EXAMINING VARIATIONS IN FATTY ACID PROFILES OF THE SEA STAR *PYCNOPODIA HELIANTHOIDES* ON THE COAST OF BRITISH COLUMBIA

by

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A THESIS

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On the Coast of British Columbia

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Pycnopodia helianthoides is a large, predatory sea star native to the northeast Pacific coast. This important intertidal and subtidal predator was historically abundant along the west coast of North America from Alaska to Baja California until the 2013-2015 sea star wasting epidemic caused drastic population declines throughout its range. The opportunistic diet of *Pycnopodia* on benthic invertebrates has previously been studied through observation, but there are no published diet analyses using fatty acids (a trophic biomarker). In order to investigate the trophic ecology of wild *Pycnopodia*, fatty acids were extracted and identified from tissues of 119 individuals, ranging in size from 2.6-46 cm in diameter, collected from different sites and habitats near Calvert Island, BC, Canada. The multivariate fatty acid signatures of the stars were compared between collection sites and substrate types, as well as size classes (5 categories). Fatty acids were significantly different between sites and substrates, but not size classes. These differences suggest that the diets of stars found at different sites and substrates could differ significantly, although very few sites featured more than one substrate type, making it difficult to distinguish the potential effects of each. Size class, however, does not appear to have an effect on the fatty acid compositions of the stars, indicating that *Pycnopodia* diets may not vary much by size, which was unexpected. Further analysis of both wild and captive stars could continue to provide more insight into the diet of this understudied species.

