PycNosh: Using Controlled Feeding Experiments to Assess Trophic Impacts of the Threatened Sea Star *Pycnopodia helianthoides*

by

Aubrey Taradash

A thesis accepted and approved in partial fulfillment of the

requirements for the degree of

Master of Science

in Biology

Thesis Committee:

Aaron Galloway, Chair

Sarah Gravem, Member

Richard Emlet, Member

University of Oregon

Spring 2025

THESIS ABSTRACT

Aubrey Taradash

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Title: PycNosh: Using Controlled Feeding Experiments to Assess Trophic Impacts of the Threatened Sea Star *Pycnopodia helianthoides*

The sunflower sea star *Pycnopodia helianthoides* is a generalist predator that impacts the population dynamics of a variety of benthic invertebrates, especially red urchins (*Mesocentrotus franciscanus*). Population declines due to sea star wasting disease and changes in macroalgal and benthic invertebrate assemblages necessitate further investigation of sunflower star diets. Observation and gut contents only provide dietary "snapshots", so controlled feeding experiments and trophic tracers such as fatty acids are promising tools for estimating long-term dietary patterns. To assess sunflower star predation habits and whether diet impacts fatty acid (FA) composition in sunflower stars, I fed sunflower stars (n=19) five monospecific diets of common prey in Sitka Sound, AK for eleven weeks: green and red sea urchins (*Strongylocentrotus droebachiensis*, *M. franciscanus)*, pinto abalone (*Haliotis kamtschatkana)*, butter clams (*Saxidomus* sp.), and dusky turban snails (*Tegula pulligo*). Sea stars were fed *ad libidum* and three size categories of prey were available at all times to measure size selection. Arm tip tissue was collected from experimental and wild sea stars for FA analysis. Sunflower stars ate 1.1 red urchins and 1.2 green urchins per day with a preference for small red urchins, while clams and abalone were eaten at lower rates (0.42-0.47/day) with no observed size preferences. Sunflower star FA profiles were overall significantly different between diet treatments ($p << 0.01$). Pairwise comparisons showed no significant differences in the fatty acids of *P. helianthoides* fed different species of urchins, indicating that the effects of closely related prey are difficult to distinguish. The FA profiles of wild *P. helianthoides* were significantly different from one another and from most captive-fed stars ($p << 0.01$), although the FA of wild stars from the kelp-dominant site were not significantly different from those of abalone-fed stars, indicating potential consumption of abalone. The predation patterns of sunflower stars indicate potentially strong trophic effects, with particular impact on small red urchins, which has implications for kelp forest health. Distinguishable effects of diet on captive *P. helianthoides* establish FA as a promising tool for estimating the diets of sunflower stars in the wild.

ACKNOWLEDGMENTS

I'd like to express my gratitude to my committee members, Drs Aaron Galloway, Sarah Gravem, and Richard Emlet for providing invaluable feedback on my research and writing. They have also played key roles in my education and have provided me with continual support and encouragement. I'd like to give special thanks to Dr. Galloway, my advisor, for supporting me both academically and professionally and providing me with many different research and career development opportunities.

I'd also like to thank the 2023 research team in Sitka, Alaska for their assistance in collecting organisms for my experiment and for assistance in keeping up with observations. I'd particularly like to thanks Miles Rough, Eliana Bower, and especially Jacob Metzger, who kept the experiment running after I left Sitka. I must also extend my thanks to the Sitka Sound Science Center for hosting us and providing the team with a research space.

Many thanks to my lab mates for their continual support, with special thanks to Mike Thomas and Jessie Masterman for mentoring me on fatty acid extraction and analysis. Lastly, I'd like to extend my thanks to the greater community of OIMB grad students, faculty, and staff for their support, mentorship, and friendship.

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CHAPTER I: PREDATION BY THE SUNFLOWER SEA STAR ON SEVERAL BENTHIC INVERTEBRATES AND ITS TROPHIC IMPLICATIONS

Introduction

In food webs, predators can exert top-down control of prey, especially when a predator applies strong predation pressure (Menge & Sanford, 2013). Trophic cascades can occur when sufficient pressure is exerted by predators as to reduce prey population densities and produce indirect effects on lower trophic levels (Paine, 1980). Trophic cascades in marine food webs are commonly attributed to generalist predators due to the predation pressure they exert on a variety of organisms in lower trophic levels (Ellingsen et al., 2020; Heithaus et al., 2008; Xu et al., 2012). Generalist predators accomplish this level of influence by having broad, adaptable diets, allowing them to function within multiple trophic roles or change their role over time. The adaptability of generalist predators may allow them to influence multiple distinct trophic cascades, and the intensity or nature of the cascades may change seasonally or spatially as the predator's diet changes (Xu et al., 2012). Shifts in diet by generalist predators also have a stabilizing effect on prey population dynamics (Vitense et al., 2016). Many generalists exhibit density-dependent functional responses (Holling type III) to shifts in prey availability (ex. seasonal shifts between planktivory and piscivory (Xu et al., 2012), which facilitates the persistence of prey populations (Dunn & Hovel, 2020; Novak et al., 2017). The roles of generalist predators are well-recognized in apex predators or those otherwise in high trophic levels. Predatory fish, birds, and mammals are common targets of research on apex predators. However, many invertebrates occupy the same or similar trophic positions and can have equally sizeable trophic effects (de la Chesnais et al., 2019; Menge & Sanford, 2013).

Sea stars often occupy upper or top trophic levels and can have notable impacts on benthic invertebrate communities, with two species being recognized as "keystone" predators: *Pisaster ochraceus* and *Stichaster australis*. Large body size and relative mobility, along with the ability to disperse and adapt to a variety of habitats across wide geographic ranges, are key factors in the trophic influence of sea stars and their ability to exert control over certain prey populations. (Menge & Sanford, 2013).

The sunflower sea star (*Pycnopodia helianthoides*) is one example of a sea star with the potential for playing a strong predatory role in the various ecological communities it's a part of.

The sunflower sea star is a large, incredibly mobile predator that occupies nearly all intertidal and subtidal (up to 435m) habitats from the Aleutian Islands, USA to Baja California, Mexico (Lambert, 2000). Due to their widespread distribution, *P. helianthoides* is exposed to a wide variety of benthic prey. The species is known to consume a variety of echinoderms, bivalves, gastropods, some crustaceans, and occasionally scavenging on vertebrate carcasses (Lambert, 2000; Shivji et al., 1983). As in other sea stars, the *P. helianthoides* diet is influenced by local prey availability and abundance. For example, the diets of sunflower sea stars is dominated by gastropods in areas occupied by sea otters and by bivalves and urchins in areas free of competition from sea otters (Herrlinger, 1983).

P. helianthoides prey regularly on bivalves and gastropods, including clams, mussels, snails, and abalone. Clams are a major component of sunflower stars in soft sediment habitats and may make up to 72% of their diet in subtidal locations with high sediment cover (Mauzey et al., 1968; Shivji et al., 1983). Sunflower stars are capable of digging deeper than other sea stars into sediment to extract infaunal clams, making them potential competitors with other sea stars for bivalve resources (Lambert, 2000; Mauzey et al., 1968). In some locations, *P. helianthoides* compete directly with sea otters for clams, each predator contributing equally to total mortality (Traiger et al., 2016). Sunflower stars also consume a wider variety of clam species than sea otters, broadening their ecological influence (Traiger et al., 2016). Although the impact of predation by *P. helianthoides* on clam populations and on other clam consumers has not been fully characterized, it is influential enough in regions with high predation activity to warrant consideration when making fishery management decisions (Traiger et al., 2016).

Pinto abalone (*Haliotis kamtschatkana*) are also an important prey species of the sunflower sea star. Although abalone are rarely mentioned as a component of *P. helianthoides* diet in existing literature and are never listed as a preferred prey item, they are nonetheless predated upon by sunflower sea stars, especially in areas without sea otters where abalone are presumably more abundant (Campbell, 2000; Herrlinger, 1983). It is possible that abalone are not listed because they are often rare, being a highly overfished group (Campbell, 2000). Despite sparse data on their preference for abalone, *P. helianthoides* has been identified as a potentially rather influential predator of pinto abalone, particularly juvenile abalone—sunflower stars were a common predator found aggregating at juvenile abalone outplanting locations (Read et al., 2013) and captive sunflower stars ate nearly a third of all juvenile abalone offered in a feeding

experiment, the second highest predation rate out of 37 potential abalone predators (Griffiths & Gosselin, 2008). Preferential consumption of small individuals has the capability to locally affect population dynamics of pinto abalone, potentially impacting other abalone predators and the already-limited pinto abalone fishery (Griffiths & Gosselin, 2008). Snails, too, are subject to predation pressure by *P. helianthoides (Shivji et al., 1983)*. Turban snails of the genus *Tegula* are common in sunflower star diets, especially in central California where sea otters outcompete sunflower stars for urchin and bivalve prey (Herrlinger, 1983). *Tegula* distribution, abundance, and behavior are impacted by *P. helianthoides* through both direct (consumptive) and indirect effects (Watanabe, 1984; Wetmore, 2022).

Sea urchins have long been noted as a significant portion of sunflower star diets. In Torch Bay, Alaska, urchins were the target of 65% of all observed predation events by *P. helianthoides* with no urchin mortality caused by any other predator across a 5-year period (Duggins, 1983). Sunflower stars prey on all three of the common urchin species in the shallow subtidal: *S. purpuratus*, *S. droebachiensis*, and *M. franciscanus* (Duggins, 1983). Snapshot surveys found that anywhere between 10-19% of *P. helianthoides* were observed consuming urchins (Duggins, 1983; Herrlinger, 1983). Rates of predation on urchins can range between 0.68-0.86 urchins per day based on laboratory studies (Duggins, 1983; Galloway et al., 2023) and do not appear to be influenced by the health (i.e. nutritional value) of the urchins (Galloway et al., 2023). Sunflower stars appear to preferentially consume urchins with test diameters between 6-8cm, which corresponds to the typical maximum sizes achieved by *S. purpuratus* and *S. droebachiensis*, while *M. franciscanus* typically lie above this preferred range outside of infrequent recruitment events and therefore achieve size refuge (Duggins, 1983). The inferred size refuge in red urchins is supported by very small observed population effects of *P. helianthoides* predation on red urchins ≥8cm compared to larger effects seen on smaller red urchins (Burt et al., 2018) and the decrease is escape response exhibited by red urchins over 8cm test diameter (Duggins, 1983).

Predation of urchins by *P. helianthoides* has received particular attention in recent years due to the dramatic increase in urchin populations and the corresponding decline of kelp coverage across the west coast of North America. Urchins are capable of clearing grazing down entire kelp beds and creating persistent “barrens” devoid of macroalgae (Galloway et al., 2023; Konar & Estes, 2003; Pearse, 2006). The predation behavior of sunflower sea stars has been identified as a notable influence on urchin distribution, creating grazer-free patches that facilitate

algal succession and promote species richness (Dayton, 1975; Duggins, 1983). Algal growth is further supported by sunflower stars through non-consumptive trait-mediated indirect interactions (TMII) whereby sunflower stars create a "landscape of fear" that causes urchins to flee and reduce their grazing rates (Watson & Estes, 2011; Whippo et al., 2024).

The recent concern regarding urchin populations and the impact of *P. helianthoides* has largely been driven by the sudden and dramatic decline—and in some cases, extirpation—of sunflower sea stars across the entirety of their range following the outbreak of sea star wasting disease (SSWD) in 2013. *P. helianthoides* population declines ranged from >87% at the northern end of their range to >99% in the southern half (Gravem et al., 2020; S. L. Hamilton et al., 2021). Estimates of abundance prior to the wasting event put estimates of overall losses on the order of billions of animals (Gravem et al., 2020). Recovery following the initial outbreak has been minimal and slow, leading to patchier population distributions (S. L. Hamilton et al., 2021), although limited re-population has been observed in Oregon and Washington, USA since 2022 (S. Hamilton, personal communication, personal observation). The decline of sunflower star populations has already been linked to declines in kelp forests in northern California and British Columbia due to the release of predation pressure on intensive grazers like urchins (Burt et al., 2018; McPherson et al., 2021; Rogers-Bennett & Catton, 2019) and long-term impacts of sunflower star extirpation have yet to be characterized, especially in regions without sea otter populations.

Sea urchins are only one of the many benthic grazers consumed by sunflower stars and while urchins may assert the strongest grazing pressure on kelp, grazing activity by other benthic invertebrates cannot be dismissed. Quantifying the potential predatory influence of *P. helianthoides* on multiple grazers is important in understanding the influence that *P. helianthoides* has on benthic invertebrate assemblages and benthic ecosystems. Most efforts to characterize and quantify direct predation pressure were conducted in a limited number of regions and habitats. It is evident that sunflower star diet varies according to region, habitat type, prey availability, and competition with other predators (Herrlinger, 1983; Lambert, 2000; Mauzey et al., 1968), necessitating further investigation of sunflower star predation habits in a variety of locations and habitats. Much of the current research efforts regarding *P. helianthoides* impacts are focused on TMIIs and the effect on grazing behavior (Wetmore, 2022; Whippo et al., 2024). While indirect effects are meaningful, measuring direct consumptive effects is important

for quantifying impacts on benthic invertebrate population density and understanding density-dependent predation. Sunflower star predation rates on purple urchins (*Strongylocentrotus purpuratus*) in an experimental setting have the potential to facilitate recovery of kelp forests (Galloway et al., 2023), providing incentive to investigate *P. helianthoides* predation rates on other grazer species.

I used a controlled feeding assay to measure *P. helianthoides* predation rates and size selection on a variety of benthic invertebrates from Sitka Sound, Alaska that are known to be preyed upon by sunflower stars: *S. droebachiensis, M. franciscanus*, *H. franciscanus*, *Saxidomus sp.*, and *T. pulligo*. I offered multiple sizes of prey and closely monitored individual grazer mortality to track consumption and size selection. I also measured prey caloric density and growth of the sea stars over the course of the experiment to explore the effects of prey type and feeding rates on sea star growth. With this feeding assay, I aimed to address 3 different research goals: (1) To investigate whether *P. helianthoides* predation rates differed between prey species, (2) compare whether prey size or sunflower star size altered predation rates to quantify size-dependent selection (3) determine whether caloric density of prey and consumption rate impacted sunflower star growth. I predicted that predation rates, size selection, and sea star growth would differ between the offered prey types, with urchins being eaten at the highest rates. I also expected to see a preference for smaller urchins overall.

Methods

Organism Collection

A total of 19 *P. helianthoides* were collected from shallow subtidal areas in and around Sitka Sound, AK in the summer of 2023. Fifteen stars were collected from within Sitka Sound and held in the lab on a maintenance diet of clams and mussels for at least three weeks before the start of the experiment. The remaining four stars were collected approximately 45km away in the Salisbury Sound at Piper Island (57.389916, -135.598046) and held for three days without food before the experiment. The stars were contained in individual plastic storage containers (approx. 17 gallon) with separate plumbing and constant flow of fresh seawater to each container. Semi-translucent lids were utilized to contain sea stars and prey and allow for exposure to a day/night cycle. Prey were kept in flow-through tanks and all except clams were regularly fed kelp. Each star was randomly assigned to one of five diets: red urchins (*Mesocentrotus franciscanus*, n = 4),

green urchins (*Strongylocentrotus droebachiensis*, n = 4), pinto abalone (*Haliotis kamtschatkana*, n = 3), clams (*Saxidomus sp.*, n = 4), and turban snails (*Tegula pulligo*, n = 4). The two urchin species were collected from the "barren" site Harris Island (57.0317, -135.2775), abalone and turban snails were collected from kelp forest and mixed habitats around DeGroff Inlet (57.176219, -135.541490), Hayward Straight (57.164967, -135.555844), and Cape Burunof (56.986619, -135.388075). Clams were collected from intertidal mudflat at the mouth of the Kaasda Héen (57.0464, -135.3122).

Feeding Assay

I fed the sea stars *ad libidum* for 35 days by maintaining availability of six prey items to each star at all times. Prey items were provided at a range of sizes. Size categories (small, medium, large) were determined separately for each prey species based on the size ranges of prey available at our collection sites. All sea stars had 2 small, 2 medium, and 2 large prey available to them throughout the experiment. Individual prey items were measured (diameter or length, height, and wet weight) before placement in a sea star tub and tests/shells were measured in the same way after egestion. Diameter and height were taken with calipers. Prey individuals were held out of water for ~15 sec before weighing to allow excess water to drip. Unique combinations of prey morphometrics allowed for tracking of specific prey individuals throughout the experiment. Sea stars were measured (radius from center of oral disc to longest arm tip) and weighed at the beginning and end of the experiment. For weight measurements, I placed each sea star on a flexible cutting board, tilted gently to drain excess water, and gently patted the aboral surface with a paper towel before weighing.

I checked the sea stars four times per day (approximately 8am, 11am, 2pm, and 5pm), recording activity (active/inactive) and feeding status (eating/not eating). The status of each prey individual (eaten/not eaten) in each tank was also recorded at every check. If empty shells/tests were found during a check, they were removed, measured, and the consumed prey individuals were identified. New prey individuals of the same size categories as the consumed individuals were selected as replacements. Time and date of removal were recorded for each shell/test, along with the time and date of the addition of each replacement prey individual. Prey individuals that were left untouched for at least half of the experiment duration (17 days) and ultimately not consumed were marked as having been "not eaten" by the sea star and were removed and

replaced with new prey items of the same size class. Three of the stars assigned to the turban snail diet and one star assigned to the green urchin diet refused to eat for the duration of the experiment and, as a result, were excluded from analysis along with the fourth snail-assigned sea star.

After the first 5 weeks (35 days), the above experiment was continued with an adjusted protocol for an additional for an additional six weeks (42 days). The extended timeframe was to continue to measure predation rates, and to maintain the diet to test long-term effects of diet on trophic biomarkers (Chapter 2). However, only prey that had been consumed (empty shells egested) were measured, and sea stars were checked only once per day. Because prey rejection was not tracked and only empty shell diameter recorded, the data from this portion of the experiment could not be included in the size selection distributions. Predation rates are also somewhat less precise due to the lower-frequency daily checks. Unfortunately, a lethal wasting event at the end of the experiment prevented final sea star measurements and weigh-ins, also removing the ability to calculate growth during this period.

