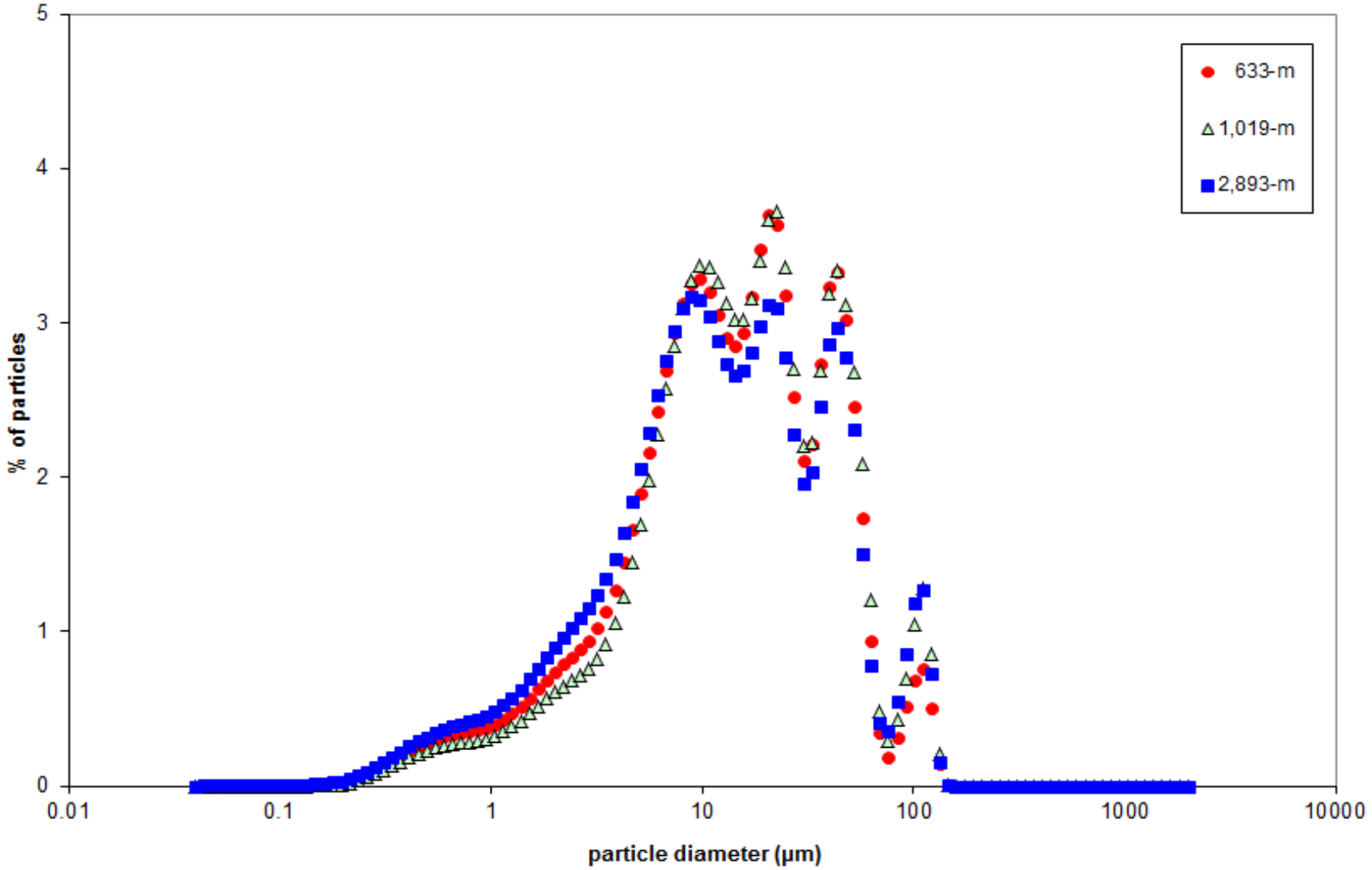


Figure 4: Distribution of total meiofauna biomass (µgC) per area (cm^2) of sediment versus distance (meters) from the carcass for the three sites, labeled by their depths. The 633-m site is also the newest, having been on the seafloor for 50 months when it was sampled. The 1,019-m site was 68 months old and the 2,893-m site was at least 112 months old (the exact age is unknown because this whale was discovered already on the seafloor whereas the other two were implanted by MBARI).

