

Vandepas, Lauren E
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Comparative Meiofaunal Diversity of Three Sandy Areas in Eagle Cove

Abstract

Meiofauna are a diverse grouping of animals less than 2mm in size that live on and between sediment grains in all oceans at all depths. Though many studies have centered on the identification and description of species within particular meiofauna assemblages, few have focused on community structure or biodiversity within this interstitial ecosystem. San Juan Island, Washington, is surrounded by nutrient-rich waters that support high seasonal productivity. Novel species have been described from meiofaunal communities on and around San Juan Island, but whole meiofaunal community structures have not previously been examined in detail. This study compares the meiofaunal communities of three distinct areas on Eagle Cove beach, each of which is exposed to different physical conditions, particularly in the form of exposure to varying intensities of wave action. There are notable differences in community structure, though nematodes make up the majority of the meiofaunal population. This study provides morphospecies classifications and abundances, as well as measures biodiversity (species richness and evenness) using the Shannon-Wiener Index. Though each sample site has differing proportions of taxa, the differences in Shannon-Wiener values were not statistically significant.

Introduction

“Meiofauna” is a collective term for microscopic animals that live between the grains of marine sediment at all ocean depths, from beaches to the deep sea. The meiofaunal community is very diverse, with over fifteen animal phyla represented (Bik et al 2012). Meiofaunal animals fulfill important roles as nutrient cyclers and trophic links between primary producers and larger secondary consumers (Pinkney et al 2003). These communities are sensitive to changes in water quality and chemistry caused by anthropogenic activities (Carman et al 2000), though few baseline population surveys have been conducted on unimpacted meiofaunal ecosystems. It is crucial to understand meiofaunal community structure and composition so that recovery can be monitored after damaging events such as oil spills, and exposure to chemical and fertilizer runoff (Carman and Todaro 1995; Carman et al 2000). It is also important to understand the eiofaunal community structure to analyze the impacts of longer-term stressors on the overall ecosystem attributed to global climate change (rising sea temperatures and decreasing seawater pH) (Worm et al 2006).

Since the members of the meiofaunal community are described as being between 60µm and 2mm in size, making it difficult to identify species based on morphology alone, the taxonomic diversity of this habitat has not been well characterized (Curini-Galletti et al 2012). One of the few extensive sampling surveys performed found over 400 species of meiofauna, with

27 being previously undescribed, over a two week sampling period in Sweden (Williams et al 2009), demonstrating how much is yet to be discovered in the sedimentary ecosystems around the world. Though meiofaunal biodiversity surveys have been conducted in the Mediterranean (Harriague and Albertelli 2011), Hong Kong (Liu et al 2011), eastern Atlantic (Williams et al 2009) and deep sea habitats (Denovaro et al 2010, Gollner et al 2010, Bik et al 2012), no studies have been published regarding the diversity of meiofaunal communities in Puget Sound.

Puget Sound is a region of high seasonal primary productivity, and the subsequent seasonal plankton blooms support fertile fisheries and aquaculture (Winter et al 1975; Beechie et al 2007). Puget Sound has also been predicted by the Census of Marine Life to have a large seafloor biomass relative to that of tropical regions, open ocean and several other temperate coastal systems such as Japan (Wei et al 2010). With several large metropolitan areas nearby, in addition to significant agricultural and aquaculture industries, ecosystems in Puget Sound are vulnerable to damage and alteration by human activities (Newton Van Voorhis 2002) though the impact of these on local meiofaunal communities is unknown. Novel species in the meiofauna have been identified and described in Puget Sound, but surveys of whole meiofaunal community compositions have not been published. Because there are no published meiofaunal community baseline surveys that have been performed in this region, it is difficult to ascertain changes in these communities in response to long-term stressors such as climate change or other more acute impacts from human activities such as agricultural runoff or pollution.

This study attempts to examine whole meiofaunal community composition of a sandy shore on San Juan Island. Sandy sediments were sampled from three physically distinct transects at Eagle Cove Beach, an intertidal beach on San Juan Island, on two days - one week apart. Meiofaunal community populations of these sites were compared by identifying the morphospecies present and comparing the proportions of taxa which comprise each of the populations, as well as the beach overall. It was surprising to find that the abundances and proportions of taxa at each sample site had changed dramatically over the course of one week.