Because some sea stars did not begin eating right away due to acclimation stress, predation rates were corrected for delay in starting date, calculated as the number of prey consumed divided by the number of days spent eating. A corrected daily consumption rate was also obtained for total biomass consumed by each star. Here, "biomass" refers to the mass of soft tissue not including shells or tests (sum of consumed individual prey weights – test/shell weights) since they are not digested by the sea stars. Biomass is measured for each prey individual by calculating the difference between the individual's initial weight and the weight of its shell after death, making tracking of individual prey critical.

Prey Sampling and Calorimetry

Ten individuals of each of the five experimental prey species were collected and sacrificed for tissue sampling. All prey items were processed separately, and all body tissues (excluding shells/tests) of each individual were homogenized as much as possible using knives, scissors, and a mortar and pestle. Mollusks were euthanized by freezing and allowed to thaw prior to tissue sampling. Urchins were processed live.

Homogenized prey tissues were lyophilized for at least 48 hours to ensure dryness and subsequently stored at -20°C. The gross energy density of each tissue sample was determined

using a Parr semi-micro bomb calorimeter. Only one stainless steel bomb was available for use, ensuring consistency in bomb calibration between samples. To maintain a constant heat capacity, the same volume of water was used for each run. A 0.2g pellet of benzoic acid standard was run as a procedural blank every 12-16 uses of the bomb to verify consistency and accuracy of readings. To ensure combustion of prey samples, approximately 0.02g of dry tissue was mixed with benzoic acid at a ratio between 1:5 and 1:7 before being manually pelleted in a punch and die set with a hammer. Each pellet was freshly made before combustion to reduce absorption of water vapor. Four replicates were run per sample and averaged to obtain a mean caloric density value for each sample.

Statistical Analysis

I performed four separate Kruskal-Wallis tests in R v(4.4.2) using the package "rstatix" to compare medians for predation rates, biomass consumption rates, estimated energy intake, and sea star growth rates. I then used Dunn's tests to conduct pairwise multiple comparisons between diet treatments following each Kruskal-Wallis test. Total energy consumed by each sea star (reported in kcal) was estimated by multiplying the average caloric density of its respective prey species by the total biomass it consumed during the first five weeks of the experiment. Total growth of each sea star over those five weeks was measured as weight gain (kg) since radius measurements were inconsistent due to the sea stars' mutable tissue. Distributions of the starting diameters of consumed and "rejected" prey were plotted as overlapping histograms to visualize size selection.

Caloric density (calories/g dry tissue) of each sample replicate was averaged to obtain a mean caloric density for each sample. The means for each sample were then further averaged within prey species to obtain an overall mean caloric density for each prey species. A Kruskal-Wallis test and Dunn's test were performed to compare the median caloric density between prey species.

For each prey species, a logistic regression was fit in R v(4.4.2) with the package "lme4" (four regressions total) to test the effects of prey size (initial diameter) on the chances of a prey individual being consumed. Chances of consumption were calculated as an odds ratio (ratio of eaten to non-eaten prey). Prey diameters were centered to the mean to simplify interpretation. The source of a sea star ("Sitka" or "Piper Island") was initially included as a random effect in

the models of treatment groups that included sea stars from both locations (red urchins and clams), but was ultimately removed because the inclusion of source as a random effect was either not beneficial to the model or caused a failure to converge due to small sample size relative to the number of factors in the model.

Results

On average, green urchins were eaten at the highest rate of all the prey types, with an average (±SE) of 1.2 (±0.08) green urchins eaten per day compared to 1.1 (±0.2) red urchins, 0.47 (±0.03) abalone, and 0.42 (±0.04) clams (Fig. 1). A Kruskal-Wallis test showed significant differences between predation rates overall (χ^2 3, N=15) = 11.16, p = 0.01), but pairwise comparisons only showed a significant difference between green urchin and clam predation rates (p = 0.02). A regression line fitted to a scatterplot of sunflower star weights and predation rates does not show a significant relationship between the two (R^2 = 0.01, p = 0.7) (Fig. 2). Mean predation rates during the second half of the experiment were lower than in the first half across all diet treatments (supplementary Figure S1). Although it was not included in analysis, the star that did accept and consume turban snails successfully ate a total of 44 snails over 35 days (1.26 snails/day) with no failed attempts.

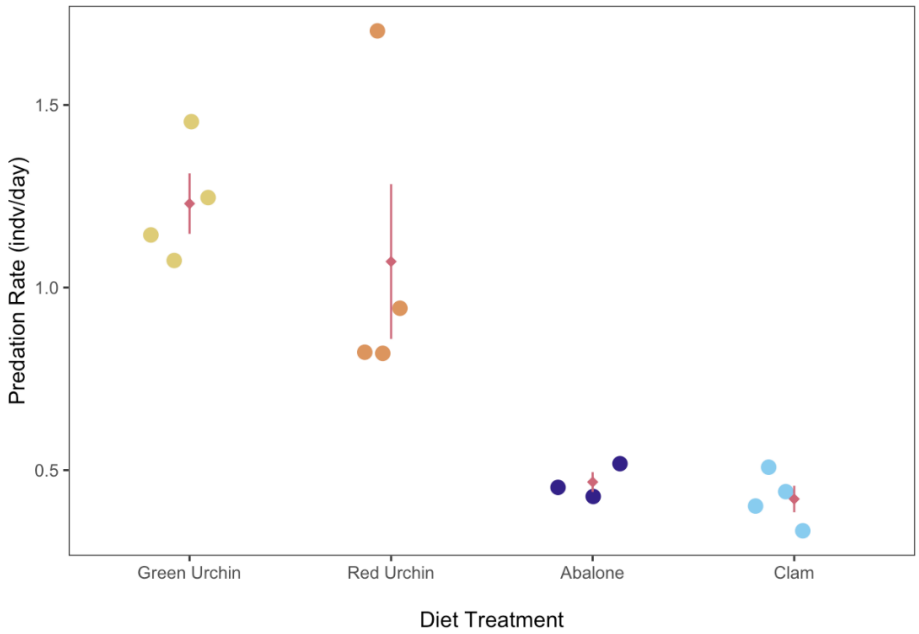

Figure 1. Predation rates on the four different prey species over 11 weeks. Means are represented by diamonds. Error bars are SE.

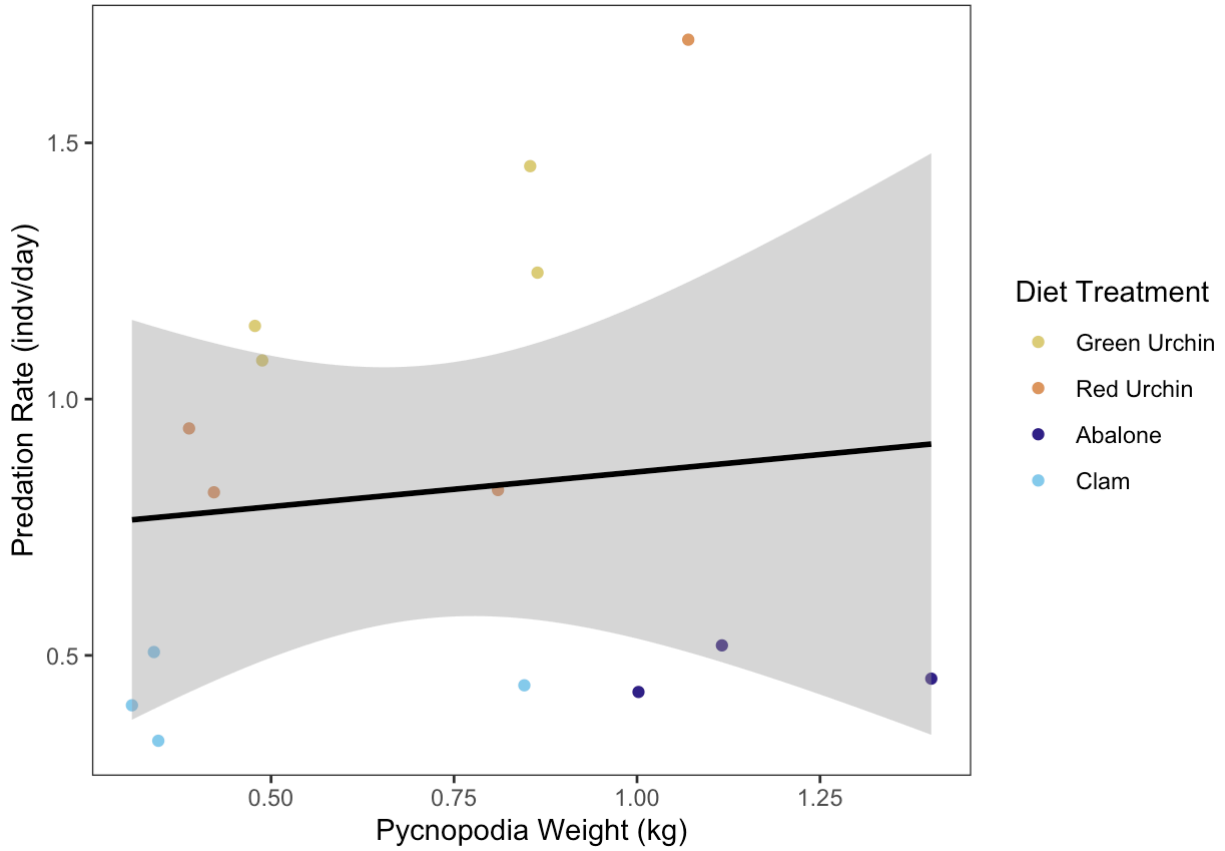

Figure 2. Scatterplot displaying the initial weights of sunflower sea stars compared to predation rates over 11 weeks. Points are colored according to assigned diet treatment. Regression line displayed with 95% CI.

Sunflower stars eating red urchins consumed the most biomass (grams of tissue) per day, on average. The mean (±SE) consumption of red urchin biomass was 28g (±8.5g) per day. Rates of biomass consumption in other treatments were lower. Average daily consumption rates were 14.2g (±1.8g) of abalone tissue, 12.4g (±2.8g) of green urchin tissue, and 7.5g (±0.3g) of clam tissue (Fig. 3). Biomass consumption rates were overall significantly different across diet treatments (χ^2 3, N=15) = 10.2, p = 0.012). However, only the consumption rates of red urchin and clam tissue were significantly different ($p < 0.01$) according to a pairwise comparison using Dunn's test.

Caloric densities (kcal/g tissue) were not significantly different across prey species according to a Kruskal-Wallis test (χ^2 3, N=39) = 3.39, p = 0.335). Median densities were 4.12 kcal/g, 4.08 kcal/g, 4.03 kcal/g, and 3.83 kcal/g in abalone, green urchins, red urchins, and clams respectively (Fig. 4). Based on the total biomass consumed by each sunflower star and the

average caloric densities of their prey, the sea stars eating red urchins consumed the most kilocalories per day at an estimated average of 113 (±34.1) kcal/day. The average (±SE) estimated energy consumption rates of the other sea star groups were much lower: 58.3 (±7.5) kcal/day for abalone-eaters, 46.5 (±10.4) kcal/day for green urchin-eaters, and 28.8 (±1.3) kcal/day for clam-eaters (Fig. 5). The estimated energy consumption rates were overall significantly different according to a Kruskal-Wallis test (χ^2 3, N=15) = 11.2, p = 0.011), but only the red urchin and clam treatment groups were found to have significantly different estimated energy consumption rates ($p < 0.01$) in pairwise comparisons.

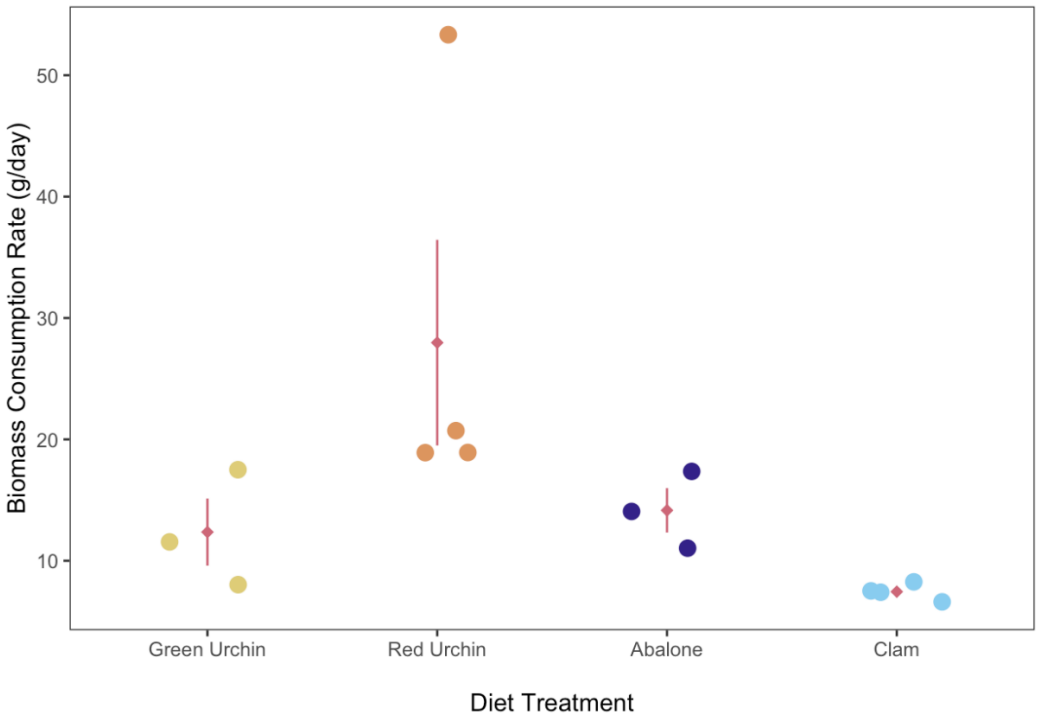

Figure 3. Rate of biomass consumption by sunflower stars in grams of tissue (wet weight) per day across diet treatments over 5 weeks. Means are represented by diamonds. Error bars are SE.

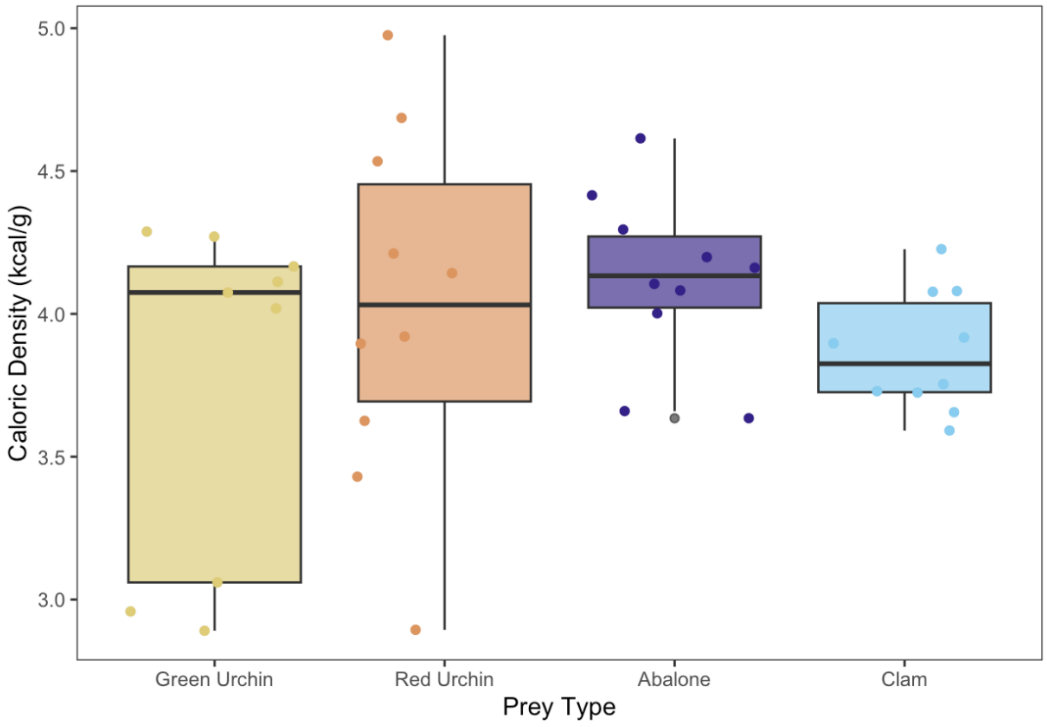

Figure 4. Boxplot showing distributions of average caloric density (kilocalories per gram of dry tissue) per sample across different prey species.

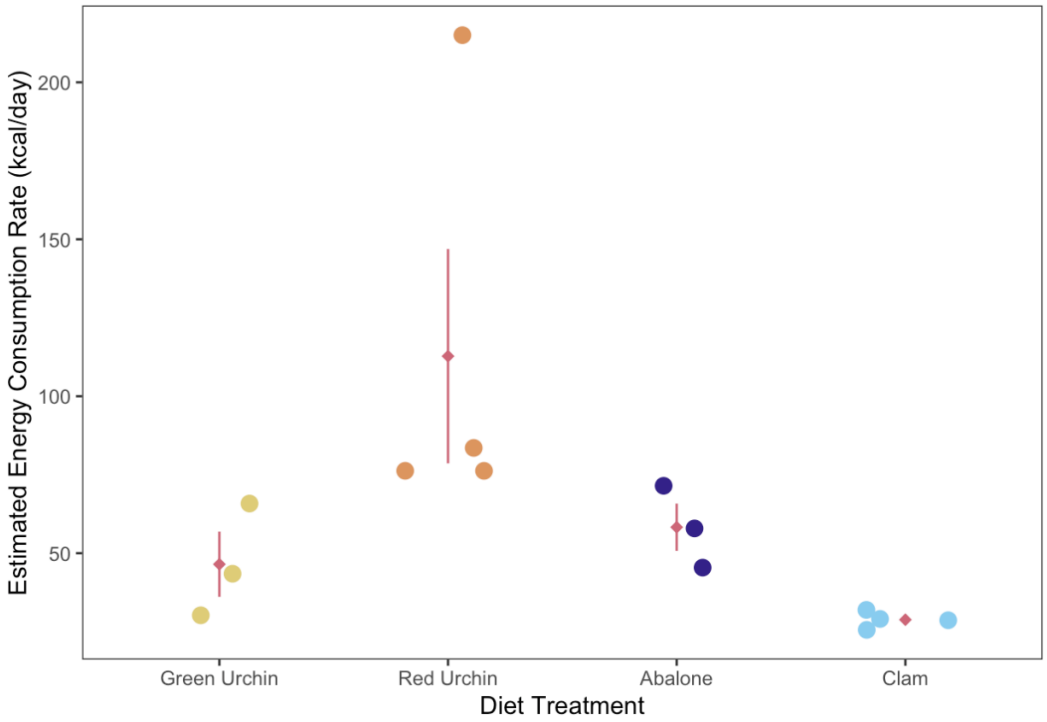

Figure 5. Rate of estimated energy consumption by sunflower sea stars over 5 weeks in terms of estimated kilocalories eaten per day. Means are represented by diamonds. Error bars are SE.