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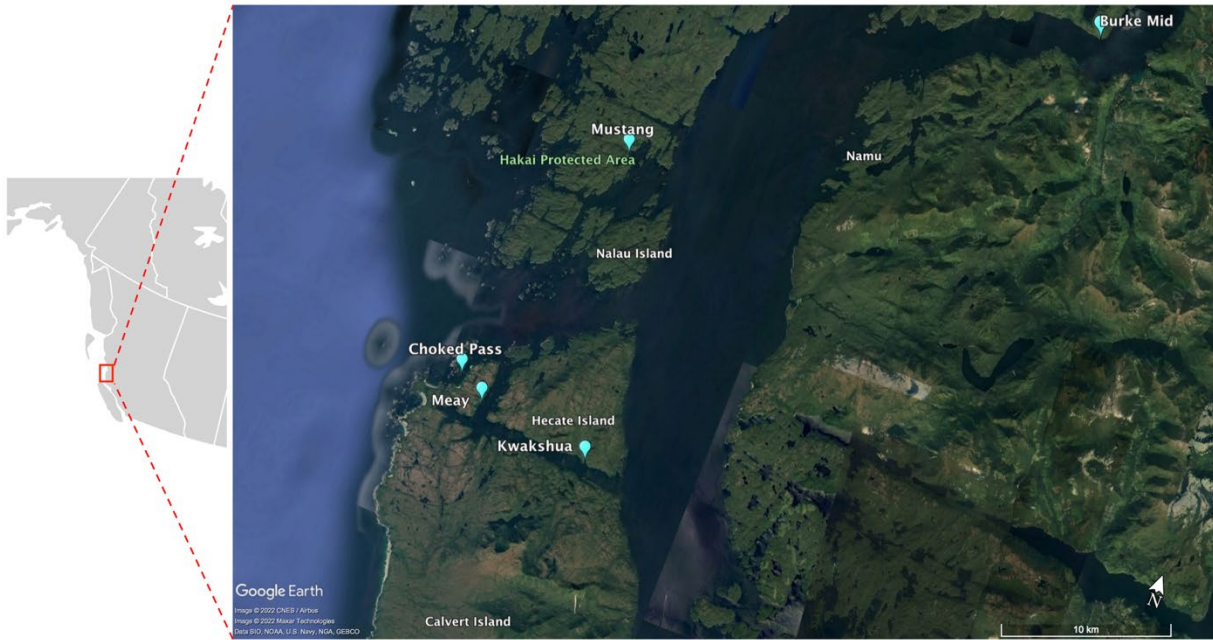
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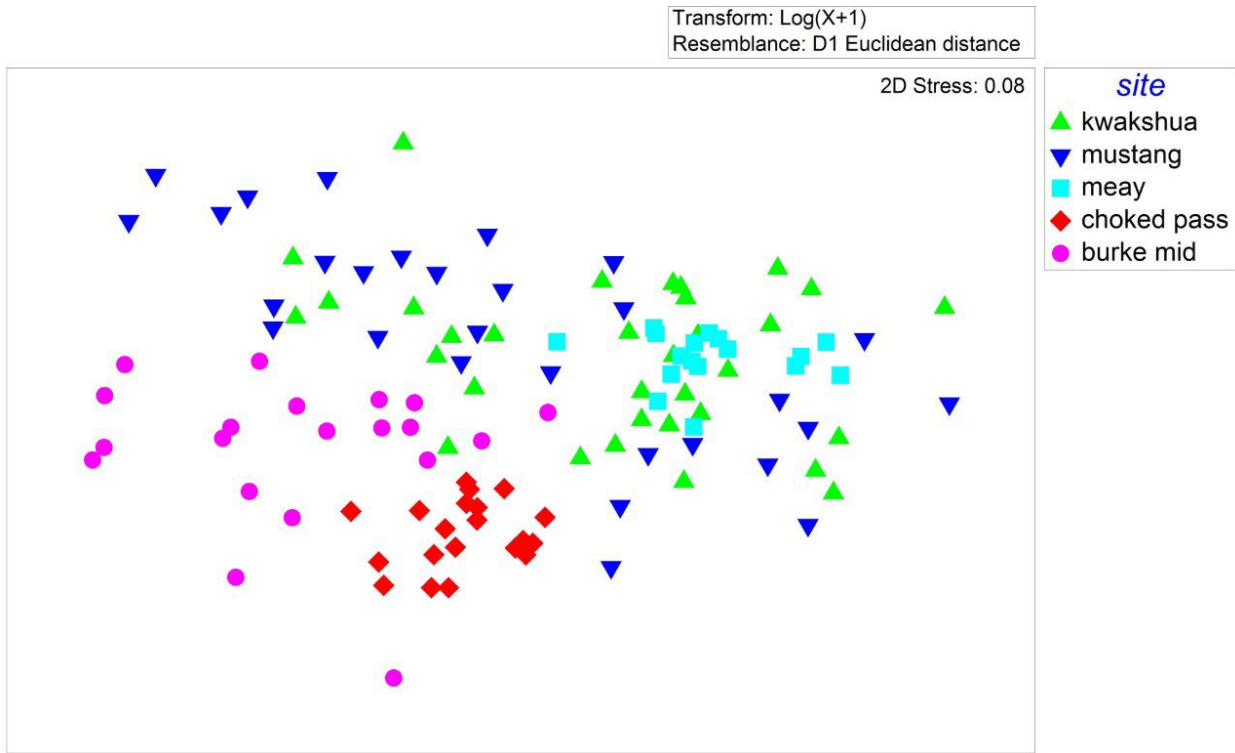