Materials and Methods

Collection sites and initial processing:

Sediment was collected from Eagle Cove on 7 July, 2012 at low tide (-18.3cm) at 1:55PM and on 15 July, 2012 at low tide (-12.2cm) at 10:00AM from 3 areas on the beach: sandy area bordered by rocky outcrops exposed to harsh wave action, a completely exposed sandy beach subjected to harsh wave action, and a sheltered sandy area surrounded by rocky outcrops (Figure 1). The sediment collected was not exposed to air at low tide and was consistently submerged under at least 10cm of seawater, regardless of wave action. Collection of each sample took approximately 5 minutes. Top layers of sand (approximately 20cm) were collected using a shovel, with approximately 1L of sand collected per site. The sand was transported to Friday Harbor Laboratories for immediate analysis. After the initial screening (one initial screening of samples collected on 7 July and two initial screenings of samples collected on 15 July), during

which time the sand was kept in the collection bucket and moistened with seawater from the incurrent flow to the laboratory (which had been passed through an initial filter to remove large organisms and plankton) sediment from each site is stored in separate open containers in a sea table with low water flow that did not house free-roaming animals.

Extraction of meiofauna from sediment:

Approximately 0.5L of sand was removed from the tray in the sea table and put in a clean bucket (rinsed with tap water and filtered sea water, FSW). FSW was added to cover the sand by several centimeters. Sand and water mix were agitated by hand and by swirling the bucket, dislodging most animals living within the sand grains. The water was poured through a 0.45µm sieve. The animals trapped in the sieve were removed by rinsing the sieve with FSW, and the runoff collected in a petri dish. Animals were extracted from the dish using forceps or a pipette, counted, and placed in a 12 well dish which was divided into broad categories: polychaetes, copepods, amphipods (large), amphipods (small), nematodes, mites, ostracods, unknowns (soft bodied), unknowns (Ecdysozoa), acoel flatworms.

Organism classification:

Animals were initially screened after removal from the sediment and placed in broad categories mentioned above. Morphospecies of copepods, rotifers, acoel flatworms and arthropod larvae were described and the numbers of individuals within each group were counted. Morphospecies taxonomic classifications were using keys by Kozloff (1996) and Carlton (2007).

Calculation of the relative proportions of taxa at each site:

The proportion of each taxon within the community was assessed by calculating the average of the number of individuals counted during each screening from each of the three sites.

Calculation of species richness, species evenness, Shannon-Wiener Index (H) and ANOVA analysis for samples collected on 15 July 2012:

The species richness (S) was determined by adding the number of taxa present in each sample (either phyla or, if possible, class, order, or morphospecies).

The Shannon-Wiener Index (H) was calculated using the standard formula (Shannon 1948):

$$H = - \sum_{i=1}^S p_i \ln p_i$$

Species evenness (E) was calculated using the formula: $E = H/\log(S)$.

To determine whether the differences in Shannon-Wiener biodiversity values of the scans between the three sample sites were statistically significant, a One-Way ANOVA analysis was performed using Sigma Plot™ (Systat Software Inc, San Jose, California).

Results

Taxonomic groups present at Eagle Cove Beach:

After screening samples collected on 7 July twice (once immediately after collection and again 26 hours later on 8 July) and screening samples collected on 15 July from Sites 2 and 3 three times and the sample from Site 1 twice over 24 hours after collection, the organisms in the meiofauna were separated into taxonomic categories (Table 1). Initial screenings misidentified a harpacticoid copepod species as an amphipod. Morphospecies of copepod, nematode, isopod, amphipod, and annelid were photographed (Figure 2, Figure 3, Figure 4).

Proportions of taxa at each study site:

Strikingly, though collection dates were eight days apart, meiofaunal communities of all three sites changed. Animals were more numerous in the samples collected on 15 July (see Supplemental Spreadsheet 1) and proportions of taxa within the communities were different from those collected on 7 July (Figure 6). Though nematodes were the dominant taxon in Site 2 and Site 3 on both collection days and on Site 1 on 7 July, copepods were the most numerous taxon in Site 1 on 15 July. Even within collection dates, each collection site, though from the same beach, had different community compositions.

Species richness, species evenness and Shannon-Wiener Index (H) comparisons between samples collected on 15 July, 2012:

Species richness was highest in Site 1 (average of 18 spp observed over two scans). Site 2 had approximately 13.3 species observed over three scans and Site 3 had 11 species observed over three scans (Table 2).

Though Site 1 had the highest number of species, Site 3 had the highest H value (Table 2).