Compared to other diets, the sunflower stars that ate abalone had the highest mean (±SE) growth rate over the course of five weeks at a rate of 19.2 (±1.23) g/day. The growth rates of sunflower stars on the other diets were notably lower and closer to one another than the sea stars that ate abalone. Sunflower stars grew 9.71 (±3.18) g/day eating red urchins, 8.01 (±0.95) g/day eating clams, and 6.6 (±3.26) g/day eating green urchins (Fig. 6). However, the sea star growth rates were not found to be significantly different across treatment groups according to a Kruskal-Wallis test (χ^2 3, N=15) = 6.02, p = 0.11). Per kilocalorie consumed, sunflower stars eating abalone gained the most weight out of any treatment group. A regression line fitted to a scatterplot of estimated consumed energy and sea star growth shows a significant relationship between the two (R^2 = 0.31, p = 0.03). Urchin diets were generally the least efficient diets—the sea stars with the least weight gained per kilocalorie consumed were in urchin treatments.

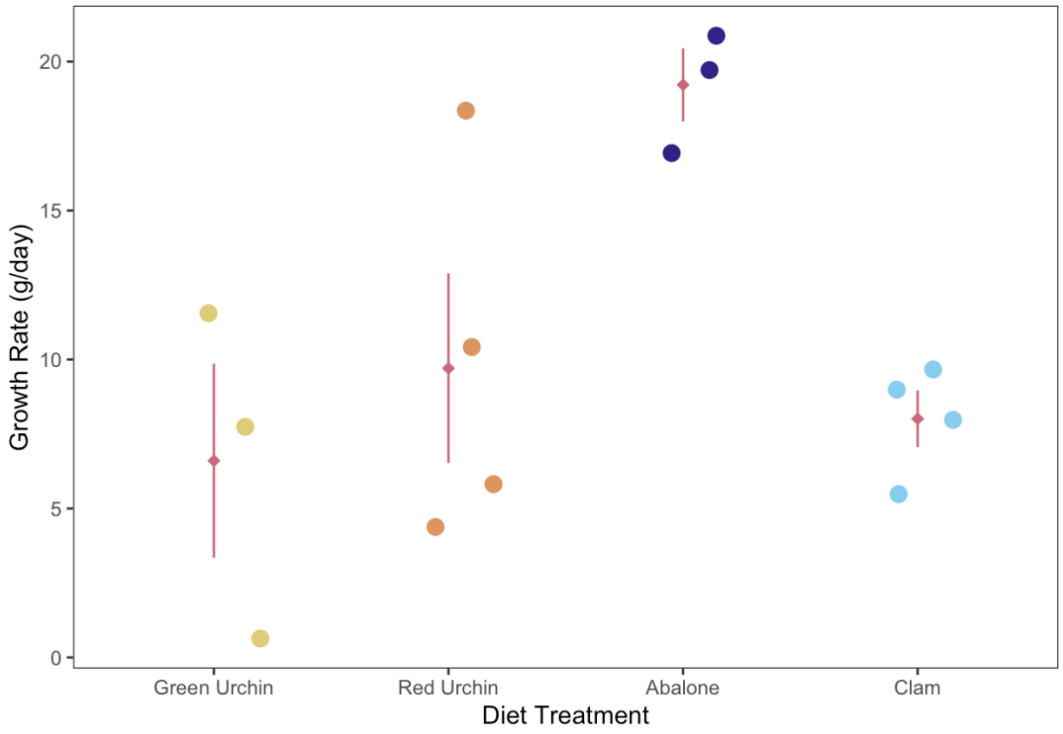

Figure 6. Growth rates of sunflower stars in grams per day over 5 weeks across different diet treatments. Means are represented by diamonds. Error bars are SE.

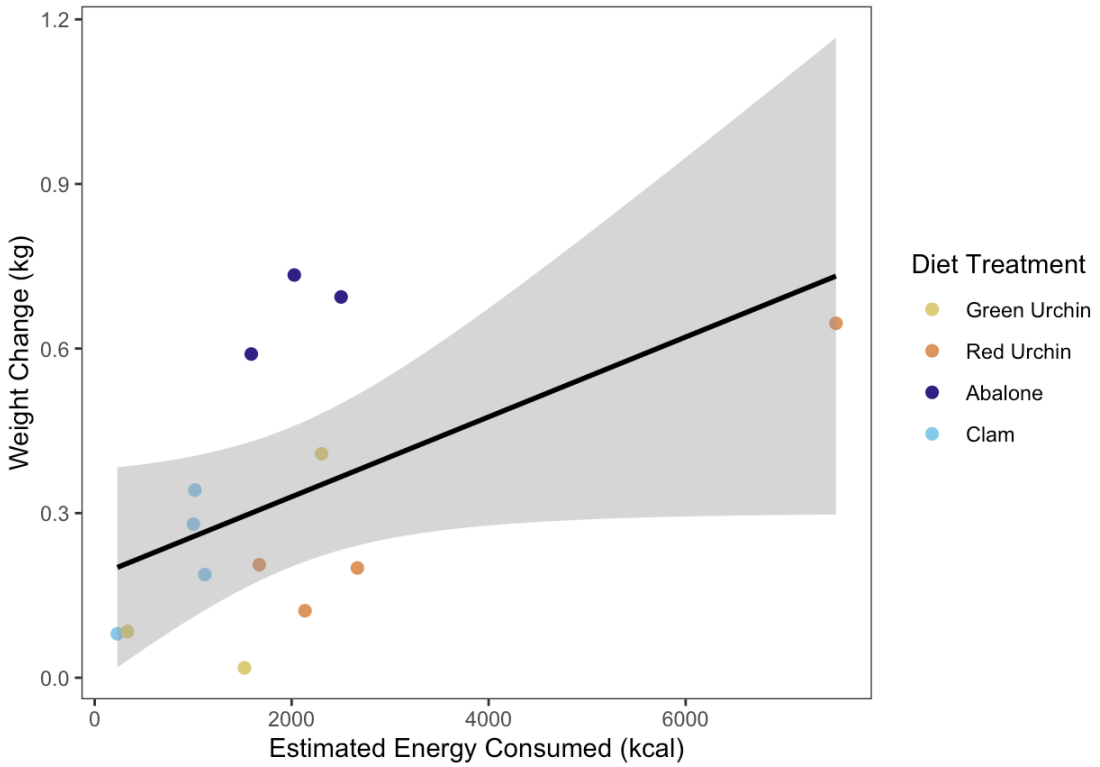

Figure 7. Scatterplot displaying estimated total energy consumed compared to kilograms of weight gain over 5 weeks. Points are colored according to assigned diet treatment. Regression line displayed with 95% CI.

For green urchins and clams, the size distribution of rejected prey sizes appears to mirror the distribution of consumed prey sizes. The distribution of rejected red urchin sizes, however, skews towards larger diameters than the distribution of sizes of consumed red urchins. No abalone were rejected over the course of the experiment (Fig. 8).

Logistic regression did not show a significant relationship between the diameter of a green urchin and its likelihood of being eaten ($z = -0.9$, $p = 0.37$). There was a relationship between red urchin size and predation likelihood ($z = 4.67$, $p << 0.001$). The odds ratio for green urchins indicated that a green urchin of average size (27.5mm) had a 15.7% chance of being eaten. For each 1mm increase in green urchin diameter from the mean, the chances of predation dropped by 4%. The odds ratio for red urchins indicated that a red urchin of average size (39.7 mm) has a 46% chance of being consumed and with each 1mm increase in diameter from the mean, the chances of a red urchin being predated are reduced by 31%. Logistic regression did not show a relationship between predation likelihood in clams and either clam diameter or sunflower star weight. No model was fitted for abalone since there were no rejected prey individuals in that group.

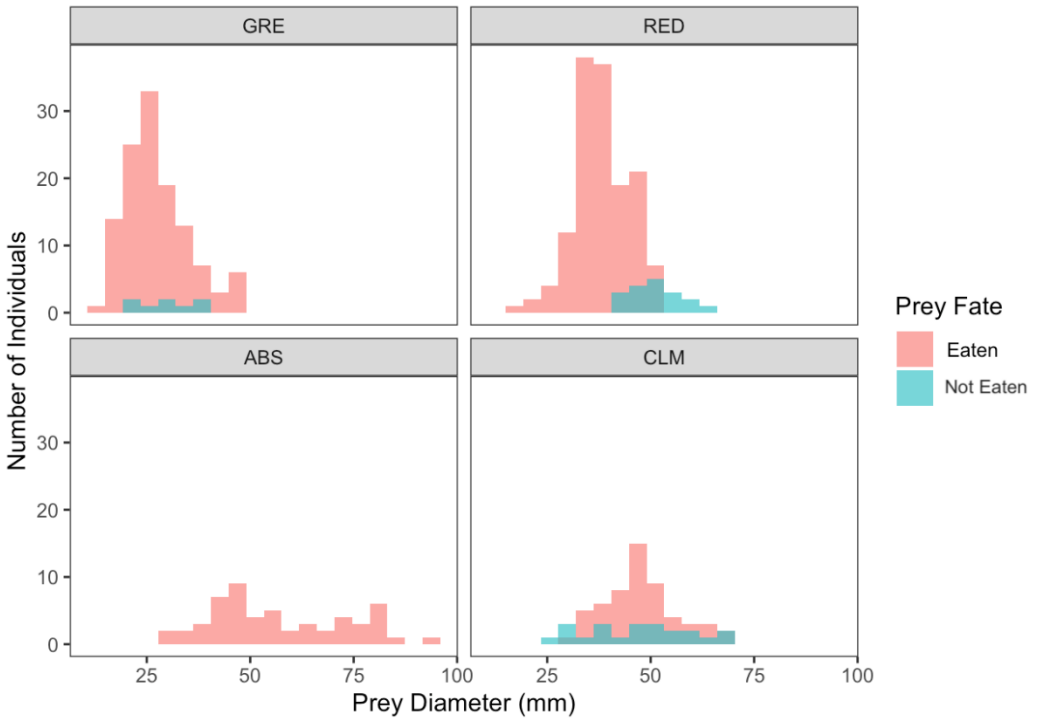

Figure 8. Histograms of the distributions of sizes of consumed individuals of all prey types, overlaid by the distributions of sizes of rejected individuals. Number of bins = 20.

Discussion

Sunflower star predation rates on urchins were higher than predation rates on mollusks, with both urchin species being consumed at a rate of approximately one individual per day while abalone and clams were consumed at a rate less than half that of urchins. The rates of consumption of both red (1.1) and green (1.2) urchins were higher than the predation rate on purple urchins (0.68 per day) reported by Galloway et al. (2023). While there could certainly be differences in geographical origin, physiology, or behavior of the sea stars that has caused the discrepancy in predation rates between urchin species, the high predation of red and green urchins remains promising for regions without abundant purple urchins. Although they are not as well-known as purple urchins for decimating kelp beds, red and green urchins nonetheless contribute to kelp forest declines in Southeast Alaska (Estes & Duggins, 1995).

Another potential benefit to kelp forest recovery is that predation rate does not appear to be influenced by sunflower star body mass, meaning consumption rates may not be closely tied to sea star size. It may be difficult to tease apart the influences of body mass and diet treatment on predation rates without additional sampling, as there is currently not a wide range of sea star

sizes represented within each diet treatment. Although mean predation rates were lower in the second half of the experiment (after the stars had grown), the decrease could have been due to non-growth-related factors such as water temperature or simply due to less frequent observation. The origin of the sea star and individual experience are other factors to consider. Benthic habitats within Sitka Sound can range from soft sand and cobble to dense kelp forest. While *P. helianthoides* is remarkably mobile and capable of traveling relatively large distances, individuals may experience a "learning curve" when introduced to prey types from unfamiliar habitats (Galloway et al., 2023). None of the sunflower stars that had were in captivity before the start of the experiment had encountered an urchin in many weeks, and yet none had difficulty hunting urchins. However, some stars from Piper Island (a site with no urchins and perhaps none or few within kilometers of the site because of the mixed rock-sand habitat) did initially struggle, evidenced by delayed starts to feeding and observations of failed hunting attempts. The effects of prior hunting experience and individual learning on predation rate are difficult to measure and not easily tested without a better understanding of sea star cognition and memory, but they may play a role in effectiveness and efficiency of hunting.

The rates of biomass consumption across diets largely reflected the pattern of predation rates—a low predation rate corresponds with a low rate of biomass consumption. However, the sea stars eating green urchins consumed very little biomass compared to the number of prey they ate. This pattern suggests that green urchins have low amounts of soft, digestible tissue compared to other prey species. Optimal foraging theory posits that it is best for consumers to pursue food sources which provide the most energy per effort expended (Brewer & Konar, 2005) and by that logic, green urchins could be a poor choice of prey for sunflower stars. However, *P. helianthoides* did not display a preference between high-quality (well-fed) versus low-quality (starved) purple urchins in a choice experiment (Galloway et al., 2023), so the ability of *P. helianthoides* to detect and/or determine the "value" of a prey item may be limited. The lack of prey value discrimination could have physiological impacts on sunflower sea stars.

Although the median caloric densities were not significantly different between prey species, the green urchins had the largest interquartile range in caloric density of all the prey groups. Because the primary lipid stores—the primary sources of caloric content—in urchins are the gonads, the caloric density of urchins is largely subject to gonad production and therefore subject to the type and quantity of algal foods available (Schram et al., 2018). The red urchins

also had a wider interquartile and overall range of caloric densities compared to the mollusks, suggesting that red urchins also had variable gonad indexes. Accordingly, the sea stars in the green urchin diet treatment had the lowest mean growth rate, albeit by a small margin. Urchins have consistently higher gonad biomass in kelp-dominated habitats compared to urchins from barren habitats (Claisse et al., 2013). The urchins collected for this experiment were from a barren location, potentially explaining the wide ranges of caloric density. It is noteworthy, however, that despite barren urchins being a more inconsistent source of nutrition, the sunflower stars that ate them still gained weight after 5 weeks.

In contrast with the urchin diets, the abalone diet facilitated much faster growth rates and greater overall weight gain in sunflower stars, despite the sea stars not exhibiting high predation rates and consumption rates. The amount of sea star growth on the abalone diet is also disproportionate to the estimated energy consumption rate. Considering that abalone did not have significantly higher caloric densities than the other prey species, the sea stars that ate abalone experienced the highest amount of growth per kilocalorie consumed, which suggests that something aside from caloric content may be making abalone more nutritionally valuable to sunflower stars. However, it must be noted that as necessitated by the protocols for tracking live prey and for prey calorimetry, there is a discrepancy in the weight measurements used for biomass consumption and caloric density. Biomass is measured as wet tissue weight and caloric density is reported in terms of dry weight. As a result, the estimated energy intake rates are likely overestimates since water weight is included in biomass measurements but not in the caloric density measurements. Intake of energy from urchins and clams is likely to be notably overestimated due to the large volumes of water held within the test/shell of living animals. A conversion factor from living (wet) biomass to dry biomass should be calculated to more accurately predict energy consumption by sea stars. Despite the caveat in estimates of energy consumption, predation rates and sea star growth rates show that it takes fewer abalone to produce the same amount of growth as the other diets, posing abalone as a potentially valuable prey source for *P. helianthoides*. Ideally, growth of sunflower stars on these diets will continue to be measured, using stars with a wider variety of starting sizes and over longer periods of time.

Although all the individual sea stars in this experiment were large enough to be considered sexually mature, the size of *P. helianthoides*, or rather its life stage, may influence diet. Newly settled sunflower stars are noted to initially consume biofilm (Lambert, 2000), but

quickly transition to eating juvenile urchins and bivalves (Hodin et al., 2021). This diet reflects what *P. helianthoides* consumes as an adult, but a key difference in the diets of young juveniles is the prevalence of cannibalism, which is not observed in older juveniles and adults (Hodin et al., 2021). Young juvenile sunflower stars grew fastest when eating conspecifics (Hodin et al., 2021), implying that cannibalism is an important factor in juvenile success, or is at least a beneficial strategy. Ontogenetic habitat shifts may also impact diet in the wild. Juvenile sunflower stars are commonly found in estuarine eelgrass beds (J. Hodin, personal communication; personal observation) where cover is greater, whereas adult sunflower stars are typically only found coastally, potentially differentiating the prey typically consumed by juveniles from that of adults. As young sunflower stars are beginning to make a resurgence in previously extirpated areas, understanding the trophic impact of the juvenile life stage and assessing whether it is different from adult sunflower stars is of interest.

Prior evidence suggests that size selection by *P. helianthoides* can impact the population dynamics of pinto abalone, as juveniles are preferentially predated (Griffiths & Gosselin, 2008; Read et al., 2013). However, in this experiment, predation of abalone was indiscriminate. All abalone were eventually consumed, regardless of size, although small abalone were typically pursued and captured more quickly than large individuals. It is noted by Griffiths and Gosselin (2008) that size selection of abalone was relative to the size of the sea star. Because the individuals that were randomly assigned to the abalone diet happened to all be larger sea stars, there was likely no need for them to discriminate by prey size, since even the largest abalone were small enough to be easily engulfed. Another possible explanation is the confined captive setting. In the wild, pinto abalone have been observed to outrun and escape sunflower stars (Lee et al., 2016), but the small tanks likely prevented successful fleeing and artificially increased predation success. Additional experimentation with a wider range of sunflower star sizes in a more natural habitat will help elucidate *P. helianthoides* size selection of abalone. However, the indiscriminate predation observed here does suggest that large sunflower stars may be more impactful predators of pinto abalone than previous knowledge of diet composition might suggest.