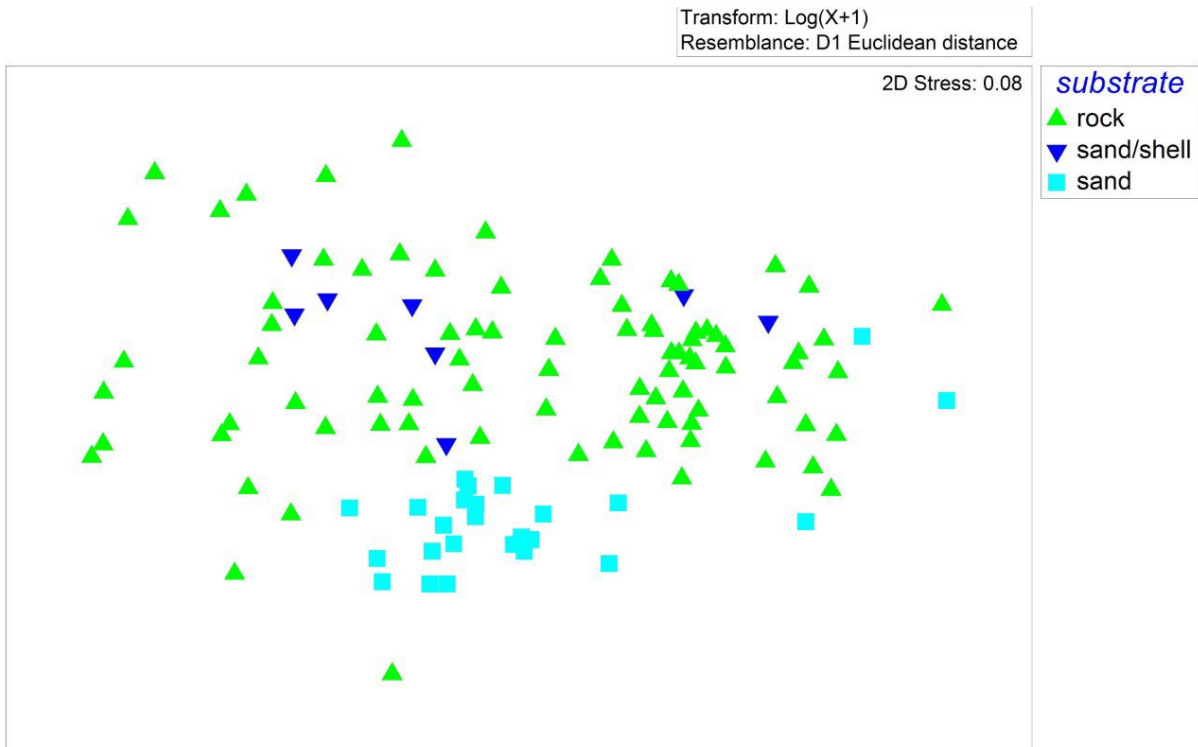Figure 2. NMDS plot with points colored by collection site

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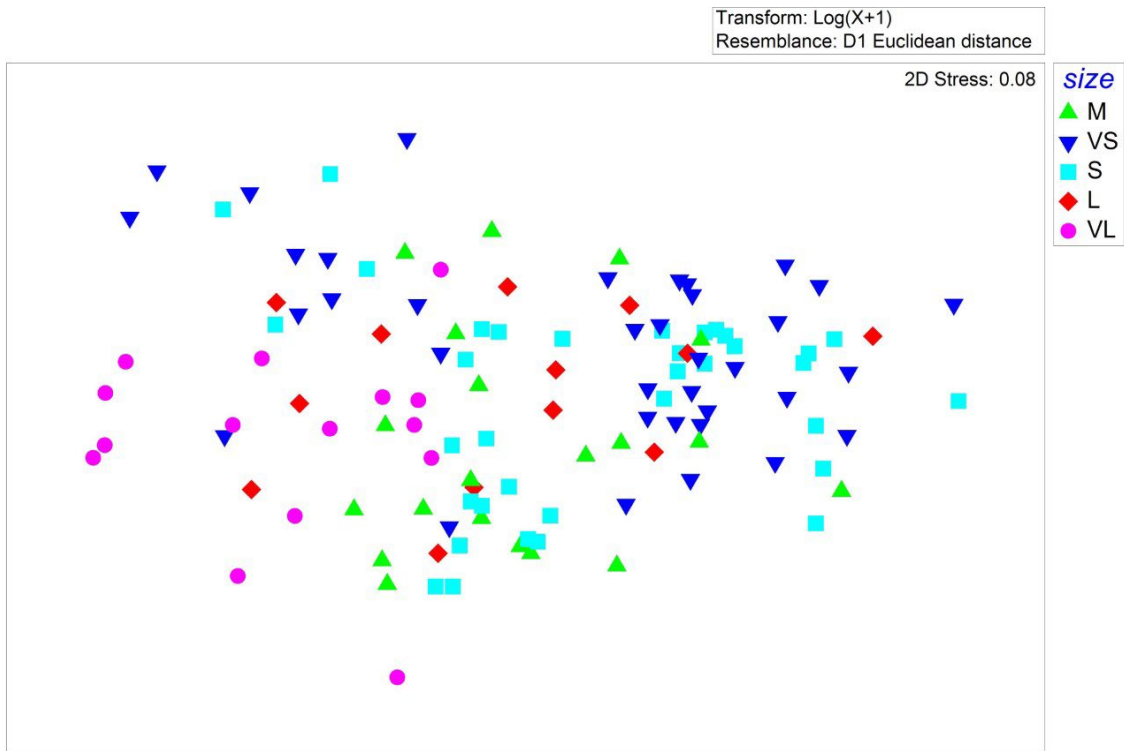
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Medium (M)	66-85	20
Large (L)	86-119	13
Very large (VL)	$\geq$120	15