However, the differences in the values of H between sites were not statistically significant ($p = 0.129$) after ANOVA analysis (Figure 4). Though the numbers of species present in each sample site are different, the sites are equally diverse.

Possible sources of error

Initial poor identification techniques and descriptions rendered detailed biodiversity calculations for samples collected on 7 July unfeasible. Were this study to be repeated, a solid background in identifying morphospecies accurately would have to be cultivated. It would also be useful to have multiple researchers analyzing the data sets simultaneously, so that delicate (especially soft-bodied) specimens could be photographed immediately and identified. Three replicate scans of samples collected on 15 July 2012 were performed, except in the case of Site 1 samples, which were examined twice. Only two scans were performed on the Site 1 sample, because it was noticed that the large population of copepods that had been observed in the two initial scans done the day of collection were almost completely absent. It is possible that the copepods escaped into the sea table, although water flow across the sample containers was very low. The container housing the sample from Site 1 had a higher surface area than the other containers, and it is

possible that this had an effect on the retention of copepod species. The counts were thus discarded and not used in this study.

Discussion

The proportion of taxa represented and the dominant presence of one taxon over others varies between collection sites on Eagle Cove Beach. This is striking because sample sites were approximately 18m apart. There may have been enough of a difference in physical conditions (turbulence caused by wave action, dissolved oxygen content, water flow, or temperature) that taxa may have a preference for settling or remaining in one area to the exclusion of others. It is also possible that certain taxa cannot survive long in a particular physical or chemical setting and perish quickly.

Interestingly, though the collection days were meant to be replicates, the species compositions of the samples obtained on collection days were too different to act as comparable surveys. This difference may be due to seasonal changes numbers of phytoplankton (grazing opportunities) or secondary consumers (predation pressures) as the summer progresses (Binet et al 1995). Nematodes, particularly of the predatory class, dominate the meiofaunal community in all three locations, as has been found in previous studies (Liu et al, 2011).

Although the proportions of broad categories of taxa differ between sites, ANOVA analysis of the Shannon-Wiener biodiversity values (which takes into account species richness and evenness) shows no significant difference between sample sites collected on 15 July, 2012. It is possible that calculations of biodiversity using other methods and statistics may paint a different picture. However, with the methods used in this study, it must be assumed that the community structures of each site are different yet equally diverse.

It should also be noted that additional screens of each sample did not always yield additional species. In fact, some initial screenings had a higher number of species found than later screenings. Perhaps some animals were not numerous in the sample site's meiofaunal populations. It is also possible that random sampling error accounted for the absence of species in some screens but not others, or the absence of the species in the sample altogether. In addition, some organisms may have been destroyed or damaged beyond identification by either the collection process or extraction from the sediment. A more comprehensive survey with more collection replicates is needed to be confident that a robust meiofaunal community survey has been completed.

The difference that 18 meters makes on what meiofaunal communities are present in beach sand is astounding. Also striking is the difference that a week can make on the presence and abundance of species in the meiofauna. This interstitial ecosystem, which is at once very small yet vast, provides a fascinating glimpse into the potential biodiversity of the oceans. Meiofaunal communities are understudied compared to their macrofaunal counterparts, but the breadth of species present warrants further study of this amazing ecological community.

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Figures and Tables

Table 1: Taxonomic groups found on Eagle Cove Beach, San Juan Island, Washington. The nematodes were the most numerous taxon at most sample sites collected on the two sampling days. However, at some sites, copepods were either the dominant taxon or were co-dominant with nematodes. Annelids, though more rare, showed a striking diversity in both body plan and size. Nine morphospecies of copepods were found in the Eagle Cove Beach meiofauna.

<u>Phylum</u>	<u>Class</u>	<u>Subclass</u>	<u>Class</u>	<u>Order</u>	<u>Species</u>
Nematoda	Enoplia				
Arthropoda	Maxillopoda	Ostracoda			
		Copepoda			
				Cyclopoida	
				Harpacticoida	
	Malacostraca			Amphipoda	
				Decapoda	
				Isopoda	
Platyhelminthes	Acoelomorpha				
Annelida	Polychaeta				
					<i>Keratella cochlearis</i>
Rotifera					

Table 2: Species richness, evenness and the Shannon-Wiener biodiversity value for all surveys of each sample site, collected on 15 July 2012. Note that species richness does not necessarily increase with additional scanning (e.g. compare Site 1 surveys 1 and 2 performed on 15 July).