Sunflower stars also did not display a clear size preference in clams or green urchins. Only red urchins displayed a pattern in size selection by the sea stars. All red urchins below 42 mm diameter were consumed and red urchins above 50 mm diameter were nearly always ignored, showing a preference for smaller individuals. Interestingly, this size refuge is lower than

the roughly 8 cm diameter maximum for red urchin consumption observed in the wild (Duggins, 1983), which may be due to the fact that the sea stars in this experiment are not very large by pre-wasting standards. In fact, all but three of the stars in this experiment would have fallen in the lower 50% of the size distribution (10-60 cm diameter) of *P. helianthoides* observed consuming urchins in the wild pre-wasting (Duggins, 1983).

Additionally, in comparison with other urchin species, red urchins have more effective defenses against sea star predation than other urchin species by virtue of their long spines, which could explain a lower size refuge threshold in red urchins than in other species. In a choice experiment, *P. helianthoides* chose more easily-subdued purple urchins over red urchins 98% of the time (Moitoza & Phillips, 1979). Spine length typically increases with red urchin size, strengthening the deterrent power of large individuals. The results of the generalized linear model of red urchin consumption odds, which state that likelihood of predation decreases with test diameter, are consistent with the pattern shown in the histograms. However, the power of the model to accurately predict predation odds may be limited by the small sample size of rejected urchins. In contrast, test diameter was not found to be a significant factor in determining predation odds in green urchins, which is consistent with the very low number of uneaten green urchins in this experiment and the size preferences of *P. helianthoides* seen across urchins in general—all green urchins were below the established 6-8 cm size refuge threshold observed by Duggins (1983).

Unlike the abalone diet treatment, the red urchin diet treatment included stars of a variety of sizes, so the persistence of a prey size selection pattern across a wider range of sunflower star size shows that preference for small red urchins might not be limited to small sunflower sea stars, evidenced by the fact that even amongst a pre-wasting population where sunflower stars could reach >50 cm in diameter, urchins over 8 cm were rarely eaten (Duggins, 1983). This size selection has the potential to impact the population dynamics of red urchins. Monitoring of red urchin population dynamics in a central California kelp bed revealed slow recruitment rates and a sudden rapid decline of 20-40 mm diameter individuals attributed to predation by *P. helianthoides* as growing recruits outgrew crevices and invaded the algal turf. As a result, red urchin density decreased over time and the remaining urchins were restricted to deep crevices (Pearse & Hines, 1987). More recently, sunflower star predation was found to more strongly impact populations of small and medium red urchins (<8 cm diameter) with little effect on larger

urchins (Burt et al., 2018), which supports the findings of pre-wasting observation and the size preference seen in this experiment. Consistent population control of this nature by *P. helianthoides* could have the capacity to regulate grazing pressure on kelp, particularly in areas where red urchins are the dominant urchin species (Burt et al., 2018).

These experimental predation rates and size preferences—or lack thereof—of *P. helianthoides* have multiple implications for trophic dynamics, particularly in kelp forests. Sunflower stars ate green and red urchins at higher rates than reported predation rates on purple urchins. If these predation rates reflect consumption patterns in the wild, *P. helianthoides* predation on green and red urchins could be an influential factor in maintenance of kelp cover alongside predation on purple urchins (Galloway et al., 2023). Sunflower stars may also have an unexpectedly large impact on pinto abalone in certain regions. While size selection was not seen in this experiment, *P. helianthoides* are clearly eager consumers of abalone and large individuals in particular are in a position to influence abalone population dynamics due to their lack of prey size discrimination. Even when co-ocurring with sea otters, sunflower stars have been experimentally shown to have a higher efficiency of abalone capture than sea otters in structurally complex substrate (Lee et al., 2016), establishing them as competent abalone predators. Abalone are also nutritionally beneficial to sunflower stars, as evidenced by the large amount of growth achieved on the diet, making them a valuable food source.

In the face of declining kelp forests, understanding the trophic impacts of *P. helianthoides* predation is crucial. Through this feeding assay, I have reported baseline predation and predator growth rates on four different prey species diets, as well as a preferential selection of small red urchins by sunflower sea stars. Although many of the patterns and relationships displayed by these results are aligned with other experimental and survey results, this feeding assay would have greatly benefited from larger sample sizes within each diet group. These initial findings will ideally facilitate further investigation of sunflower star predation impacts on a variety of prey species, leading to additional experimentation and more robust conclusions about the potential trophic influence of sunflower sea stars.

CHAPTER II: FATTY ACIDS AS INDICATORS OF SUNFLOWER STAR DIET

Introduction

In the face of changing habitats and ecosystems, estimation of diet in consumers can become a continuous task as food type, availability, and distribution shift over time (Frelat et al., 2022; Gladics et al., 2014; Gulka et al., 2017). Changes in consumer diets have been associated with shifts in community assemblages in marine habitats (Frelat et al., 2022; Gulka et al., 2017; Provencher et al., 2012). Consumption patterns suggest that for generalist consumers in changing environments, past snapshot estimates of diet composition may no longer be accurate or may be too simplistic. In rapidly changing coastal marine habitats, dietary shifts have already been observed in top predators such as seabirds and large fish (Howells et al., 2017; Olson et al., 2014; Provencher et al., 2012); updated investigations into the diets of other consumers may reveal similar trends. Diet estimation is traditionally done by observing feeding behavior or analyzing gut contents; while relatively simple to conduct, these methods can be limited in scope, only reflecting prey that has been recently consumed and potentially biasing against quickly digested soft-bodied prey. Moreover, unless gut contents can be evaluating using gastric lavage (Elston et al., 2015), gut content analysis usually requires sacrificing the subject of the study, which is not a good option for species which are rare or endangered. In instances where a more complete picture is needed, trophic "biomarkers" can offer a time-integrated reflection of diet (Dalsgaard et al., 2003; Parrish et al., 2000).

Fatty acids (FA) are physiologically important lipids that play roles in membrane structure, cellular signaling, hormone regulation, and—most importantly—energy storage (Kainz et al., 2009). Fatty acids can be influenced by changing environmental conditions and the corresponding physiological changes in the organism, but this is a secondary factor—the primary driver of FA differences between organisms being phylogeny (Galloway & Winder, 2015). The types and concentrations of FA created or consumed tend to be similar within taxa, resulting in similar overall fatty acid profiles. These taxonomic FA "signatures" can carry influence from primary producers and lower-level consumers to the FA profiles of consumers in higher trophic levels (Budge et al., 2006; Iverson, 2009), making it possible to potentially trace diet back to

specific taxa rather than the broad trophic categories provided by stable isotopes (Kelly & Scheibling, 2012; Pitt & Purcell, 2009).

Primary producers must synthesize all their fatty acids; marine algae are notably rich in long-chain polyunsaturated fatty acids (PUFA), for example, and are largely responsible for supplying marine trophic systems with these FA (Galloway & Winder, 2015). Animals, however, cannot synthesize the n-3 and n-6 FA they need *de novo* and therefore must obtain these FA (deemed "essential fatty acids") from dietary sources (Kainz et al., 2009). Although animals are capable of some FA modification, synthesis from dietary precursors, and even de novo synthesis of some PUFA (Kabeya et al., 2018; Wei et al., 2019), their limited capacity to do so means that FA have been generally assumed to be incorporated into body tissue with relatively little modification occurring (Iverson, 2009). As such, FA can provide a direct trophic link to diet. However, even with minimal modification, the FA profiles of consumers often do not completely match their diets due to phylogenetic differences, modification of precursor FA, or selective uptake of specific FA (González-Durán et al., 2008; Jardine et al., 2020).

To address the differences in FA profiles between consumers and diets, controlled feeding experiments that directly examine how diet influences consumer FA profiles have gained notable attention and importance in trophic biomarker research (Galloway & Budge, 2020; Kelly & Scheibling, 2012). Invertebrate consumers in particular are highly capable of selecting and significantly modifying specific FA from their diets, adding importance to the use of feeding trials when studying their trophic dynamics (Galloway & Budge, 2020; Schram et al., 2018; Thomas et al., 2020). Quantitative analysis of the outcomes from these feeding experiments, utilizing a wide array of FA (i.e., complete FA profiles rather than individual biomarkers) can illuminate the relationships between consumers and diets while accounting for variations in FA attributed to phylogeny or physiology (Schram et al., 2018; Thomas et al., 2020). The FA profiles of consumers fed known diets can be used along with the FA data from the diets themselves to inform predictive models such as QFASA (Quantitative Fatty Acid Signature Analysis; (Iverson et al., 2004)), which estimate the relative proportional contributions of different dietary components in organisms with unknown diets (Happel et al., 2016; Zhang et al., 2020). This quantitative FA analysis application is particularly useful for organisms that are elusive, rare, protected, or for which using traditional diet estimation methods is otherwise impractical (Zhang et al., 2020).

One such organism for which fatty acids could be a useful tool is the sunflower sea star (*Pycnopodia helianthoides*). Sunflower stars are large, predatory asteroids found in the Northeast Pacific. Sunflower stars are known to be generalist predators and scavengers of a wide variety of taxa, including sea urchins, sea cucumbers, gastropods, bivalves, barnacles, decapods and amphipods, vertebrate carcasses, and occasionally conspecifics as juveniles (in captivity) (Hodin et al., 2021; Lambert, 2000; Shivji et al., 1983). Sunflower stars inhabit both intertidal and subtidal rocky and soft-bottom habitats (Shivji et al., 1983). Their wide-ranging habitats and generalist diet give sunflower stars the potential to meaningfully influence benthic invertebrate populations and community assemblages. The influence of sunflower star predation within kelp forests has received particular attention since the decline or loss of the sea otter (*Enhydra lutris*) along much of the west coast of North America (Burt et al., 2018; Estes et al., 1978). However, the potential for trophic influence by sunflower stars also declined in many coastal habitats following the sea star wasting disease (SSWD) epidemic that began in 2013. Populations across North America's west coast suffered declines > 90% with seemingly complete extirpation south of the Salish Sea (S. L. Hamilton et al., 2021; Harvell et al., 2019). The sunflower star was designated as "critically endangered" by the IUCN (International Union for the Conservation of Nature) in 2021 (Gravem et al., 2020) and is currently slated to be listed as "threatened" under the Endangered Species Act. Limited recruitment of juveniles has been noted in Oregon in 2022-2023 (S. Hamilton et al., 2024) but sightings of large adults remain scarce. In regions that have maintained populations of adult sunflower stars post-SSWD, such as Southeast Alaska, densities generally remain below pre-wasting levels and can vary greatly year to year (Gravem et al., 2020). The loss of a once-abundant generalist predator, particularly of urchins, has been linked to the dramatic increase in urchin populations and corresponding decline in kelp density in parts of British Columbia and northern California (Burt et al., 2018; McPherson et al., 2021).

Previous research on sunflower star diet relied on observation of behavior and stomach contents and were limited to small populations in specific locations (Herrlinger, 1983; Shivji et al., 1983). Only within the past decade has sunflower star diet and feeding behavior received renewed scientific attention due to their large-scale decline (Galloway et al., 2023), which may reveal differences in sunflower diet due to changes in invertebrate communities. Increased ocean warming events and decline in habitats like kelp forests and seagrass beds have altered benthic invertebrate assemblages over the past 40 years (Meunier et al., 2024; Rogers-Bennett & Catton,

2019; Smale et al., 2017). While the broad categories of invertebrates consumed by sunflower stars may remain the same, shifts in the density and distribution of prey species may alter the proportional composition of sunflower star diet. Changes in prey availability and the trophic influence of sunflower stars as generalist predators suggests the need for updated research on the diets of these stars. Fatty acids are a promising new approach for studying sunflower star diet due to their ability to reflect specific taxa within diets. Additionally, sampling tissue for FA analysis can be minimally invasive in sea stars and does not rely on finding individuals who are actively hunting or feeding, which are key benefits when working with a threatened species. To date, only one study has examined the FA of sunflower sea stars (Taradash 2022, Taradash et al. in prep) and few other studies have used FA to examine diets of other asteroids (Howell et al., 2003; Latyshev et al., 2001). To model diet composition using FA, the FA profiles of sunflower stars and the impact of known diets on the predator's FA signature must first be characterized.

I used a controlled feeding assay to measure and compare the FA profiles of sunflower stars fed five known single-species diets in captivity: green and red sea urchins, pinto abalone, butter clams, and dusky turban snails. Each of the prey species used in the assay, along with two additional prey species, were collected and sampled for FA analysis to compare to the FA of sunflower stars and to begin building a "library" of prey FA data. I aimed to address four different research goals: (1) To add to existing data regarding general FA composition of sunflower stars, (2) assess how known diets affect sunflower star FA composition, (3) compare FA profiles of prey species to one another, and (4) investigate FA of wild sunflower stars with unknown diets and compare them to those fed known diets. I hypothesized that the sunflower star FA would differ between diets, with the most notable distinctions being reflective of the phylum level of the prey, and that wild sunflower stars from two different locations/habitat types would have different FA profiles.

Methods

Feeding Assay

The feeding assay described in Chapter I was also used for measuring dietary impact on fatty acids. *P. helianthoides* were fed one of the described five diets *ad libidum* for 35 days and subsequently sampled, which involved weighing, measuring radius, and snipping 1-2cm of tissue

from one arm tip for fatty acid analysis. Three of the stars assigned the turban snail diet and one star assigned the green urchin diet refused to eat for the first five weeks of the experiment.

Feeding resumed for an additional six weeks—the four starved stars were randomly re-assigned either turban snail (n=3) or green urchin (n=1) diets. The turban snails were euthanized by freezing and were thawed and crushed before being fed out to incentivize consumption by the sea stars. At the conclusion of the full ten-week period, arm tip tissue was again collected from each star. Unfortunately, a lethal wasting event prevented weighing and measuring of the stars at the ten-week time point.

Wild *P. helianthoides* and Prey Sampling

Two sets of arm tip tissue samples were collected from wild sunflower stars. Seven stars were collected on SCUBA and sampled in July 2021 from Magic Island (57.0980, -135.401) in Sitka Sound. At the time of collection, Magic Island was characterized by dense kelp forest habitat on complex, rugose substrate (S. Gravem, personal communication). Likely prey available to sunflower stars at this site include sea urchins, abalone, snails, and potentially barnacles. An additional 25 stars were collected and sampled in August 2024 from Piper Island, a mixed-habitat site with sand, boulders, cobble, and occasional patches of kelp (A. Galloway, personal communication). Potential prey available at Piper Island include clams, barnacles, snails, abalone, and sea urchins. The wild sea stars collected were primarily smaller “young adults”.

Ten individuals from each of the five experimental prey species were collected and sacrificed for tissue sampling. The two urchin species were collected from the “barren” site Harris Island (57.0317, -135.2775), abalone and turban snails were collected from kelp forest and mixed habitats around DeGroff Inlet (57.176219, -135.541490), Hayward Straight (57.164967, -135.555844), and Cape Burunof (56.986619, -135.388075). I collected clams from intertidal mudflat at the mouth of the Kaasda Héen (57.0464, -135.3122). All prey items were processed separately, and all body tissues of each individual prey sample were homogenized as much as possible using knives, scissors, and a mortar and pestle. This approach was taken so that whole body tissues analyzed would be reflective of the actual whole-body digestion of the predator, i.e., rather than sampling specific tissue types only. Mollusks were euthanized by freezing and allowed to thaw prior to tissue sampling.

I sampled two non-experimental prey types known to be eaten by sunflower stars to provide additional fatty acid data for a "prey library". I collected acorn barnacles (*Balanus glandula*) from three locations around Coos Bay, OR: South Cove and Sunset Bay at Cape Arago, and the Charleston Marina. Small aggregations of 10-20 barnacles were lumped and treated as individual samples to obtain sufficient tissue mass for lipid extraction. I extracted whole bodies from shells and homogenized them in a stainless-steel mortar and pestle. I also gathered black rockfish (*Sebastes melanops*) carcasses originating offshore from Cape Arago from fish cleaning stations at the Charleston Marina. Carcasses were chosen over fresh fish to more closely reflect the form in which fish are typically encountered and eaten by sunflower stars in the wild. I sampled lean muscle tissue from the head above the gill arch and along the spine using scissors. The muscle tissue was finely minced to homogenization. All tissue samples were stored at -20°C following collection.

Fatty Acid Analysis

Tissue samples were stored at -20°C until they were lyophilized for a minimum of 48hrs. Tissues were returned to -20°C until extraction. I extracted all samples within 8 months of collection, with the exception of those from 2021. Before extraction, each sample was ground into a homogenous powder using a stainless-steel mortar and pestle. For especially fibrous tissue, I also utilized spatulas to help break and cut the tissue. The homogenized tissues were digested in chloroform, sealed under nitrogen at -20°C, for a minimum of 24 hrs. Total lipids were extracted using a modified Folch method and derivatized into fatty acid methyl esters (FAME) using a modified method described by Taipale (2016).

Following tissue digestion, lipids were extracted using a 2:1:0.75 mixture of $ChCl_3$:MeOH:NaCl (0.9%). Nonmethylated nonadecanoic acid (19:0) was added as an internal standard. Samples were sonicated, vortexed, and centrifuged to separate the denser organic phase, which was removed and evaporated to dryness under nitrogen gas. The extraction process was repeated after replacing 2mL of chloroform to each sample. To produce FAME, the dried lipid extracts were resuspended in a 1:2 ratio of toluene and 1% sulfuric acid in methanol and incubated at 90C for 90 minutes. Following transesterification, the samples were allowed to cool and were neutralized with 2% $KHCO_3$. Hexane was added and the samples were vortexed and centrifuged to capture the FAME in the hexane layer, which was removed and evaporated to

dryness under nitrogen. The FAME extraction process was repeated after adding additional hexane. Dried FAME were resuspended in 1.5mL hexane and stored at -20C.

FAME were analyzed with a gas chromatograph-mass spectrometer (Shimadzu GCMS model QP-2020) fitted with a DB-23 column (30 × 0.25 mm × 0.15 μm, Agilent, Santa Clara, CA, USA), using helium as the carrier gas. The heating program described in Taipale (2016) was utilized to ensure sufficient separation between chromatogram peaks. Fatty acids were identified by relative retention time and specific ions. Identifications were checked against a FAME standard (GLC 566C, Nu-Chek Prep, Elysian, MN, USA).