Table 1. Body size ranges and sample sizes of each defined category

Site	Sample Size
Kwakshua	33
Mustang	29
Meay	17
Choked Pass	20
Burke Mid	20

Table 2. Number of individuals sampled from each site

Substrate Type	Sample Size
Rock	86
Sand	25
Sand/shell	8

Table 3. Number of individuals sampled from each substrate type

Introduction

Pycnopodia helianthoides, the sunflower sea star, is a large species of sea star native to the northeast Pacific coast. Compared to most sea stars, it is remarkably mobile and fast, making it one of the most formidable invertebrate predators in the rocky intertidal and subtidal zones (Lambert 2000). It is an opportunistic predator, having been observed to prey and/or scavenge on a large variety of small invertebrates (Lambert 2000). *P. helianthoides* predation upon purple urchins (*Strongylocentrotus purpuratus)* is particularly noteworthy because, when left unchecked, purple urchin populations can have a substantial negative effect on kelp forests through overgrazing, potentially eliminating all kelp in an area and creating urchin barrens (Hamilton and Caselle 2015).

The important role of the sunflower sea star in kelp forest ecosystems is supported by the finding that their predation of *S. purpuratus* in Torch Bay, Alaska, directly affected subtidal algal distributions and densities (Duggins 1983). In the case of Torch Bay, *P. helianthoides* was the sole predator of *S. purpuratus* (Duggins 1983)*.* However, even in regions where other urchin predators exist, the loss of *P. helianthoides* has caused noticeable shifts in kelp forest ecosystems; In British Columbia, declines in the sunflower sea star population were correlated with a 311% increase in medium-sized urchins and a 30% decrease in kelp densities (Burt et al. 2018). This "deforestation" has become more apparent and widespread in recent years, at least in part due to the rapid decline and/or loss of *P. helianthoides* populations along much of North America's west coast, caused by the ongoing sea star wasting disease (SSWD) epidemic (Burt et al. 2018).

The SSWD epidemic has led to the recent classification of *P. helianthoides* as "critically endangered" on the IUCN Red List (Gravem et al. 2020). As *P. helianthoides* can have such a