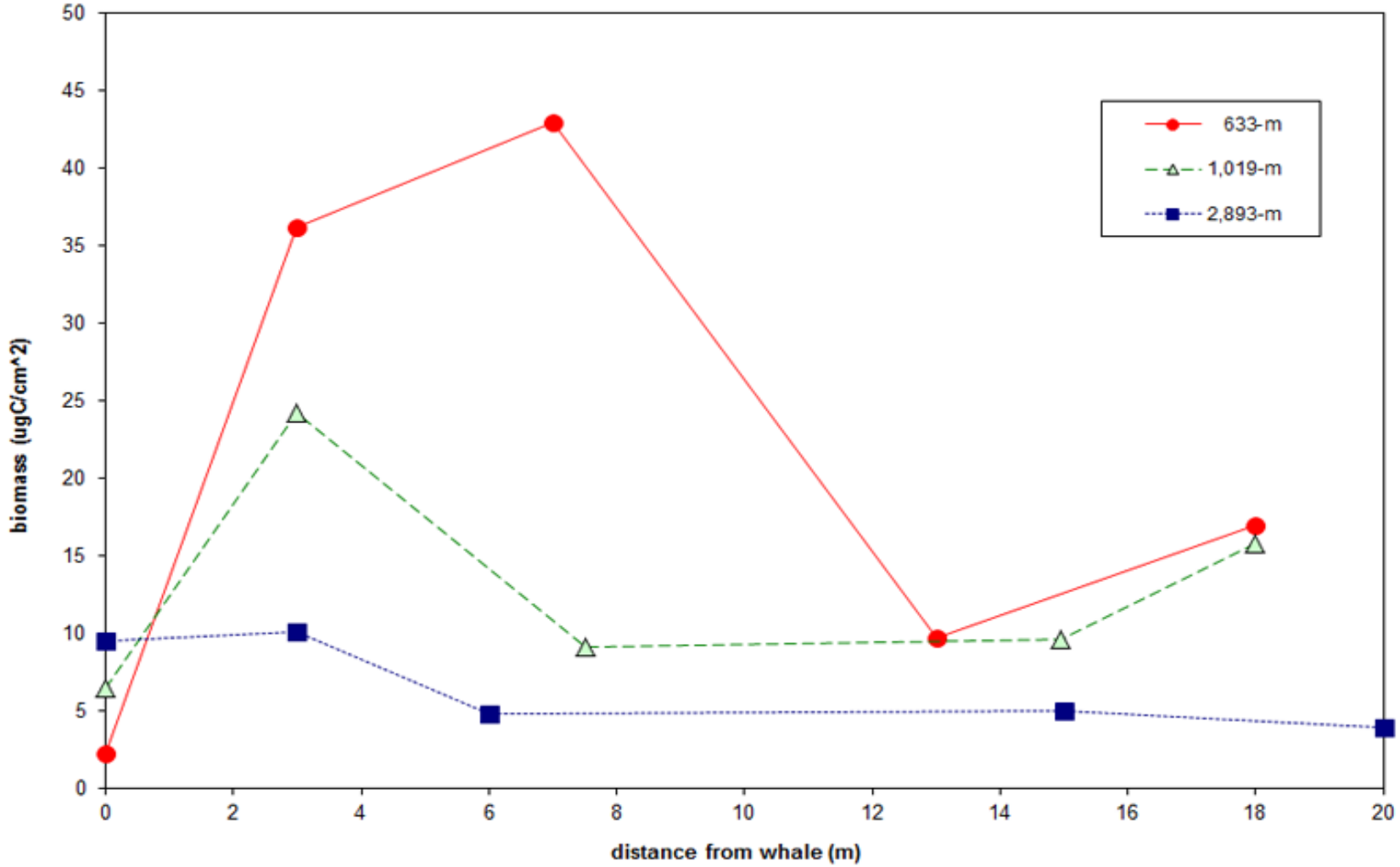
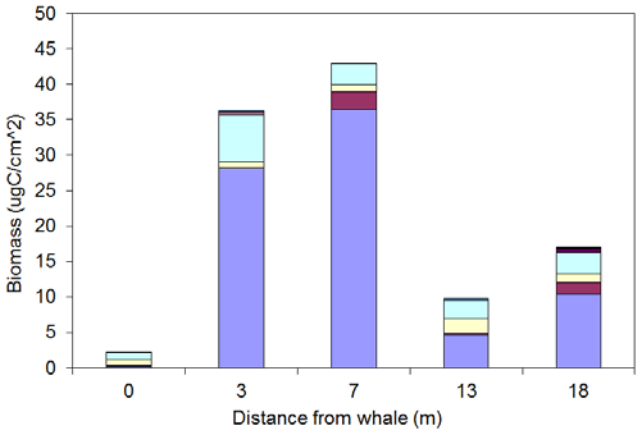
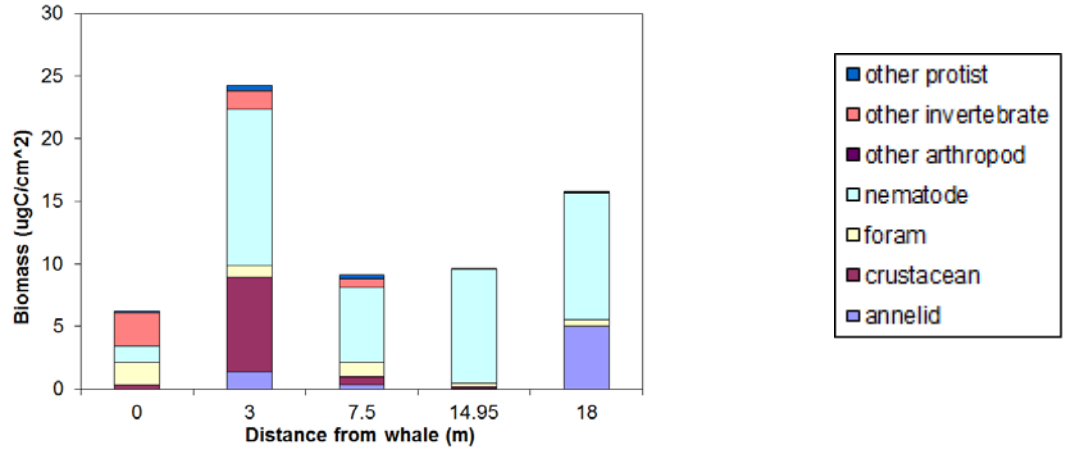