	<u>Site 1</u>	<u>Site 1</u>	<u>Site 1</u>	<u>Site 2</u>	<u>Site 2</u>	<u>Site 2</u>	<u>Site 3</u>	<u>Site 3</u>	<u>Site 3</u>
<u>July 15, 2012 Collection</u>	<u>Survey1</u>	<u>Survey2</u>	<u>Survey3</u>	<u>Survey1</u>	<u>Survey2</u>	<u>Survey3</u>	<u>Survey1</u>	<u>Survey2</u>	<u>Survey3</u>
	<u>7/15/2012</u>	<u>7/15/2012</u>	<u>7/17/2012</u>	<u>7/15/2012</u>	<u>7/15/2012</u>	<u>7/17/2012</u>	<u>7/15/2012</u>	<u>7/15/2012</u>	<u>7/17/2012</u>
-									
<u>Species Richness</u>	20	16	x	14	13	13	10	13	10
-									
<u>Species Evenness</u>	1.113572421	1.072481766	x	1.34094094	0.7819577	0.806416	1.69037	1.3673765	1.63115
-									
<u>Shannon-Weiner</u>	1.448791122	1.291396726	x	1.53689	0.8710566	0.898302	1.69037	1.52318	1.63115

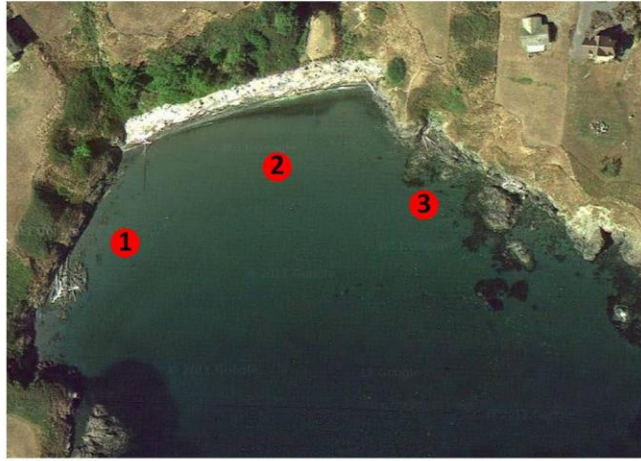


Figure 1: Eagle Cove beach, San Juan Island. Submerged sand was collected at three distinct sites on the beach at low tide: (1) sheltered by rocky outcrops with little wave action, (2) exposed with substantial wave action, (3) surrounded by rocky outcrops with substantial wave action. Image: Google Earth



Figure 2: Copepod diversity in the Eagle Cove Beach meiofauna. Nine morphospecies were identified over three sample sites along the beach.

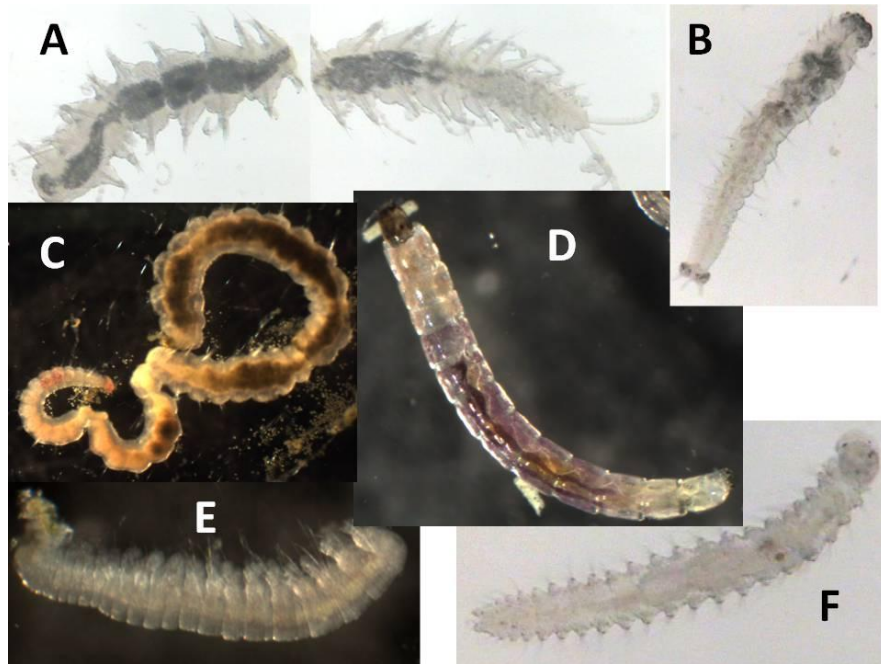


Figure 3: Polychaete worms found in the meiofauna of Eagle Cove Beach. Five species were present. Species (E) was broken and was unable to be identified.