large impact on kelp forest ecology, it is crucial to gain as much information about the species as possible, particularly in regard to their feeding ecology. The diet of the sunflower sea star is relatively well-known in the literature, primarily through behavioral observations and gut content analyses (Shivji et al. 1983, Lambert 2000, Hodin et al. 2021). Diet varies between regions but overall, the sunflower sea star is a generalist (Lambert 2000). Off the coast of British Columbia, *P. helianthoides* was observed eating species from eleven different taxa, including gastropods, bivalves, crustaceans, echinoderms, and occasionally even carcasses of seabirds, dogfish, and herring (Shivji et al. 1983). In many regions, urchins and bivalves are the preferred prey types (Lambert 2000). In terms of urchins specifically, one food preference study found that although *P. helianthoides* was equally attracted to the urchins *S. franciscanus* and *S. purpuratus*, the sea stars ultimately preyed upon *S. purpuratus* 90% of the time (Lambert 2000). Another food preference study found that the sea stars greatly preferred dead or damaged prey over live prey, even choosing to pass by live prey en route to a damaged individual (Brewer and Konar 2005). Such preferences provide further support for the role of *P. helianthoides* as an important influence on kelp distribution and a potential aid in reducing urchin barrens.

Studies suggest that the diets of juvenile and adult *P. helianthoides* may differ, suggesting ontogenetic shifts in diets (Lambert 2000, Hodin et al. 2021). Juveniles are commonly observed in more sheltered habitats, mainly feeding on microflora/fauna and detritus (Shivji et al. 1983, Lambert 2000). In a captive setting, juveniles were successfully fed various biofilms, juvenile echinoderms (*S. purpuratus* and *Dendraster excetricus*), and juvenile bivalves (Hodin et al. 2021). The "juvenile" age class, however, is not well defined and current knowledge of juvenile diet is still reliant upon behavioral observations (Hodin et al. 2021). Therefore, actual ontogenetic shifts in the diets of wild individuals, if present, are uncertain. Overall, although

much is known about *P. helianthoides* diet, especially of the adults, the current knowledge base is by no means comprehensive or complete.

Trophic biomarkers such as fatty acids (FAs) can help to close the knowledge gap created by the exclusive use of gut content analyses and behavioral observations. Fatty acids—hydrocarbon chains with terminal carboxyl groups—can be important tools in the study of trophic ecology due to their highly transferable nature (Budge et al. 2006). FAs are typically created by primary producers and taken up by consumers, although it should be noted that de novo FA synthesis by consumers is possible and that consumers may also modify their dietary FAs, once obtained, to form new FAs according to the needs of the organism (Kelly and Scheibling 2012, Galloway and Budge 2020). Frequently, however, FAs are passed from prey to predator unchanged, making them easy to trace through trophic relationships and therefore extremely useful in identifying and confirming those relationships (Budge et al. 2006).

Fatty acids have been utilized in trophic ecology studies of other sea star species and have been shown to be fairly accurate in determining their diets (Latyshev et al. 2001, Kelly and Scheibling 2012). One study of the arctic sea stars *Lepasterias groenlandicus* and *L. polaris* found, through both stable isotope and FA analysis, that gastropods, echinoderms, polychaetes, and bivalves all contributed significantly to the sea stars' diets and that diet varied based on location and prey availability (North et al. 2019). Fatty acid analysis of three deep-sea predator/scavenger sea star species found that there is reliance on both photosynthetic and bacterial sources of carbon (Howell et al. 2003). Although these studies form a good baseline of sea star FA research, the featured species are primarily scavengers and detritivores, not predators like *P. helianthoides*, which supports the need for further study of *P. helianthoides* specifically.

At time of publishing, only one study utilizing trophic biomarkers (in this case, stable isotopes) to analyze the diet of *P. helianthoides* exists, which found that the species derived more that 60% of its essential amino acids from subtidal kelps in south central Alaska (Smith et al. 2018). Such dependence is reflective of the key role *P. helianthoides* plays as a predator in kelp forest ecosystems. However, stable isotope analysis alone does not make for a reliable approach to examining trophic interactions, especially amongst benthic invertebrate communities, which can be highly diverse and trophically intertwined (Kelly and Scheibling 2012). Therefore, fatty acid analysis of *P. helianthoides* can serve to expand and strengthen the understanding of their diets through trophic biomarkers.