Statistical Analysis

Fatty acids were quantified by integrating chromatogram peaks in the Shimadzu LabSolutions Insight software. Peak areas were then converted to proportions, representing the % contribution of all identified FA within each sample. Data were log-transformed to reduce heteroscedasticity. Statistical separation of the proportional fatty acid profiles was tested using one-way permutational analysis of variance (PERMANOVA, $\alpha \leq 0.05$, 9999 permutations, Type III sums of squares, Euclidean distance) in Primer v6.1.13 with the PERMANOVA+ v.1.0.3 add-on (Anderson et al., 2008). Post hoc pairwise comparisons were also conducted and in instances where the number of possible unique permutations was too low, Monte Carlo p-values were calculated. To identify FA contributing to differences between species and diets, similarity percentage (SIMPER) analysis was conducted using the R (v4.4.2) package “vegan”. Data were visualized with non-metric multidimensional scaling (nMDS) plots in R using the “vegan” package and plotted using the “ggplot2” package. Vector overlays of the FA identified by SIMPER were mapped onto the nMDS plot to illustrate the directional influence of individual FA on the relationships between groups. This series of analyses (PERMANOVA, SIMPER, nMDS) was completed three times: (1) for the full dataset, including prey species and sea stars, (2) a subset that includes only sunflower star data (including from wild individuals), and (3) a further subset of only the sea stars from the experimental feeding assay. The stars that were in the turban snail treatment and/or starved during the first half of the experiment were ultimately excluded from the 11-week timepoint analyses due to the difference in feeding duration from the other diet groups. Data from the 5-week timepoint were analyzed using PERMANOVA and visualized using nMDS.

To further visualize the relationships between the experimental diet groups and wild sea stars, I conducted univariate comparisons of the untransformed proportions of the five FA that contributed to the most difference between groups, as identified by SIMPER analysis. Potential consumer FA modification was quantified by calculating the ratio between the mean proportions of each FA in consumer tissue to the mean proportions in their diets, for each diet treatment.

Results

Prey FA Composition

A total of 38 FA shared by laboratory-fed sea stars and experimental prey species were identified, with an additional 10 FA identified in wild sea star populations and non-experimental prey. The FA signatures of the prey groups were significantly different from the sea stars and from each other (one-way PERMANOVA, *post hoc* pairwise comparisons; Table 1a and supplementary Table S3) and showed notable clustering in multidimensional NMDS space (Fig. 1). Rockfish and clams had significantly higher proportions of DHA (22:6ω3, docosahexaenoic acid) than all other species, while pinto abalone had the lowest. Compared to grazer species (abalone, sea urchins, and turban snails), non-grazers (rockfish, clams, and barnacles) had comparatively low proportions of ARA (20:4ω6, arachidonic acid). The two urchin species had the highest proportions of SDA (18:4ω3, stearidonic acid) compared to all other species (Supplementary Table S1).

Table 1. Results of one-way PERMANOVA tests (Euclidean distance) of log-transformed FA proportions from (a) all *P. helianthoides* individuals and all potential diets (both experimental and non-experimental), (b) all *P. helianthoides* individuals, and (c) *P. helianthoides* fed mono-specific diets.

Comparison	Variable	d.f.	MS	Pseudo-F	P (perm.)	Unique perm.
(a) Combined	Identity	7	0.767	168.79	0.0001	9901
	Residual	108	0.0045			
(b) All sea stars	Treatment	5	0.022	10.676	0.0001	9926
	Residual	40	0.002			
(c) Experimental sea stars	Treatment	3	0.0098	5.69	0.0022	9887
	Residual	10	0.0017			

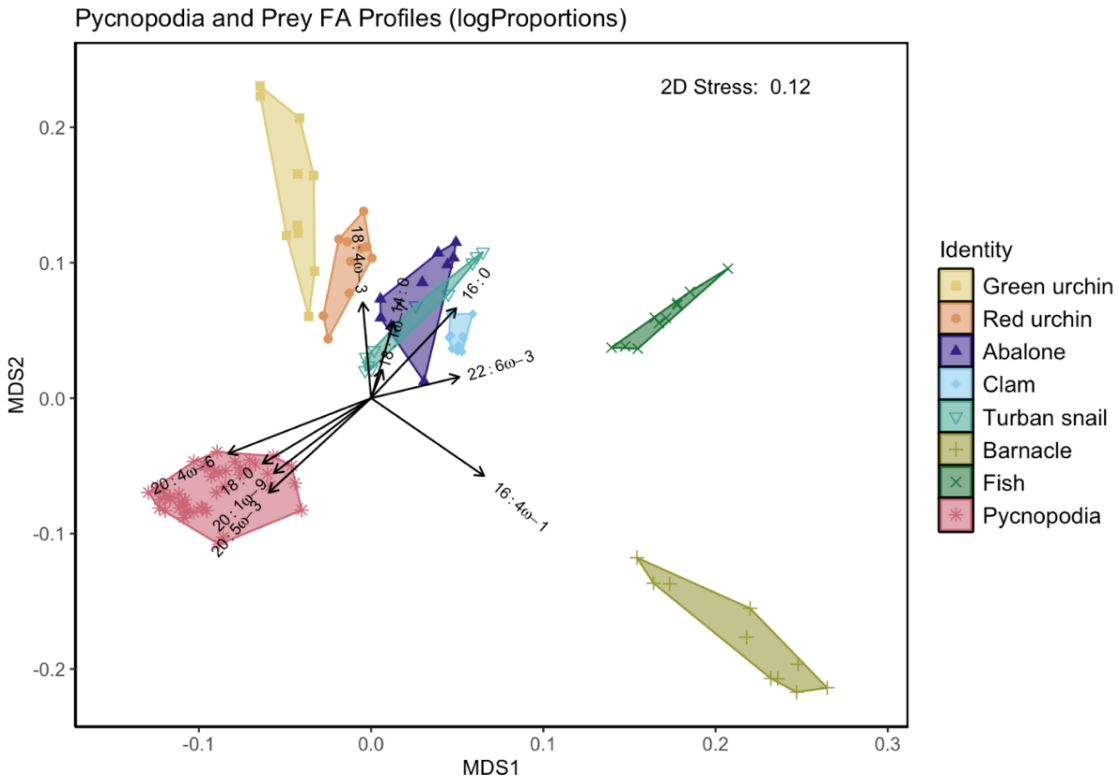

Figure 1. NMDS plots (Euclidean distances) of the log-transformed proportions of FA of all *P. helianthoides* individuals and all potential diets (both experimental and non-experimental).

Experimental *P. helianthoides* FA and Trophic Modification

After five weeks of feeding P. helianthoides single-species diets, the FA profiles of the sea stars were not significantly different (one-way PERMANOVA, $p \leq 0.0934$) according to their diet (supplementary Figure S1). After eleven weeks of feeding, the FA profiles of *P. helianthoides* differed significantly (one-way PERMANOVA, Table 1b) in multivariate space according to their diet (Fig. 2). *Post hoc* pairwise comparisons (with Monte Carlo permutations) showed that the green and red urchin diet treatments did not produce significantly different predator FA signatures ($P \leq 0.6855$, supplementary Table S5). The FA signatures of sea stars that ate abalone were also not significantly different from those that ate clams or either urchin species, although the p-values are marginal (Table S5). The clam diet produced predator FA signatures that were significantly different from those produced by both urchin diets ($P < 0.05$; Table S5). SIMPER analysis (Table 2) identified five FA contributing to a majority (>60%) of the differences separating each group from all others. All but one FA identified by SIMPER are also abundant (>5% of total FA) in the sea star profiles (Table 2).

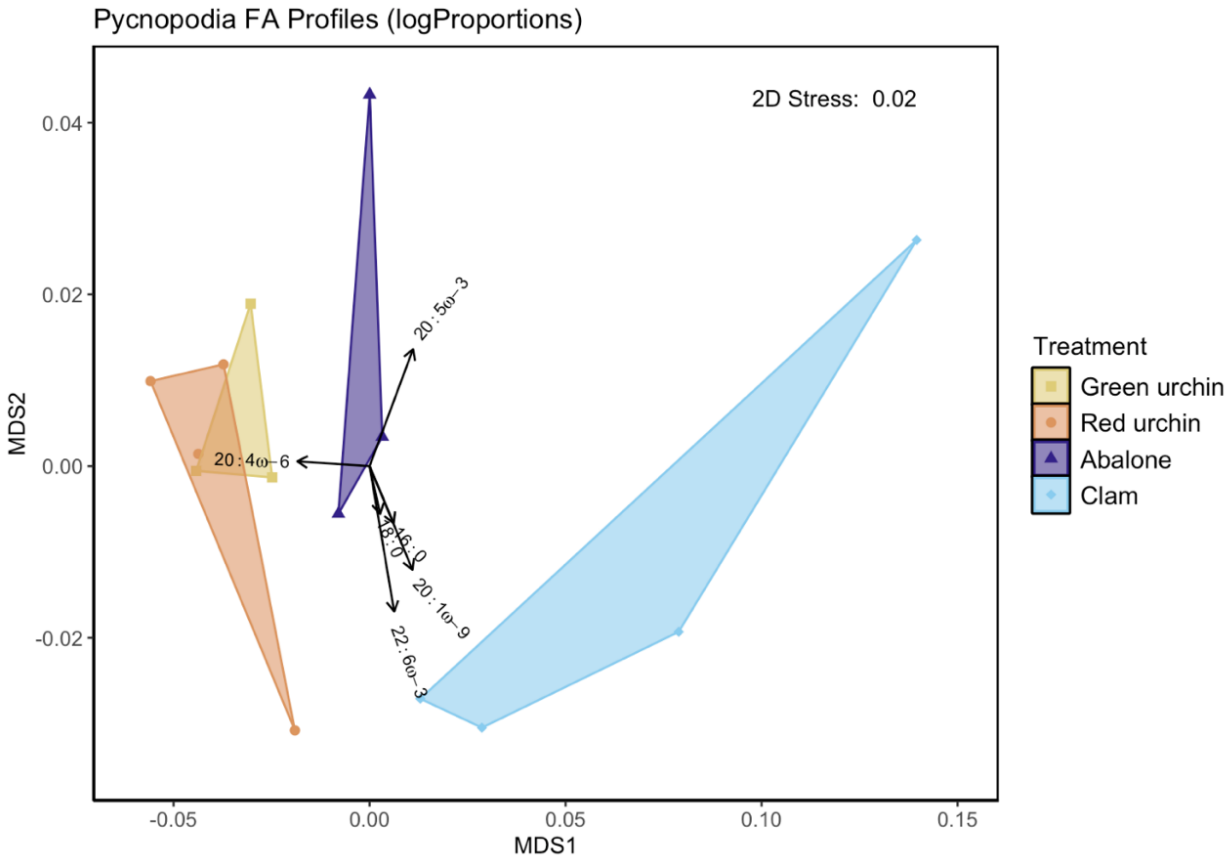

Figure 2. NMDS plots (Euclidean distances) of the log-transformed proportions of FA of *P. helianthoides* fed mono-specific diets.

Table 2. SIMPER (similarity percentage analysis) results for analysis of FA of *P. helianthoides* in each diet treatment, paired against grouped sea star FA profiles from all other diets. "Contrib %" is the average between-group dissimilarity due to each FA.

Diet Treatment	FA	Mean FA %	Contrib %
H. kamtschatkana	20:4ω6	23.42	19.7
	20:5ω3	26.97	13.2
	22:6ω3	4.49	12.2
	22:5ω3	3.45	11.3
	20:1ω9	9.35	5.7
Saxidomus sp.	20:4ω6	16.35	30.1
	20:5ω3	30.87	15.1
	22:6ω3	8.51	14.5
	20:1ω9	10.45	6.6
	22:5ω3	1.66	3.8

Table 2. (continued).

Diet Treatment	FA	Mean FA %	Contrib %
S. droebachiensis	20:4ω6	26.34	23.8
	20:5ω3	26.86	12.7
	22:6ω3	4.74	12.2
	20:1ω9	8.46	7.3
	22:5ω3	0.91	5.9
M. franciscanus	20:4ω6	26.52	23.8
	20:5ω3	25.39	14.4
	22:6ω3	4.54	13.2
	20:1ω9	8.15	7.8
	22:5ω3	1.11	5.1

Sunflower sea stars preferentially integrated moderately abundant (at least 1% of total FA) PUFA >C20 when compared with the FA profiles of their diets. DHA was an exception and was found in slightly higher relative proportion in clams, which had the highest DHA proportion of all the diets (Fig. 3; Table S1). Most other FA were found in greater proportions in prey species, or in nearly equal proportions between prey and predator. Compared to their diets, sunflower sea stars had notably higher median proportions of EPA (Fig. 4a) and ARA (Fig. 4b), with relative proportions between treatment groups reflecting that of the diets. As evidenced by Fig. 3, the median proportions of DHA were also higher in sea star groups than in their corresponding diets, except for those that ate clams, which had lower proportions of DHA than their diet (Fig. 4c). The sea stars that were fed clams did have higher proportions of DHA compared to the stars that consumed grazers (urchins, abalone), however. The clam-eaters also had higher proportions of EPA, although the grazer-eaters had higher proportions of ARA than clam-eaters (Supplementary Table S2; Fig. 4a-c).

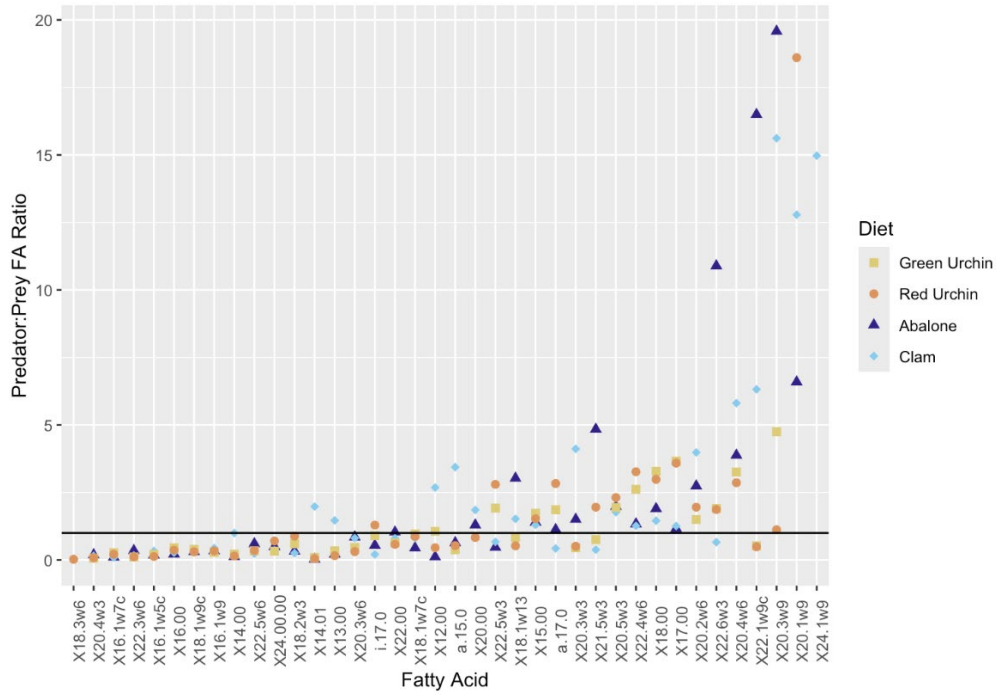

Figure 3. Means of $\log_{10}$ ratios of FA proportion in sea stars/FA proportion in experimental diets, ranked by ratio. Values above/below the black line indicate FA found in higher/lower proportion in sea stars than in their respective diet.

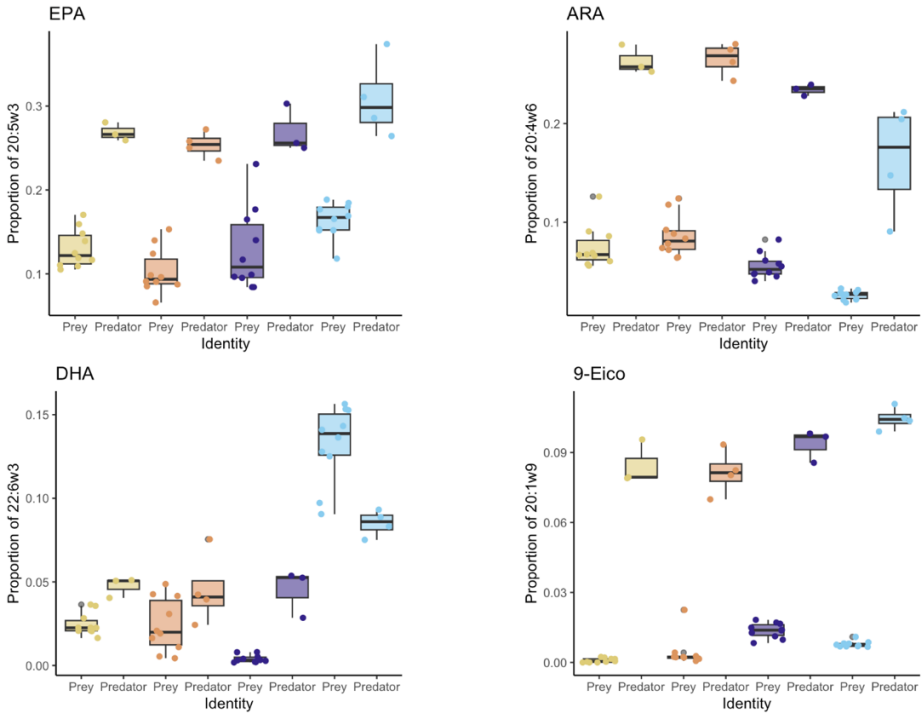

Figure 4. Boxplots comparing proportions of (a) EPA, (b) ARA, (c) DHA, and (d) 20:1w9 between laboratory-fed sea stars and their corresponding diet treatments.

Wild *P. helianthoides* FA

The FA of the wild sunflower stars differed significantly in multivariate space (one-way PERMANOVA, Table 1c; Fig. 5) between the two collection sites. The wild FA signatures were significantly different from those of most laboratory-fed animals (*post hoc* pairwise comparisons, Table S4). However, the sea stars from Magic Island did not have significantly different FA profiles from the laboratory sea stars fed abalone (Table S4).