Figure 5: Meiofauna community composition by biomass of broad taxonomic categories in (a) the 633-m site, (b) the 1,019-m site, and (c) the 2,893-m site.

a. 633-m site



b. 1,019-m site



c. 2,893-m site

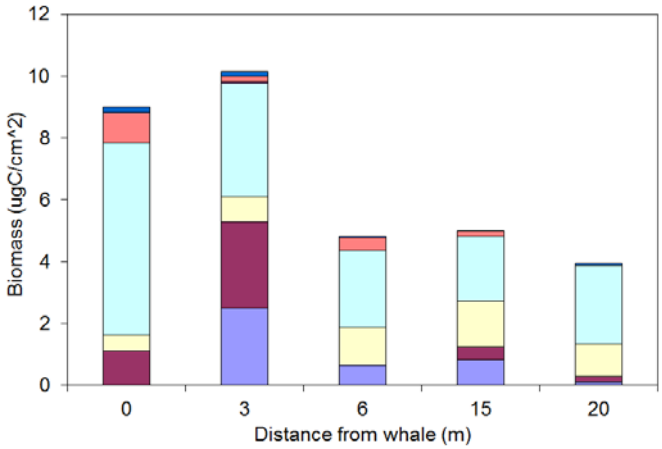


Figure 6: Alpha diversity (Shannon-Weiner diversity index) for the three sites, labeled by their depths.