Without prior studies to establish a baseline, hypotheses regarding how the fatty acid profiles of *P. helianthoides* are affected by variables such as collection site, body size, or substrate were difficult to make. However, body size was expected to have at least some effect, given the observed ontogenetic changes in diet reported in the literature (Hodin et al. 2021). Substrate was also expected to affect fatty acid profiles due to the differences in benthic invertebrate communities associated with different substrate types (Thorson 1966). In order to investigate the physiological and environmental factors that may affect fatty acid profiles in *P. helianthoides*, I extracted the fatty acids from tube feet/arm tip tissue from 119 wild individuals collected on the coast of British Columbia and analyzed the overall fatty acid profiles using collection site, substrate, and body size category as variables.

Materials and Methods

Tube feet and arm tip tissue were taken from 119 *Pycnopodia helianthoides* individuals collected from five sites around Calvert Island, BC, using SCUBA (Fig. 1). The collections were done in August and October 2020 by divers at the Hakai Institute at five sites: Choked Pass, Meay, Kwakshua, Mustang, and Burke Mid. Choked Pass is an exposed outer coast site along the northwestern edge of Calvert Island. Meay is located in the narrow Meay Channel and Kwakshua is located in the similarly narrow Kwakshua Channel, which runs perpendicular to the Meay Channel. Mustang is a protected site in a cove north of Calvert Island, and Burke Mid lies approximately 15 km inland from the mouth of a fjord. All sites are characterized almost entirely by rocky substrate, with the exception of Choked Pass, which only features sand.

The tissue samples were kept frozen at -20 °C until shipment to the Oregon Institute of Marine Biology in February 2021. The tissue was lyophilized for 48 hours, then ground with an isopropyl alcohol pre-washed stainless-steel mortar and pestle. Extractions were completed in batches of ten, along with a procedural blank. One week before extraction, 10-20 mg from each tissue sample were transferred, along with 2 mL of chloroform, to rinsed graduated test tubes, which were then sealed under nitrogen. Tissue samples weighing below 10 mg were not processed.

The day of extraction, 1 mL of methanol and 70 μl of C19 standard (GLC Reference Standard 566 C, Nu-Check-Prep, Elysian, MN) were added to each sample. The samples were vortexed for 10 seconds and sonicated for 10 minutes. Afterwards, 0.75 mL of 0.9% NaCl water solution were added to each sample. Test tubes were centrifuged for 5 minutes at 3000 rpm to separate the phases. The lower organic phase was removed to a rinsed 8 mL scintillation vial and

an additional 2 mL of chloroform were added to the original samples. The process of sonication, vortexing, and centrifugation was performed again to extract the organic layer a second time.

The extracted organic layers from each sample were evaporated to dryness under nitrogen and 1.5 mL of chloroform were added back to each sample. One milliliter from each sample was transferred to rinsed graduated test tubes and the samples were again evaporated to dryness under nitrogen. After evaporation, 1 mL of toluene and 2 mL 1% solution of sulfuric acid in methanol were added and the samples were vortexed. The samples were then placed in a 90 °C water bath for 90 minutes to allow for methylation of the fatty acids. Once cooled, 1.5 mL of 2% $KHCO_3$ solution and 2 mL of hexane were added to each sample. The test tubes were vortexed and centrifuged for two minutes at 1500 rpm to separate the phases.

The upper organic phase, containing fatty acid methyl esters (FAME), was transferred to a rinsed ungraduated test tube and 1.5 mL of hexane were added to the original samples. Vortexing and centrifugation were repeated to extract any remaining FAME. The samples were evaporated to dryness under nitrogen in a 30 °C water bath, after which 1.5 mL hexane were added back to each sample. The FAME layers were transferred to 1.5 mL vials, flushed with nitrogen, sealed with parafilm, and stored at -20 °C.