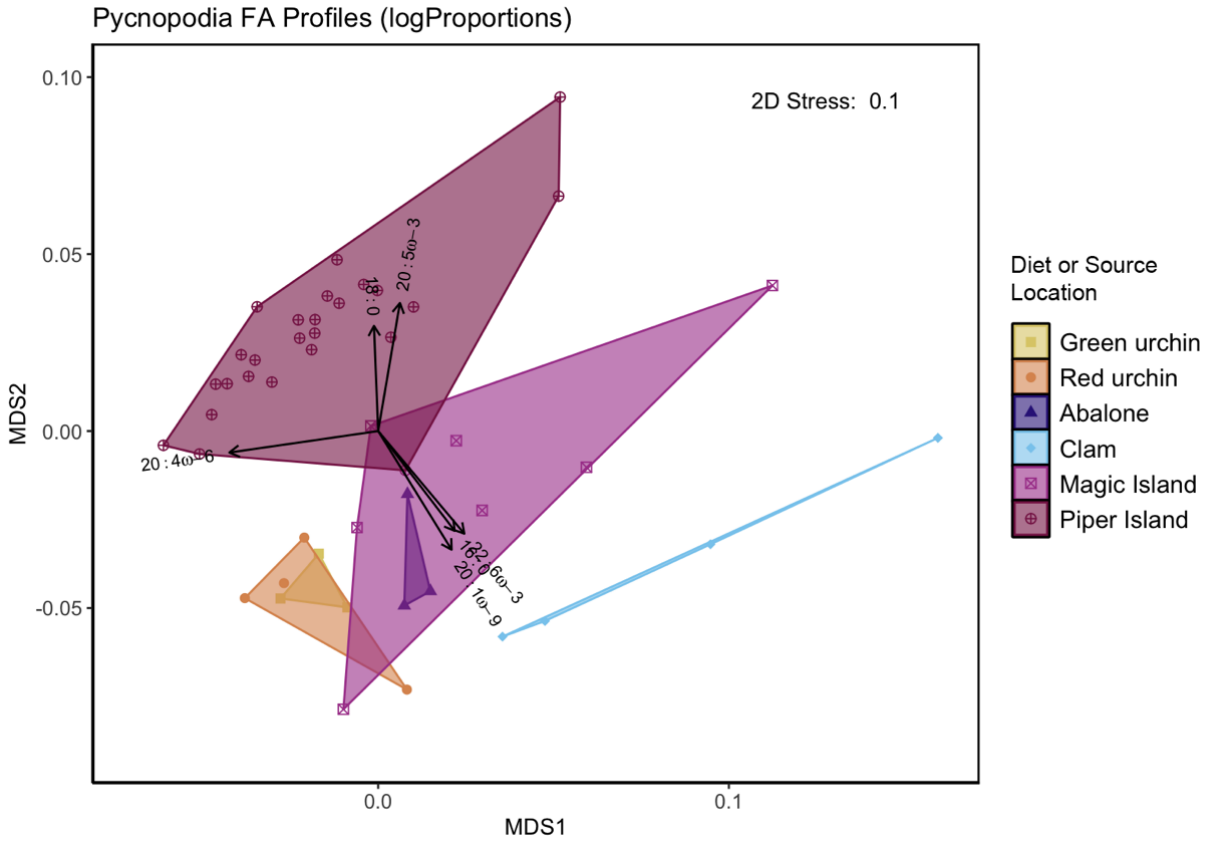

Figure 5. NMDS plots (Euclidean distances) of the log-transformed proportions of FA of all *P. helianthoides* individuals. Wild *P. helianthoides* are displayed overlaying the experimental individuals.

Compared to the stars from Piper Island, the stars from Magic Island had higher proportions of DHA (Fig. 6b), but lower proportions of EPA and ARA (Fig. 6a,c); Table S2). Both groups of wild sea stars had comparable proportions of EPA and ARA to the experimental individuals, but the sea stars from Piper Island were relatively deficient in DHA compared to the laboratory-fed stars (Fig. 6a-c; Table S2). All sea stars had abundant 20:1ω9 compared to the prey species (Fig. 4d), but the wild individuals had lower proportions of it than the laboratory-

fed sea stars (Fig. 6d; Table S2). The sum of bacterial indicator FA was highest in the wild individuals, however (Fig. 7; Table S2).

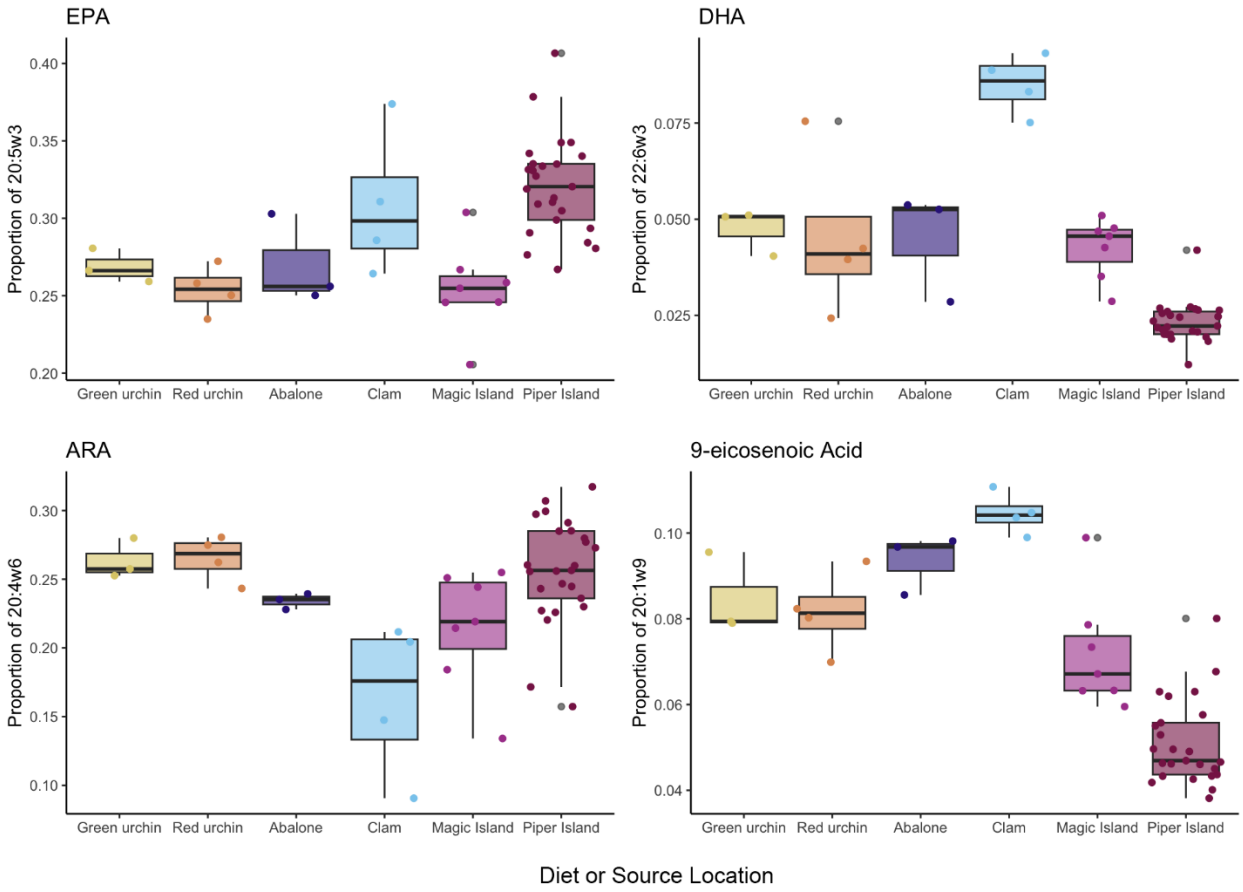

Figure 6. Boxplots comparing proportions of (a) EPA, (b) DHA, (c) ARA, and (d) 20:1w9 between laboratory-fed sea stars (n=14) and wild sea stars (n=25).

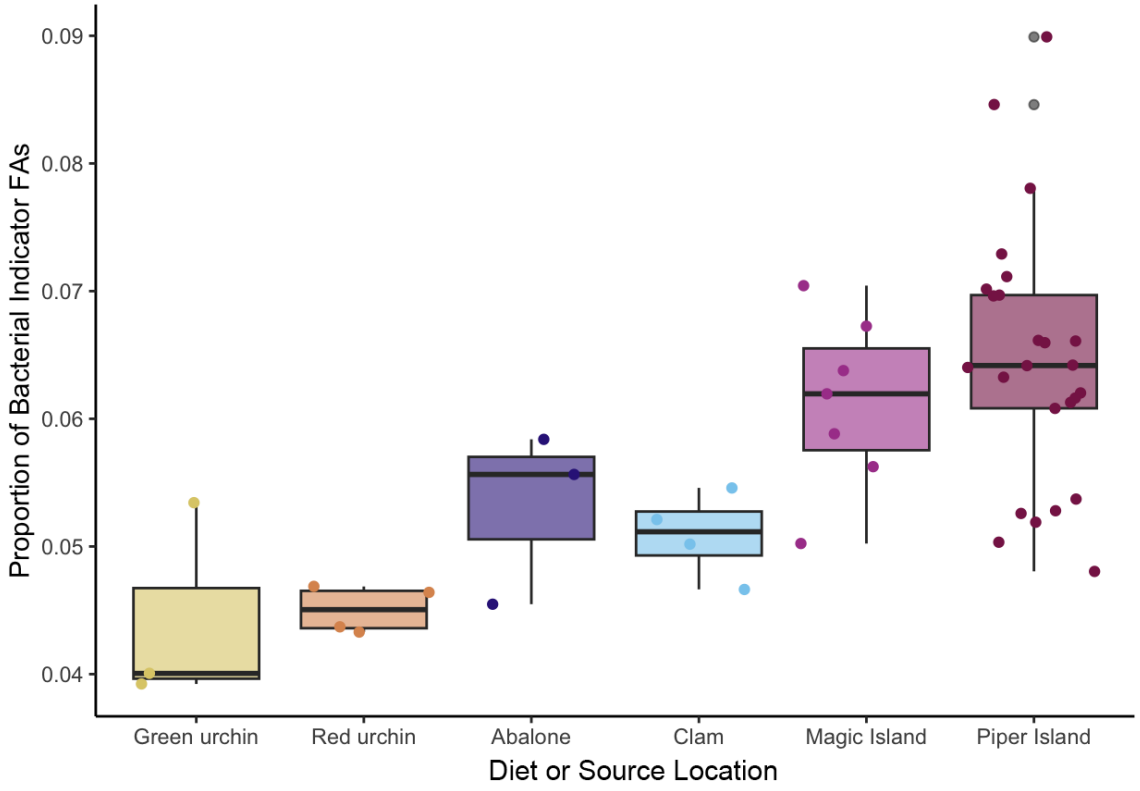

Figure 7. Boxplot comparing proportions of the sum of bacterial indicator FA (*a*15:0, 16:1w5, *a*17:0, *i*17:0, 17:0, 18:1w7) between laboratory-fed sea stars (n=14) and wild sea stars (n=25).

Discussion

The FA profiles of sunflower sea stars were strongly influenced by diet, showing clear differentiation after 10 weeks on specific controlled diets. Although there are no other examples of sea stars being used in FA feeding assays, the findings here are consistent with results from similar experiments using other marine invertebrates, including other echinoderms and arthropods (Galloway et al., 2014; Schram et al., 2018, 2019; Thomas et al., 2020) (Galloway et al. 2014, Schram et al. 2018, 2019, Thomas et al. 2020), in that the fatty acids of invertebrate consumers are generally and consistently depended on and reflective of their experimental diets (Schram et al., 2019). The sea star FA profiles also showed distinct separation from the various prey/diet species, being strongly characterized by abundant FA like EPA, ARA, and 20:1w9. However, phylogeny is one of the primary determinants of FA profile (Kelly & Scheibling, 2012) and may be the factor driving differences between groups at this scale, rather than differences in assimilation/modification of dietary FA.

FA profiles of prey groups generally differed as well, likely also primarily due to clustering based on phylogeny. Some degree of trophic influence is evidenced by NMDS vector overlays identifying the directional impact of abundant FA. For example, the position of the two urchin species (and perhaps grazers in general) in multivariate space is primarily driven by SDA (18:3w4), a noteworthy marker for kelp and other brown algae (Parrish, 2013). The position of the rockfish—bio-accumulators of many LCPUFA—is unsurprisingly driven by DHA and the barnacles' position is influenced by 16:4w1, a known marker for diatoms (Parrish, 2013). Amongst the prey species that were fed to sea stars in the experimental feeding assay, clams had the highest proportions of FA markers of dinoflagellates and diatoms (DHA and EPA), but the lowest proportions of an FA associated with red and brown algae (ARA) compared to the grazing species. These patterns reflect the respective filter feeding and grazing strategies and corresponding FA intake of these prey species (Zhukova, 2019).

Following the same pattern as their diet, the sea stars fed clams also had the highest proportions of EPA and DHA amongst the experimental sea stars. However, the clam-eating group had lower DHA proportions than the clams that they ate, whereas all other experimental diets facilitated higher proportions of DHA in the predators compared to their prey. Additionally, despite abalone having the lowest proportion of DHA out of all the prey, the abalone-eating sea stars had comparable proportions of DHA to the other sea stars that had eaten grazers (urchins). This pattern of relative DHA proportion between predator and prey—the reduction in the clam-eating group in particular—suggests that there may be an "ideal" proportion of DHA within *P. helianthoides* tissues and that the sea stars internally regulate that proportion through synthesis and/or mobilization, depending on their dietary intake of DHA. Other organisms, including marine organisms, have been shown to regulate biosynthesis of LCPUFA in response to varying levels of nutrition and dietary FA intake (Fernandes et al., 2016; Hillgartner et al., 1995; Morais et al., 2015), although the degree to which sea stars can accomplish such regulation is unknown. All *P. helianthoides* had much higher proportions of EPA and ARA than their prey, with the sea stars' FA proportions again following the pattern of proportions in their respective prey. This difference between predator and prey suggests that *P. helianthoides* selectively assimilates and concentrates EPA and ARA obtained from their diets. This result is consistent with findings from other shallow-water echinoderms, which are known to contain high proportions of EPA and

ARA, and lower proportions of DHA (Allen, 1968; Bell & Sargent, 1985). The accumulation and concentration of ARA, specifically, may be characteristic of echinoderms (Takagi et al., 1980).

Along with EPA and ARA, *P. helianthoides* appears to accumulate and concentrate nearly all abundant LCPUFA in higher proportions than their diets, the one exception being DHA in the clam-eating individuals. In addition, the sea stars had undetectable or negligible proportions of common precursor FA: 18:2w6 (linoleic acid, LA), 18:3w3 (α-linoleic acid, ALA), and 18:4w3 (stearidonic acid, SDA). The presence of these FA in the prey suggests that *P. helianthoides* use precursors acquired from their diets to synthesize LCPUFA, especially EPA. Most invertebrates are capable of PUFA synthesis (Kabeya et al., 2018; Monroig et al., 2013), although it is unclear whether invertebrates are capable of de novo synthesis in physiologically relevant amounts and the capacity for such biosynthesis by sea stars is unknown. The selective enrichment of *P. helianthoides* in LCPUFA indicates that they are physiologically important, but the nature of their importance and the degree to which they impact health and growth remains unclear. Controlled manipulation of dietary FA intake may elucidate some of these relationships.

While there is some evidence for trophic modification and potential de novo synthesis in *P. helianthoides*, it is clear that their FA profiles are impacted by their diet. Phylogeny of the prey species appears to play a role here; the two urchin species diets did not produce significantly different FA profiles in the sea stars, a result that was not unexpected given the very close phylogenetic relationship between red and green urchins. However, the diet of the prey items also appears to contribute to differences in predator FA profiles; the FA profiles of sea stars fed abalone were marginally not significantly different in multivariate space to those that ate urchins, creating a loose cluster of "grazer-eaters". In contrast, the clam diet produced predator FA profiles that were significantly different from the sea stars in the urchin treatments and nearly significantly different from those that ate abalone, a fellow mollusk.

The separation of clam-eater FA profiles from the grazer-eaters may reflect the difference in the types of primary producers consumed by clams (a filter feeder) and the corresponding differences in dietary FA passed from primary consumer to predator. Indeed, the multivariate positions of FA profiles of urchin-eaters and abalone-eaters were directionally influenced by ARA (macroalgae biomarker) and EPA (macroalgae/diatom biomarker) respectively, while the position of clam-eater FA profiles was influenced by DHA, a well-known biomarker of

dinoflagellates and zooplankton (Parrish, 2013). These influences are supported by the proportions of these FA within *P. helianthoides* profiles, where clam-eaters had the highest proportions of DHA and grazer-eaters had the highest proportions of ARA. It is worth noting, however, that the small sample sizes within each diet treatment do not allow for confident conclusions. Furthermore, arm tip tissue does not capture the full distribution of FA across the body. Arm tips do not contain any lipid stores and have a higher turnover rate than other tissues due to arm growth originating from arm tips. Therefore, arm tips may not be the most metabolically meaningful tissue for FA analysis. However, significant differences in arm tip FA profiles between diets demonstrates that dietary effects can be seen even in tissue not used for digestion or lipid storage. A major strength of arm tips in this case is that sampling is minimally invasive and doesn't require sacrificing the animal, which is a key factor for endangered or threatened species such as *P. helianthoides*. The many arms of *P. helianthoides* also allow for sampling of an individual multiple times. Additional experimentation using these diet treatments and potential exploration of other tissues will allow for more robust comparisons between groups and stronger conclusions regarding the impacts of diet on *P. helianthoides* FA.