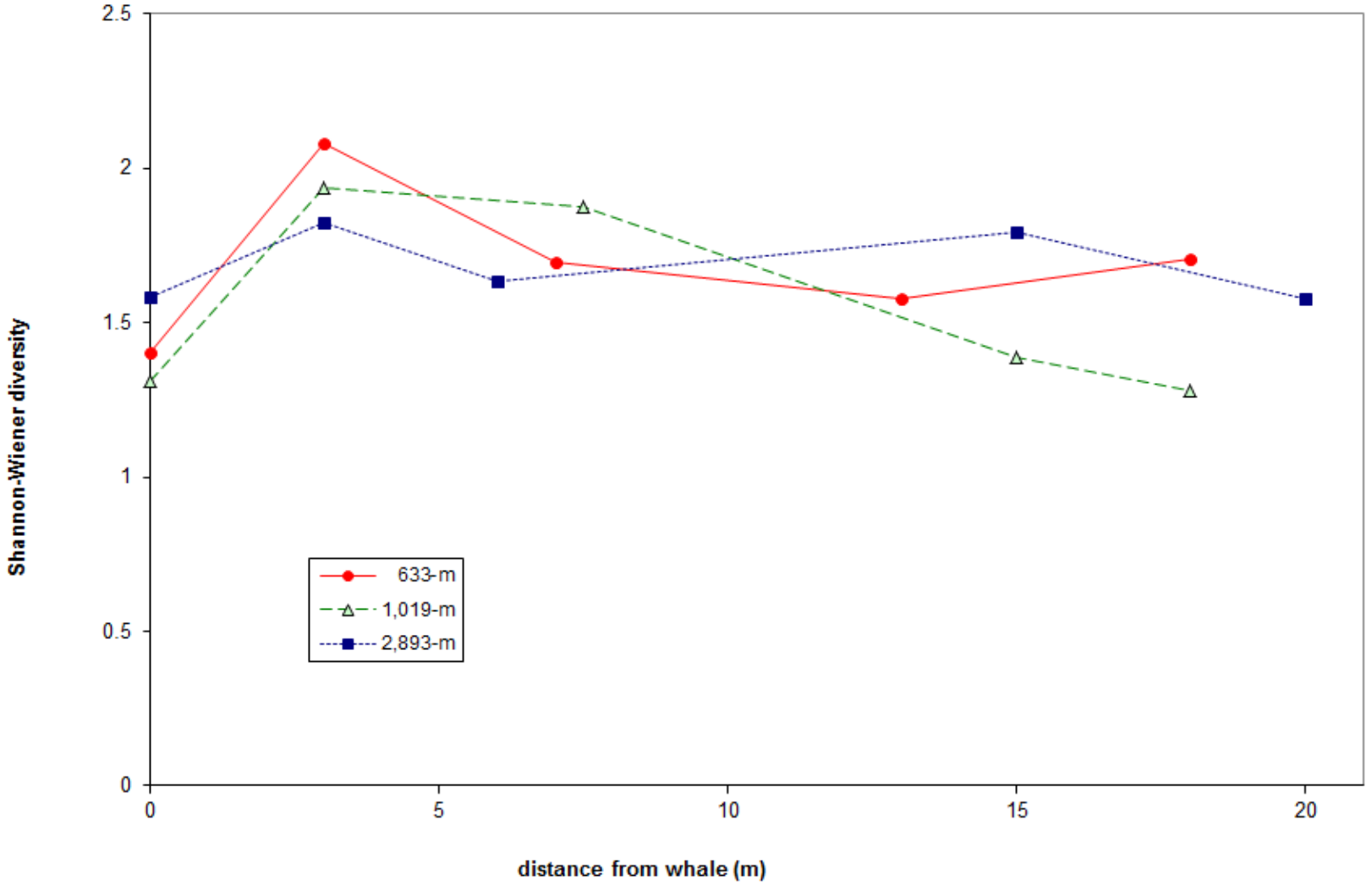