The FAME samples were run through a gas chromatograph/mass spectrometer (GCMS) in batches of approximately 40. Four standards of increasing concentration were run at the beginning of each batch. Fatty acid peaks in the resulting chromatograms were identified using GCMS PostRun Analysis Software (v.4.41, Shimadzu Corporation). GCMS Quantitation Browser Software (v.4.41, Shimadzu Corporation) was used to determine the concentrations of each identified fatty acid and the initial weights of the samples were used along with concentrations to determine the proportions of each fatty acid within the samples. An NMDS

(non-metric multidimensional scaling) plot was created in the Primer 6 & PERMANOVA+ software using the fatty acid concentration data. The points were colored according to three variables: collection site, size category, and substrate, resulting in three iterations of the plot. PERMANOVA tests were run according to the same variables, using the same software.

Size category (radius in mm) was also split into five categories: very small, small, medium, large, and very large (Table 1). Collection site had five designated categories—one per site (Table 2). Substrate had three defined categories: rock, sand, and sand/shell (Table 3).

Results

When colored according to collection site, the NMDS plot showed some distinct groupings, particularly Choked Pass and Burke Mid (Fig. 2). NMDS plots display similarity between points, where the distance between points indicates similarity—short distances between points indicate high similarity. A PERMANOVA test revealed that overall, the fatty acid profiles of the individuals were significantly different between sites ($p = 0.0001$). Pair-wise tests revealed that each site pairing showed significant difference except for Kwakshua/Meay ($p = 0.21$). The difference between Mustang and Burke Mid, while technically significant ($p = 0.0317$), was less so than the other pairings.

In terms of substrate type, the NMDS plot showed that the rock and sand groups were quite visually separated (Fig. 3). This pattern is supported by the PERMANOVA test results which suggest that the substrate types as a whole are significantly different from one another ($p = 0.0001$). Not all pair-wise tests of the substrate treatments were completed; the sand/shell and sand pairing could not be tested for due to small sample sizes, but rock/sand was significantly different ($p = 0.0001$), as well as rock and sand/shell ($p = 0.0069$).

There was no apparent grouping according to size category within the NMDS plot (Fig. 4). This result is supported by the PERMANOVA tests, which reveal that there was no significant difference in fatty acid compositions of individuals of different body size categories ($p = 0.4823$). No pair-wise tests were performed for size category.

Discussion

The significant differences found in the fatty acid profiles between the collection sites suggests that the sea stars found at each site had significantly different fatty acid compositions, which may imply that the stars at each of the sites have different diets. Any potential differences in diet would be influenced by prey availability and selection by the predator. Although *P. helianthoides* is known to have regional prey preferences (Lambert 2000), the survey area of this study is smaller than the scale on which those regional preferences vary. Therefore, prey availability would likely be the primary driver of diet in the case of this study.

The five collection sites around Calvert Island, while contained in an area roughly only 750 square kilometers, are situated in a variety of environmental contexts. For example, Choked Pass lies on the outer coast of the island while Kwakshua is located in a narrow channel and Burke Mid is father inland in the wider Burke Channel. Each of these locations are subject to different physical environmental factors, the most important of which is wave action. Wave exposure has a notable effect on subtidal benthic invertebrate communities. High levels of wave action in sedimented locations tend to exclude tube/burrow dwellers and other sessile organisms, instead primarily hosting small, mobile organisms like crustaceans (Oliver et al. 1980). In rocky habitats, sessile animals—suspension feeders—tend to be favored in more wave-exposed areas (Ricciardi and Bourget 1999). These differences in benthic invertebrate communities based on varying levels of wave exposure provide evidence for differences in communities, and thus prey availability, between the five collection sites.

Substrate also has a substantial effect on benthic invertebrate community composition. Sandy substrate tends to host a higher proportion of deposit feeders and burrowing suspension feeders, whereas rocky substrates have higher proportions of sessile suspension feeders and

mobile grazers and carnivores (Ricciardi and Bourget 1999). Rocky habitats also generally have higher macroinvertebrate diversity and biomass (Ricciardi and Bourget 1999). Therefore, it is reasonable to assume that sea stars that frequent rocky habitats would have different diets from those that inhabited primarily sandy sites, an assumption supported by the significant difference in fatty acid profiles of stars found on rock versus sand.