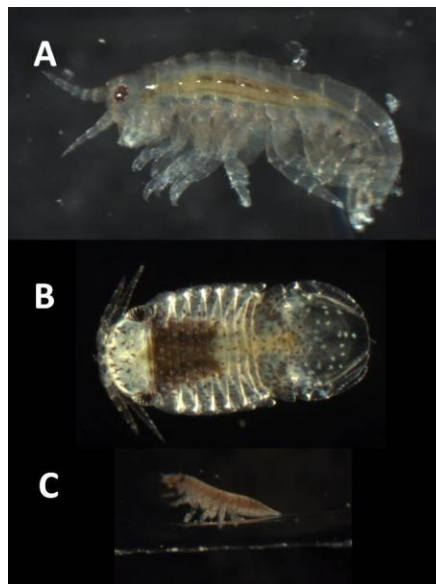
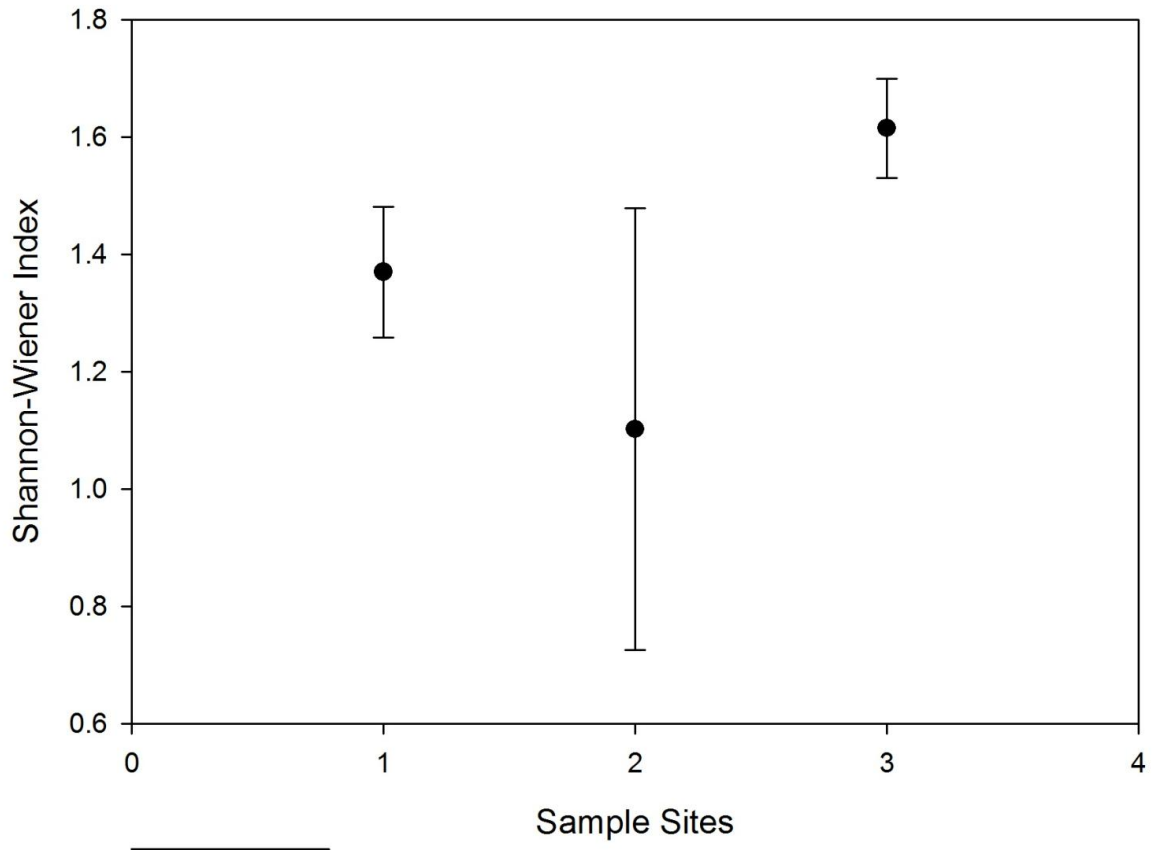


Figure 4: Amphipod (A) and isopods (B,C) found in the meiofauna at Eagle Cove Beach.