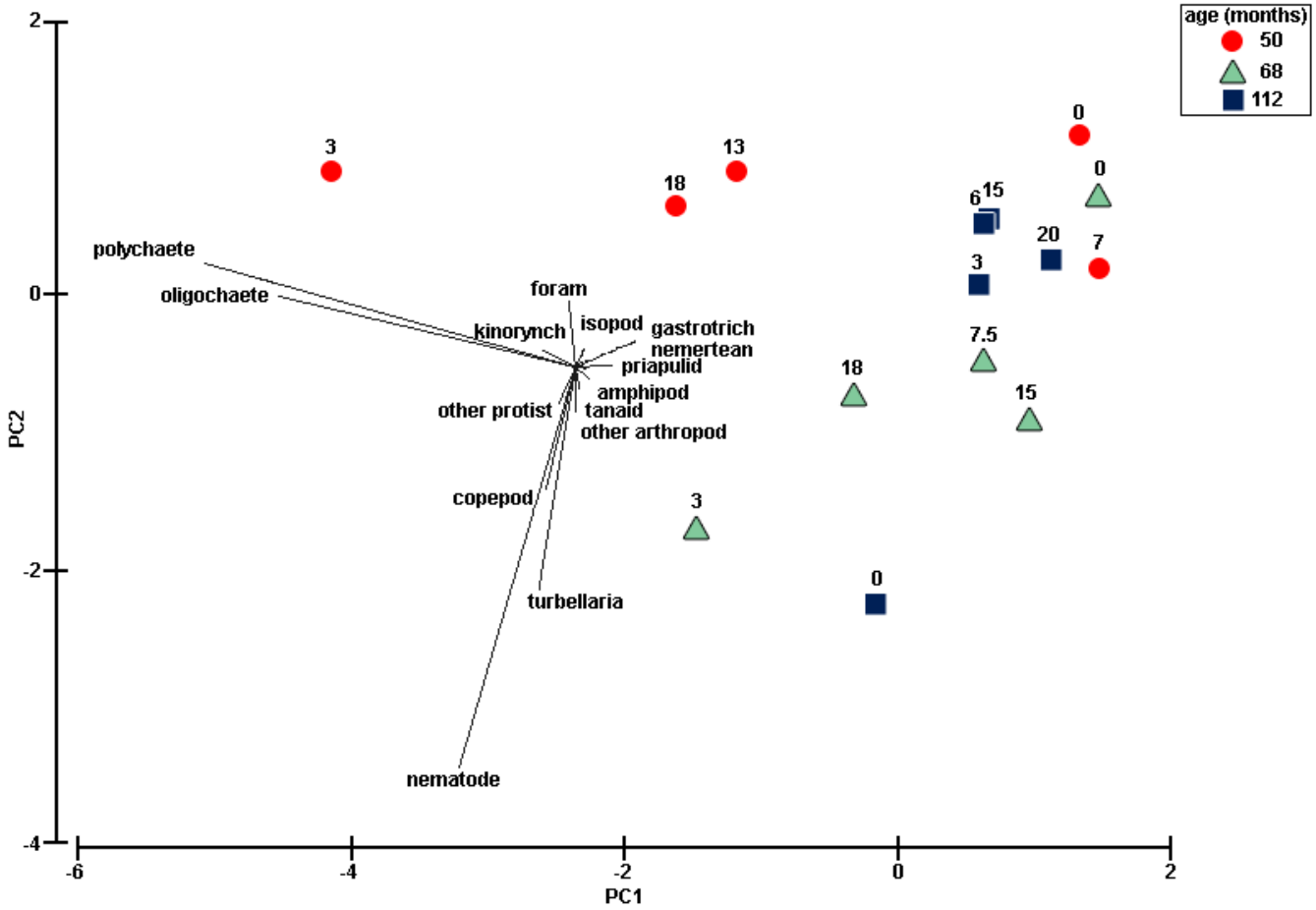