The third substrate type—sand/shell—was underrepresented in this study and it is unclear how the inclusion of shell hash may affect the benthic invertebrate communities of otherwise sandy environments, although the presence of certain tubeworms, bivalves, and crustaceans appears to be inhibited by shell hash (Peterson et al. 2006, Raineault et al. 2012). The fatty acid profiles of stars found on sand/shell substrate were significantly different from those found on rock, but without a higher sample size of sand/shell stars, the potential effect of sand/shell substrate on diet, especially in comparison with pure sand, remains unclear. Additionally, many of the collection sites featured only one substrate type, making it difficult to distinguish the effects of substrate from collection site. Inclusion of additional sites that feature more than one substrate type would be valuable in potential future analyses.

The most surprising finding of this study was that the difference between body size categories was insignificant. Current literature states that there is at least some level of ontogenetic change in the diet of *P. helianthoides*, with juveniles feeding primarily on macrofauna/flora and detritus (Shivji et al. 1983, Lambert 2000), which would imply a difference in fatty acid composition between juveniles and adults. The lack of significance between the fatty acid compositions of stars in the five different size categories suggests that diet does not meaningfully change as individuals grow. Assuming this to be the case, the establishment of potential diet preferences at a young age may be important. Ultimately, the lack of understanding

of how diet shifts with body size and age beyond early juvenile stages limits the capacity to make inferences regarding differences, or lack thereof, in fatty acid compositions.

Alternatively, the lack of significant difference may suggest that the size distribution of adults within a population has inconsequential effect on diet and therefore prey choice. That is, a population of small *P. helianthoides* may exert predatory pressure on the same groups of prey as a population of large individuals. However, according to one definition of "adult" for *P. helianthoides*—an arm tip-to-arm tip diameter >20 cm (Hodin et al. 2021)—there are relatively few adults represented in this study. Only 20 of the 119 individuals had a radius over 100 mm (10 cm). Whether this definition of age class is universally applicable to all *P. helianthoides* populations is unknown. Further analysis using this definition may be helpful, although the "large" and "very large" categories (>85 mm radius) defined and used in this study still did not exhibit any significant differences from the smaller size categories. Ultimately, a lack of significant difference between juvenile groups and between juveniles and adults may suggest that individuals eat similar types of prey throughout their ontogeny, perhaps simply in different ways (ex. transitioning from eating juvenile bivalves to adult bivalves).

One of the major assumptions that needs to be taken into account when working with fatty acids is the fact that in certain cases, they may not reflect diet very accurately. Many consumers, especially those in higher trophic levels are able to alter or synthesize fatty acids de novo according to their physiological needs. This process can potentially render FAs taken up through diet unrecognizable, or at least complicate the picture of the individual's overall fatty acid profile. As a result, fatty acid analysis is best utilized in tandem with other types of diet analysis and/or a controlled diet study (Galloway and Budge 2020). A controlled diet study would allow for the identification of actual components of the diets of the wild sea stars, an

analysis which would work synergistically with gut content and behavioral analyses as well as other trophic biomarker studies. Another potential extension of this study would be to include wild stars from other regions within their native range along the west coast in order to compare fatty acid profiles across a larger geographical range.

In conclusion, the sunflower sea stars of the Calvert Island region of British Columbia display distinct and significant differences in their fatty acid profiles across the populations at each collection site, as well as across different substrate types. These differences have potential trophic implications in that they suggest that each of these groups of stars have differences in their diets. Ultimately, differences in diet may reveal certain features of the species' natural history, particularly feeding habits, prey selection, and potentially habitat preference and distribution, all of which may be helpful in informing management and reintroduction.

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