The MUFA 20:1w9 was also abundant in all *P. helianthoides* groups and was identified as an influential FA for all groups by SIMPER analyses. This 20:1 isomer is a well-known copepod biomarker (Parrish, 2013) and was found in a variety of deep-sea sea stars in similar proportions (~7-11%) to *P. helianthoides* (Howell et al., 2003). Howell et al. attribute the abundance of 20:1w9 in suspension-feeding sea stars to likely consumption of copepods, although it was also found in non-suspension feeders. Of the experimentally fed *P. helianthoides*, the clam-eaters had the highest proportion of 20:1w9. Clams are known to graze on copepods (Kimmerer & Lougee, 2015) and may be a source of 20:1w9 in higher consumers feeding on bivalves (Howell et al., 2003). It must be noted, though, that echinoderms may have the ability to synthesize various isomers of 20:1, including 20:1w9 (Sargent et al., 1983; Takagi et al., 1980). De novo synthesis may explain the abundance of 20:1w9 in the *P. helianthoides* that ate urchins and abalone, neither of which could be reasonably expected to be consumers of copepods. However, both groups of wild *P. helianthoides* had lower average proportions of 20:1w9 than all diet treatment groups. The potential cause of this result is unclear; the flow-through seawater filters theoretically limited the ability for captive prey and sea stars to directly or indirectly

consume copepods, but copepods may have been introduced to prey holding tanks through feeding with wild-collected kelp.

The FA profiles of the two wild groups of *P. helianthoides* were significantly different from each other and from all experimental *P. helianthoides* groups with the exception of the individuals from Magic Island and the abalone-eaters, which overlapped in multivariate space (Figure 5). The diets of all wild individuals are unknown, but the lack of significant difference between the Magic Island individuals and abalone-eaters suggests that abalone may have been a component of the diets of Magic Island *P. helianthoides*. While pinto abalone may occasionally be found in "barren" habitat, the dominance of kelp forest habitat at Magic Island in 2021 could have supported high abalone populations. Despite the overlap with abalone-eaters, the high multivariate dispersion of the Magic Island FA profiles indicates that those sea stars likely had other, unknown dietary sources. The group from Piper Island also had high dispersion, indicating varied diets in stars from that location as well. The significant difference of FA profiles between the two groups provides evidence for the diets of Piper Island *P. helianthoides* being different from those at Magic Island. This evidence is further supported by Piper Island being a mixed habitat site with bare rocky reef, kelp, cobble, and sand, which may support different diet opportunities for *P. helianthoides*.

Sea stars from both sites also had elevated proportions of bacterial indicator FA compared to the experimental groups. Naturally occurring biofilms in sediments are hypothesized to be the primary contributors to bacterial FA presence in deposit-feeding sea stars (Howell et al., 2003). Incidental ingestion of biofilms may be the cause for the elevated bacterial indicator FA proportions in the wild *P. helianthoides* compared to the captive individuals, whose tanks were cleaned regularly. The relative complexity of the FA profiles of wild *P. helianthoides* and the significant difference of the Piper Island sea stars supports the need for further captive experimentation and greater sampling of wild *P. helianthoides* populations.

One of the goals of this experiment is to lay groundwork for future efforts in predictive diet modeling using QFASA (Iverson et al. 2004, Bromaghin 2017). This method of diet prediction directly compares the FA composition of predators and their potential prey. In marine systems, QFASA is primarily used to estimate the diets of high trophic level organisms like marine mammals and birds; its application in marine invertebrate consumers is extremely limited and there are currently no published examples of QFASA modeling for invertebrates (Zhang et

al., 2020). Controlled feeding experiments are ideal for QFASA modeling because the effect of specific dietary items on consumer FA profiles can be directly quantified (Zhang et al., 2020). Invertebrates lend themselves well to captive experiments compared to most birds and mammals, making invertebrate predators promising subjects for future applications of QFASA. By conducting a controlled feeding experiment and sampling a variety of prey, I hope to lay the foundation for future efforts in *P. helianthoides* diet estimation. Testing more *P. helianthoides* within the experimental diet treatments used here and expanding to include additional diet treatments will certainly aid in creating a robust base of knowledge on *P. helianthoides* FA composition.

Fatty acids are an incredibly promising tool for diet estimation in *P. helianthoides*. The dramatic decline of a once incredibly abundant generalist predator across the entirety of its range necessitates a more comprehensive understanding of its trophic impacts. In the face of persisting scarcity of *P. helianthoides*, fatty acids provide a minimally invasive method for assessing long-term diet that can complement alternative diet estimation tools like stable isotope and DNA analysis. The development and continual improvement of quantitative methods of diet estimation in *P. helianthoides* may ultimately make diet estimation possible for any wild individual, regardless of region or habitat, thereby expanding opportunities for investigating localized and range-wide impacts of *P. helianthoides* predation and improving our understanding of a previously overlooked marine predator.

APPENDIX A

CHAPTER I SUPPLEMENTAL FIGURES

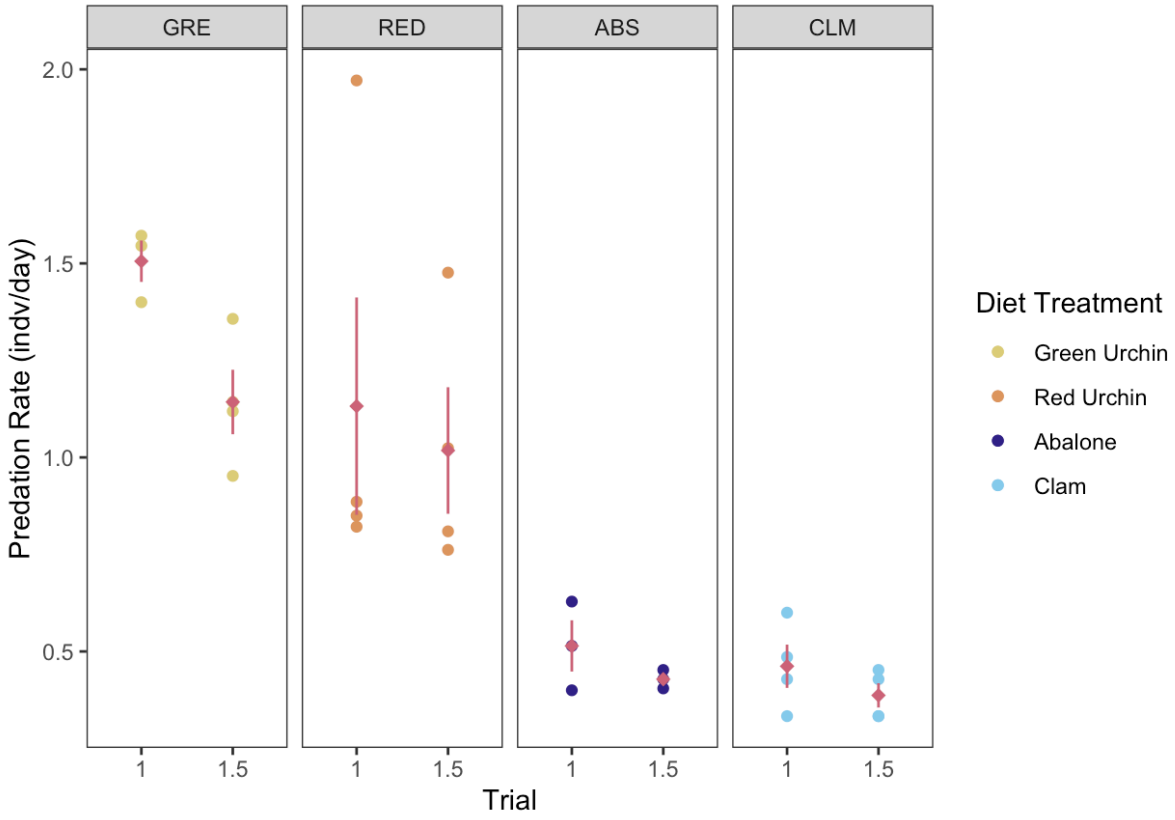

Figure S1. A dotplot comparing the predation rates of the different prey species between the first 5 weeks (Trial 1) and the following 6 weeks (Trial 1.5) of feeding. Means are represented by diamonds. Error bars are SE.

APPENDIX B

CHAPTER II SUPPLEMENTAL FIGURES AND TABLES

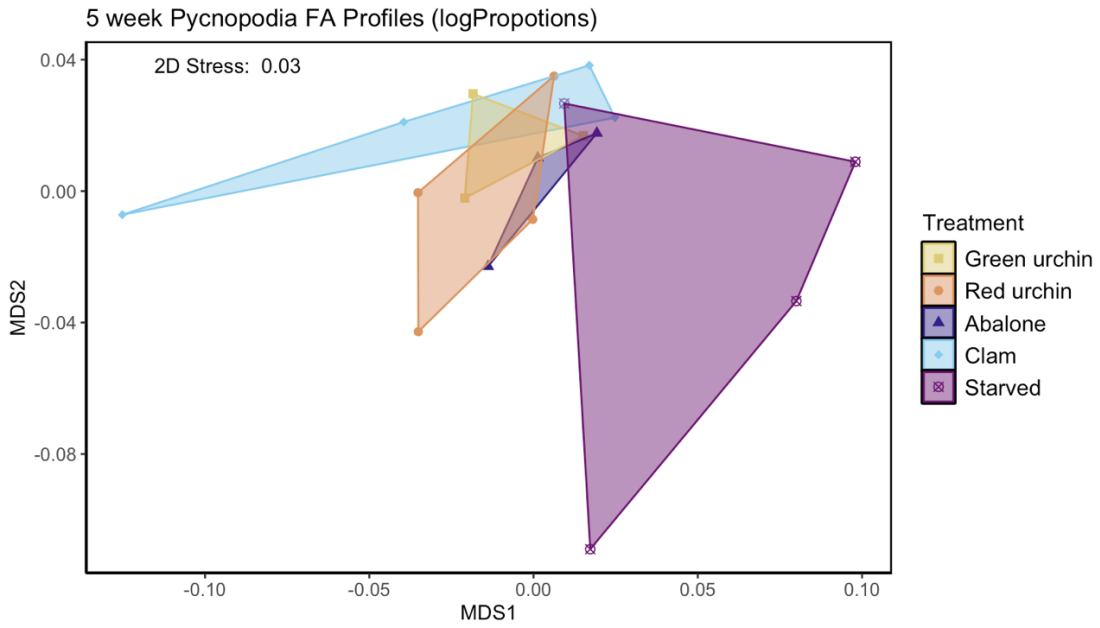

Figure S1. NMDS plots (Euclidean distances) of the log-transformed proportions of FA of *P. helianthoides* fed mono-specific diets at the 5-week timepoint.

Table S1. Fatty acid composition of all prey species. Numbers are mean percent of all FA identified ± SD. SAFA = saturated FA, MUFA = monounsaturated FA, PUFA = polyunsaturated FA, Bacterial = bacterial indicator FA. Codes: ABS = *H. kamtschatkana*, CLM = *Saxidomus sp.*, GRE = *S. droebachiensis*, RED = *M. franciscanus*, TEG = *T. pulligo,* BAR = *B. glandula*, FSH = *S. melanops*, --- indicates FA proportions <0.001

	ABS	CLM	GRE	RED	TEG	BAR	FSH
8:0	---	---	---	---	---	---	---
12:0	0.24±0.19	0.02±0.01	0.03±0.004	0.07±0.02	0.05±0.04	0.38±0.12	0.02±0.01
13:0	0.04±0.01	0.01±0.002	0.03±0.01	0.07±0.03	0.03±0.01	0.01±0.002	0.004±0.002
14:0	7.49±3.71	1.67±0.54	6.31±0.97	12.4±2.4	3.47±3.1	3.48±0.5	0.96±0.28
15:0	0.67±0.25	0.52±0.06	0.61±0.13	0.68±0.23	0.72±0.08	0.18±0.03	0.22±0.04
16:0	24.7±2.1	21.8±1.94	11.6±1.64	15.2±1.1	25.2±2.72	8.4±1.2	20.7±1.02
18:0	5.4±1.8	6.73±1.13	2.64±0.87	3.1±0.46	5.5±1.78	2.0±0.55	6.49±0.38
20:0	0.07±0.03	0.07±0.01	---	0.07±0.22	0.1±0.03	0.12±0.04	0.16±0.02
22:0	0.02±0.007	0.03±0.005	0.04±0.02	0.03±0.01	0.04±0.01	0.11±0.05	0.05±0.01

Table S1. (continued).

	ABS	CLM	GRE	RED	TEG	BAR	FSH
23:0	---	---	---	---	---	0.02±0.02	0.01±0.002
24:0	0.02±0.01	0.04±0.01	0.05±0.02	0.01±0.004	0.05±0.01	0.07±0.03	0.06±0.02
Σ SAFA	38.6	30.9	21.4	31.6	35.2	14.7	28.7
13:1	---	---	---	---	---	---	---
14:1	0.08±0.08	0.004±0.01	0.45±0.25	1.43±0.62	0.24±0.31	0.11±0.07	0.02±0.01
16:1ω7	5.5±4.6	7.11±1.35	4.03±1.77	3.66±0.73	5.4±5.5	4.6±0.7	1.7±0.28
16:1ω9	0.21±0.08	0.4±0.05	0.6±0.16	0.47±0.3	0.29±0.04	0.16±0.06	0.21±0.06
17:1ω7	---	---	---	---	---	0.05±0.01	0.11±0.02
18:1ω13	0.14±0.15	0.42±0.09	0.68±0.42	1.45±0.54	0.13±0.05	0.02±0.02	0.08±0.04
18:1ω5	---	---	---	---	---	1.12±0.24	3.1±0.37
18:1ω9	4.7±1.6	2.9±0.44	3.03±2.0	4.86±1.14	4.57±3.55	3.34±0.65	5.45±0.54
20:1ω9	1.37±0.32	0.78±0.13	0.09±0.09	0.42±0.65	0.32±0.08	1.04±0.27	0.53±0.09
22:1ω9	0.05±0.007	0.15±0.03	2.25±0.48	2.32±0.44	0.04±0.02	0.18±0.03	0.18±0.04
24:1ω9	---	0.12±0.02	0.04±0.01	0.02±0.01	0.02±0.02	0.17±0.06	0.77±0.07
Σ MUFA	12.7	11.9	11.2	14.6	11.0	10.8	12.1
16:4ω1	---	---	---	---	---	45.4±7.07	3.53±1.54
18:2ω3	0.33±0.11	0.97±0.19	0.23±0.15	0.14±0.04	0.22±0.04	0.31±0.04	0.13±0.02
18:2ω6	1.5±0.44	0.56±0.11	1.11±0.37	1.47±0.45	1.83±0.55	0.42±0.06	0.87±0.1
18:3ω3	1.4±0.36	0.22±0.04	1.21±0.23	1.13±0.24	0.77±0.15	0.21±0.06	0.18±0.04
18:3ω6	0.11±0.11	0.1±0.02	0.22±0.08	0.16±0.04	0.04±0.02	0.12±0.01	0.05±0.01
18:4ω3	5.87±3.28	9.85±1.4	30.64±7.86	16±2.12	3.25±1.47	1.1±0.14	0.22±0.1
20:2ω6	0.66±0.32	0.42±0.04	1.57±0.43	1.12±0.26	0.37±0.26	0.23±0.1	0.28±0.05
20:3ω3	0.26±0.13	0.04±0.01	1.31±0.42	0.96±0.27	0.07±0.06	0.06±0.03	0.07±0.01
20:3ω6	0.2±0.06	0.23±0.05	0.49±0.07	0.6±0.13	0.19±0.07	0.06±0.01	0.12±0.02
20:3ω9	0.05±0.03	0.05±0.01	0.2±0.14	1.54±0.37	0.04±0.01	---	---
20:4ω3	0.39±0.2	0.4±0.04	1.4±0.71	0.7±0.21	0.24±0.16	0.24±0.03	0.28±0.05
20:4ω6	5.58±1.29	2.63±0.45	7.46±2.13	8.59±2.07	11.7±3.84	0.56±0.17	1.56±0.17
20:5ω3	12.89±4.87	16.4±2.11	13.02±2.28	10.31±2.71	11.0±2.98	16.66±2.88	11.0±1.8
21:5ω3	0.02±0.01	1.12±0.2	0.18±0.09	0.08±0.03	0.07±0.01	0.63±0.08	0.23±0.05
22:2ω6	---	---	---	---	---	---	---
22:3ω6	0.03±0.02	0.01±0.01	0.18±0.08	0.11±0.04	---	---	---
22:4ω6	0.25±0.05	0.19±0.04	0.06±0.02	0.06±0.02	0.47±0.08	0.01±0.003	0.06±0.02
22:5ω3	7.5±1.5	2.49±0.47	0.47±0.13	0.4±0.18	6.64±1.94	0.29±0.1	2.69±0.38
22:5ω6	0.09±0.1	0.52±0.1	0.19±0.06	0.24±0.14	0.26±0.07	0.08±0.03	0.62±0.1
22:6ω3	0.4±0.23	13.2±2.28	2.47±0.67	2.41±1.6	2.09±0.78	4.12±1.23	34.1±2.92
Σ PUFA	37.5	49.4	62.4	46.0	39.2	70.4	56.0
*a*15:0	0.04±0.03	0.02±0.003	0.1±0.04	0.07±0.03	0.08±0.02	0.02±0.01	0.01±0.004

Table S1. (continued).

	ABS	CLM	GRE	RED	TEG	BAR	FSH
16:1ω5	0.88±0.66	0.24±0.04	1.34±0.54	4.2±1.15	0.29±0.12	0.09±0.01	0.13±0.02
*i*17:0	0.27±0.11	1.98±0.3	0.14±0.04	0.12±0.06	1.61±0.37	0.05±0.01	0.13±0.02
*a*17:0	0.09±0.04	0.78±0.16	0.05±0.01	0.04±0.02	0.59±0.1	0.03±0.01	0.04±0.02
17:0	0.91±0.58	0.78±0.3	0.23±0.07	0.3±0.16	1.86±0.66	0.16±0.06	0.28±0.04
18:1ω7	8.86±4.88	4.0±0.62	3.18±1.0	3.02±0.32	10.1±4.27	3.6±0.94	2.64±0.23
Σ Bacterial	11.1	7.78	5.03	7.74	14.5	3.97	3.23

Table S2. Fatty acid composition of all *P. helianthoides*. Numbers are mean percent of all FA identified ± SD. SAFA = saturated FA, MUFA = monounsaturated FA, PUFA = polyunsaturated FA, Bacterial = bacterial indicator FA. Codes are as in S1. MAG = Magic Island sea stars, PIP = Piper Island sea stars.