Figure 7: PCA on (a) square root transformed biomass and (b) square root transformed counts of meiofauna per sediment area. Each shape/color represents a different site: the 50-month/633-m depth site, the 68-month/1,019-m depth site, and the 112-month/2,893-m depth site. The number labels on the points are the distances (meters) from the carcass. The factors are the taxonomic groups (groups that contributed the least to either PC1 or PC2 were not labeled, for readability). The plot for counts (b) was generated using the data in Table 2 and the plot for biomass was generated using the same matrix but with biomass per sediment (µg C per cm^2) in place of counts.

a.



b.

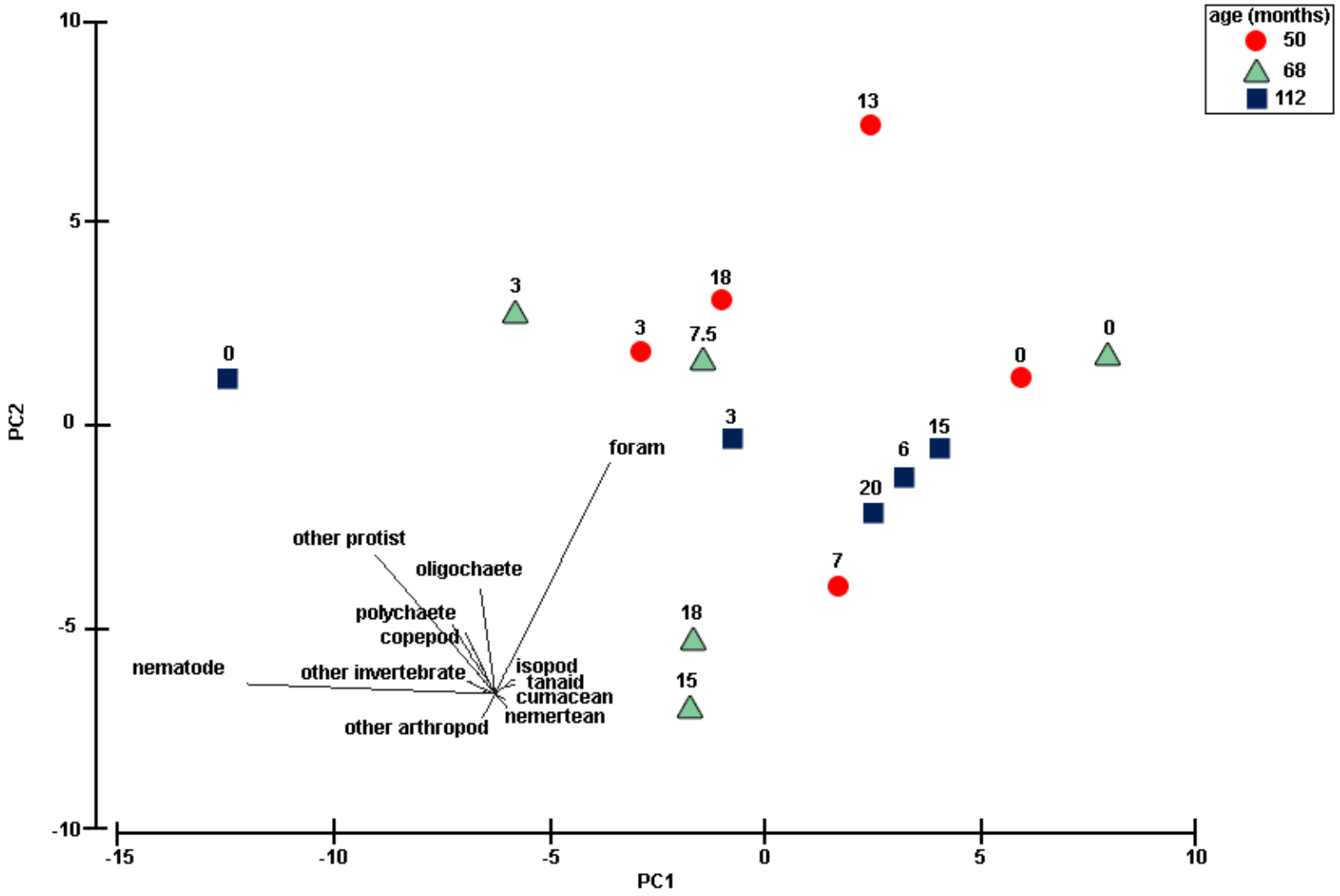

age (months)
50
68
112
PC2
PC1
10
5
0
-5
-10
-15
-10
-5
0
5
10
13
18
3
3
7.5
0
0
0
3
15
6
20
7
18
15
0
foram
other protist
oligochaete
polychaete
copepod
nematode
other invertebrate
isopod
tanaid
cumacean
nemertean
other arthropod

Figure 8: Simpson's evenness for the three sites labeled by their depth (meters), using the formula E = (1/D)/s, where D = Simpson's Diversity Index ($D = \sum p_i^2$) and s = the total number of taxa in the sample, calculated using the data in Table 2.