Shannon-Wiener Diversity Index Comparison



One Way Analysis of Variance

Normality Test

(Shapiro-Wilk) Passed (P = 0.251)

Equal Variance Test: Passed (P = 0.573)

Group Name	N	Mean	Std Dev	SEM		
15 July Site 1	2	1.37	0.111	0.0787		
15 July Site 2	3	1.102	0.377	0.218		
15 July Site 3	3	1.615	0.0848	0.0489		
Source of Variation	DF	SS	MS	F	P	
Between Groups	2	0.395	0.197	3.176	0.129	
Residual	5	0.311	0.0621			
Total	7	0.705				

Figure 5: The means of the Shannon-Wiener values for each scan for each sample site are shown in the graph with standard deviation. Through analysis with ANOVA, it was found that the differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.129).

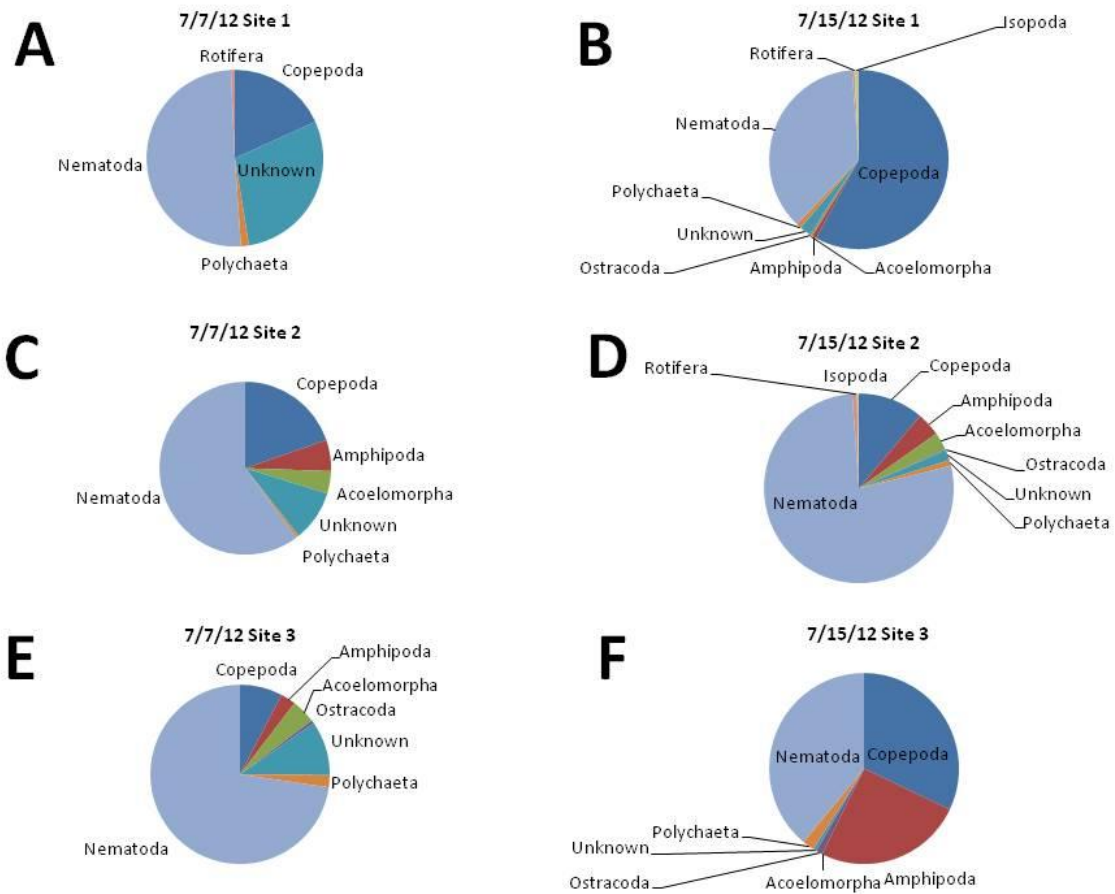


Figure 6: Proportions of taxa represented in each sample site, with comparisons of the two collection dates – Site 1 (A, B), Site 2 (C, D), Site 3 (E, F). Proportions stayed relatively similar in Site 2 across both dates, but changed substantially in Sites 1 and 3.