	ABS	CLM	GRE	RED	MAG	PIP
8:0	0.02±0.01	0.03±0.01	0.06±0.02	0.03±0.02	0.01±0.003	---
12:0	0.03±0.01	0.04±0.02	0.03±0.002	0.03±0.02	0.15±0.06	0.03±0.01
13:0	0.01±0.001	0.01±0.002	0.01±0.001	0.01±0.002	0.03±0.02	0.01±0.002
14:0	0.88±0.15	1.66±0.6	1.35±0.16	1.69±0.07	0.57±0.21	0.67±0.33
15:0	0.95±0.19	0.68±0.13	1.06±0.05	1.05±0.02	0.76±0.21	0.66±0.2
16:0	4.87±0.36	5.44±0.65	5.11±0.19	5.24±0.11	5.6±1.4	3.44±0.59
18:0	10.5±0.79	9.9±0.57	8.93±0.34	9.53±0.33	15.5±1.74	14.3±0.97
20:0	0.09±0.003	0.12±0.03	0.14±0.02	0.06±0.02	0.19±0.06	0.41±0.2
22:0	0.02±0.01	0.2±0.003	0.02±0.01	0.02±0.004	0.03±0.01	0.04±0.02
23:0	---	---	---	---	---	0.02±0.01
24:0	0.01±0.003	0.01±0.004	0.02±0.002	0.01±0.0004	0.04±0.06	0.02±0.006
Σ SAFA	17.4	17.9	16.7	17.7	22.9	19.6
13:1	0.01±0.001	0.01±0.002	0.01±0.001	0.01±0.003	0.02±0.01	---
14:1	0.02±0.004	0.01±0.004	0.04±0.01	0.07±0.01	0.01±0.004	0.02±0.02
16:1ω7	0.54±0.05	0.63±0.08	1.05±0.32	0.76±0.24	1.46±1.2	0.67±0.26
16:1ω9	0.07±0.01	0.18±0.04	0.17±0.01	0.16±0.01	0.12±0.03	0.24±0.15
17:1ω7	---	---	---	---	---	---
18:1ω13	0.41±0.05	0.65±0.18	0.56±0.03	0.76±0.16	0.71±0.26	0.4±0.1
18:1ω5	---	---	---	---	---	---

Table S2. (continued).

	ABS	CLM	GRE	RED	MAG	PIP
18:1ω9	1.41±0.15	0.97±0.24	1.17±0.13	1.43±0.52	1.15±0.6	0.46±0.11
20:1ω9	9.35±0.69	10.45±0.49	8.46±0.95	8.15±0.96	7.2±1.36	5.1±1
22:1ω9	0.91±0.18	0.94±0.11	1.17±0.11	1.12±0.08	0.91±0.22	0.6±0.14
24:1ω9	2.07±0.38	1.82±0.26	2.52±0.22	2.45±0.12	1.76±0.3	1.25±0.3
Σ MUFA	14.8	15.6	15.2	14.9	13.4	8.75
16:4ω1	---	---	---	---	---	---
18:2ω3	0.11±0.01	0.24±0.08	0.14±0.03	0.13±0.01	0.16±0.06	0.22±0.06
18:2ω6	---	---	---	---	0.95±2.33	---
18:3ω3	---	---	---	---	---	---
18:3ω6	---	---	---	0.004±0.008	0.05±0.03	---
18:4ω3	---	---	---	---	---	0.09±0.06
20:2ω6	1.83±0.22	1.67±0.31	2.36±0.09	2.2±0.17	1.73±0.15	2.19±0.21
20:3ω3	0.39±0.004	0.18±0.06	0.59±0.05	0.48±0.1	0.43±0.13	0.36±0.2
20:3ω6	0.17±0.03	0.19±0.04	0.23±0.03	0.18±0.04	0.11±0.02	0.15±0.03
20:3ω9	1.01±0.53	0.75±0.2	0.97±0.49	1.73±0.38	0.22±0.07	0.26±0.14
20:4ω3	0.07±0.01	0.06±0.02	0.09±0.04	0.06±0.01	0.13±0.11	0.11±0.02
20:4ω6	23.42±0.57	16.35±5.65	26.34±1.46	26.52±1.65	21.46±4.33	25.62±3.85
20:5ω3	26.97±2.89	30.87±4.74	26.86±1.09	25.39±1.56	25.44±2.93	32.11±3.19
21:5ω3	0.12±0.02	0.43±0.12	0.14±0.03	0.15±0.01	0.15±0.05	0.25±0.07
22:2ω6	0.07±0.01	0.05±0.01	0.08±0.02	0.11±0.03	0.05±0.01	0.06±0.01
22:3ω6	0.01±0.002	0.002±0.003	0.02±0.003	0.01±0.004	0.01±0.01	---
22:4ω6	0.34±0.05	0.24±0.02	0.15±0.02	0.21±0.05	0.28±0.05	0.05±0.01
22:5ω3	3.45±0.66	1.66±0.29	0.91±0.12	1.11±0.22	2.18±0.41	1.19±0.23
22:5ω6	0.06±0.01	0.12±0.01	0.07±0.003	0.08±0.01	0.07±0.02	0.16±0.08
22:6ω3	4.49±1.42	8.51±0.78	4.74±0.6	4.54±2.16	4.25±0.79	2.33±0.52
Σ PUFA	62.5	61.3	63.7	62.9	57.6	65.1
*a*15:0	0.03±0.004	0.06±0.02	0.04±0.002	0.04±0.005	0.05±0.03	0.18±0.11
16:1ω5	0.16±0.07	0.08±0.04	0.28±0.05	0.52±0.03	0.03±0.01	0.12±0.08
*i*17:0	0.15±0.02	0.4±0.06	0.12±0.01	0.15±0.04	0.32±0.08	0.46±0.2
*a*17:0	0.1±0.02	0.33±0.06	0.09±0.01	0.11±0.02	0.19±0.03	0.41±0.25
17:0	1.06±0.11	0.98±0.1	0.86±0.02	1.06±0.11	1.34±0.14	1.5±0.14

Table S2. (continued).

	ABS	CLM	GRE	RED	MAG	PIP
18:1ω7	3.82±0.76	3.24±0.3	3.04±0.78	2.63±0.31	4.2±0.64	3.79±0.65
Σ Bacterial	5.32	5.09	4.42	4.51	6.12	6.46

Table S3. Results of pairwise comparisons for one-way PERMANOVA (Euclidean distance) of FA (n=48) proportions from all *P. helianthoides* and all diet species. Codes are in S2. Pycno = *P. helianthoides*

Groups	t	P (perm)	Unique permutations
Pycno, BAR	20.616	0.0001	9936
Pycno, FSH	19.039	0.0001	9936
Pycno, ABS	12.922	0.0001	9934
Pycno, CLM	14.714	0.0001	9942
Pycno, GRE	15.635	0.0001	9948
Pycno, RED	14.751	0.0001	9935
Pycno, TEG	11.982	0.0001	9942
BAR, FSH	20.594	0.0001	9342
BAR, ABS	11.761	0.0001	9384
BAR, CLM	18.289	0.0001	9314
BAR, GRE	15.135	0.0001	9381
BAR, RED	17.205	0.0001	9359
BAR, TEG	11.866	0.0001	9425
FSH, ABS	9.9849	0.0001	9375
FSH, CLM	13.013	0.0001	9372
FSH, GRE	15.269	0.0001	9386
FSH, RED	17.924	0.0001	9333
FSH, TEG	9.5233	0.0001	9355
ABS, CLM	5.0132	0.0001	9398
ABS, GRE	6.6575	0.0001	9416
ABS, RED	4.9514	0.0001	9435
ABS, TEG	1.8645	0.0259	9447

Table S3. (continued).

Groups	t	P (perm)	Unique permutations
CLM, GRE	8.7802	0.0001	9420
CLM, RED	9.6372	0.0001	9424
CLM, TEG	5.2826	0.0001	9437
GRE, RED	4.902	0.0001	9444
GRE, TEG	7.2739	0.0001	9380
RED, TEG	5.7554	0.0001	9462

Table S4. Results of pairwise comparisons for one-way PERMANOVA (Euclidean distance) of FA (n=44) proportions from all *P. helianthoides*. Codes: GRE = *S. droebachiensis*, ABS = *H. kamtschatkana,* RED = *M. franciscanus*, CLM = *Saxidomus sp.*, WLD21 = *P. helianthoides* from Magic Island, WLD24 = *P. helianthoides* from Piper Island.

Groups	t	P (perm)	Unique permutations	P (MC)
GRE, ABS	1.8557	0.101	10	0.0656
GRE, RED	0.85884	0.6952	35	0.5294
GRE, CLM	2.5765	0.0534	35	0.0333
GRE, WLD21	2.2151	0.0288	120	0.0219
GRE, WLD24	3.102	0.0016	3107	0.0006
ABS, RED	1.8232	0.0809	35	0.0588
ABS, CLM	2.0211	0.0603	35	0.0606
ABS, WLD21	1.5978	0.0497	120	0.0842
ABS, WLD24	2.9937	0.0017	3114	0.0006
RED, CLM	3.0373	0.0292	35	0.0116
RED, WLD21	2.389	0.0048	330	0.0087
RED, WLD24	3.6961	0.0001	8117	0.0001
CLM, WLD21	2.5721	0.0034	330	0.0043
CLM, WLD24	4.8291	0.0001	8149	0.0001
WLD21, WLD24	3.7322	0.0001	9951	0.0001

Table S5. Results of pairwise comparisons for one-way PERMANOVA (Euclidean distance) of FA (n=41) proportions from laboratory-fed *P. helianthoides.* Codes are as in S2.

Groups	t	P (perm)	Unique permutations	P (MC)
GRE, ABS	1.8557	0.0978	10	0.0686
GRE, RED	0.85884	0.6855	35	0.5177
GRE, CLM	2.5765	0.0578	35	0.0335
ABS, RED	1.8232	0.0863	35	0.0589
ABS, CLM	2.0211	0.0528	35	0.0671
RED, CLM	3.0373	0.0281	35	0.0121

REFERENCES CITED

Allen, W. V. (1968). Fatty-acid synthesis in the echinoderms: *Asterias rubens*, *Echinus esculentus* and *Holothuria forskali*. *Journal of the Marine Biological Association of the United Kingdom*, *48*(2), 521–533. https://doi.org/10.1017/S0025315400034640

Anderson, M. J., Gorley, R. N., & Clarke, K. R. (2008). *PERMANOVA+ for PRIMER: Guide to software and statistical methods*. PRIMER-E Ltd. https://learninghub.primer-e.com/books/permanova-for-primer-guide-to-software-and-statistical-methods

Bell, M. V., & Sargent, J. R. (1985). Fatty acid analyses of phosphoglycerides from tissues of the clam *Chlamys islandica* (Muller) and the starfish *Ctenodiscus crispatus* (Retzius) from Balsfjorden, Northern Norway. *Journal of Experimental Marine Biology and Ecology*, *87*(1), 31–40. https://doi.org/10.1016/0022-0981(85)90189-3

Brewer, R., & Konar, B. (2005). Chemosensory responses and foraging behavior of the seastar *Pycnopodia helianthoides*. *Marine Biology*, *147*(3), 789–795. https://doi.org/10.1007/s00227-005-1608-7

Bromaghin, J. F. (2017). QFASAR: Quantitative fatty acid signature analysis with R. *Methods in Ecology and Evolution*, *8*, 1158–1162.

Budge, S. M., Iverson, S. J., & Koopman, H. N. (2006). Studying trophic ecology in marine ecosystems using fatty acids: A primer on analysis and interpretation. *Marine Mammal Science*, *22*(4), 759–801. https://doi.org/10.1111/j.1748-7692.2006.00079.x

Burt, J. M., Tinker, M. T., Okamoto, D. K., Demes, K. W., Holmes, K., & Salomon, A. K. (2018). Sudden collapse of a mesopredator reveals its complementary role in mediating rocky reef regime shifts. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1883). https://doi.org/10.1098/rspb.2018.0553

Campbell, A. (2000). Review of northern abalone, *Haliotis kamtschatkana*, stock status in British Columbia. In *Workshop on rebuilding abalone stocks in British Columbia*. https://publications.gc.ca/site/eng/9.615332/publication.html

Claisse, J. T., Williams, J. P., Ford, T., Pondella II, D. J., Meux, B., & Protopapadakis, L. (2013). Kelp forest habitat restoration has the potential to increase sea urchin gonad biomass. *Ecosphere*, *4*(3), 1–18. https://doi.org/10.1890/ES12-00408.1

Dalsgaard, J., St. John, M., Kattner, G., Müller-Navarra, D., & Hagen, W. (2003). Fatty acid trophic markers in the pelagic marine environment. *Advances in Marine Biology, 46*, 225–340. https://doi.org/10.1016/S0065-2881(03)46005-7

Dayton, P. K. (1975). Experimental evaluation of ecological dominance in a rocky intertidal algal community. *Ecological Monographs*, *45*(2), 137–159. https://doi.org/10.2307/1942404

de la Chesnais, T., Fulton, E. A., Tracey, S. R., & Pecl, G. T. (2019). The ecological role of cephalopods and their representation in ecosystem models. *Reviews in Fish Biology and Fisheries*, *29*(2), 313–334. https://doi.org/10.1007/s11160-019-09554-2

Duggins, D. O. (1983). Starfish predation and the creation of mosaic patterns in a kelp-dominated community. *Ecology*, *64*(6), 1610–1619. https://doi.org/10.2307/1937514

Dunn, R. P., & Hovel, K. A. (2020). Predator type influences the frequency of functional responses to prey in marine habitats. *Biology Letters*, *16*(1), 20190758. https://doi.org/10.1098/rsbl.2019.0758

Ellingsen, K. E., Yoccoz, N. G., Tveraa, T., Frank, K. T., Johannesen, E., Anderson, M. J., Dolgov, A. V., & Shackell, N. L. (2020). The rise of a marine generalist predator and the fall of beta diversity. *Global Change Biology*, *26*(5), 2897–2907. https://doi.org/10.1111/gcb.15027

Elston, C., von Brandis, R.G., & and Cowley, P. (2015). Gastric lavage as a non-lethal method for stingray (Myliobatiformes) diet sampling. *African Journal of Marine Science*, *37*(3), 415–419. https://doi.org/10.2989/1814232X.2015.1076519

Estes, J. A., & Duggins, D. O. (1995). Sea Otters and Kelp Forests in Alaska: Generality and variation in a community ecological paradigm. *Ecological Monographs*, *65*(1), 75–100. https://doi.org/10.2307/2937159

Estes, J. A., Smith, N. S., & Palmisano, J. F. (1978). Sea otter predation and community organization in the western Aleutian Islands, Alaska. *Ecology*, *59*(4), 822–833. https://doi.org/10.2307/1938786

Fernandes, T., Fernandes, I., Andrade, C. A. P., & Cordeiro, N. (2016). Changes in fatty acid biosynthesis in marine microalgae as a response to medium nutrient availability. *Algal Research*, *18*, 314–320. https://doi.org/10.1016/j.algal.2016.07.005

Frelat, R., Kortsch, S., Kröncke, I., Neumann, H., Nordström, M. C., Olivier, P. E. N., & Sell, A. F. (2022). Food web structure and community composition: A comparison across space and time in the North Sea. *Ecography*, *2022*(2). https://doi.org/10.1111/ecog.05945

Galloway, A. W. E., & Budge, S. M. (2020). The critical importance of experimentation in biomarker-based trophic ecology. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *375*(1804), 20190638. https://doi.org/10.1098/rstb.2019.0638

Galloway, A. W. E., Gravem, S. A., Kobelt, J. N., Heady, W. N., Okamoto, D. K., Sivitilli, D. M., Saccomanno, V. R., Hodin, J., & Whippo, R. (2023). Sunflower sea star predation on urchins can facilitate kelp forest recovery. *Proceedings of the Royal Society B: Biological Sciences*, *290*(1993), 20221897. https://doi.org/10.1098/rspb.2022.1897

Galloway, A. W. E., Taipale, S. J., Hiltunen, M., Peltomaa, E., Strandberg, U., Brett, M. T., & Kankaala, P. (2014). Diet-specific biomarkers show that high-quality phytoplankton fuels herbivorous zooplankton in large boreal lakes. *Freshwater Biology*, *59*(9), 1902–1915. https://doi.org/10.1111/fwb.12394

Galloway, A. W. E., & Winder, M. (2015). Partitioning the relative importance of phylogeny and environmental conditions on phytoplankton fatty acids. *PLOS ONE*, *10*(6), e0130053. https://doi.org/10.1371/journal.pone.0130053

Gladics, A. J., Suryan, R. M., Brodeur, R. D., Segui, L. M., & Filliger, L. Z. (2014). Constancy and change in marine predator diets across a shift in oceanographic conditions in the Northern California Current. *Marine Biology*, *161*(4), 837–851. https://doi.org/10.1007/s00227-013-2384-4

González-Durán, E., Castell, J. D., Robinson, S. M. C., & Blair, T. J. (2008). Effects of dietary lipids on the fatty acid composition and lipid metabolism of the green sea urchin *Strongylocentrotus droebachiensis*. *Aquaculture*, *276*(1), 120–129. https://doi.org/10.1016/j.aquaculture.2008.01.010

Gravem, S., Heady, W., Saccomanno, V., Alvstad, K., Gehman, A., Frierson, T., & Hamilton, S. (2020, August). *Sunflower sea star (*Pycnopodia helianthoides*)*. International Union for Conservation of Nature.

Griffiths, A. M., & Gosselin, L. A. (2008). Ontogenetic shift in susceptibility to predators in juvenile northern abalone, *Haliotis kamtschatkana*. *Journal of Experimental Marine Biology and Ecology*, *360*(2), 85–93. https://doi.org/10.1016/j.jembe.2008.04.004

Gulka, J., Carvalho, P. C., Jenkins, E., Johnson, K., Maynard, L., & Davoren, G. K. (2017). Dietary niche shifts of multiple marine predators under varying prey availability on the northeast Newfoundland coast. *Frontiers in Marine Science*, *4*. https://doi.org/10.3389/fmars.2017.00324

Hamilton, S., Calvanese, T., Gravem, S., Galloway, A., Chabot, D., Vidusic, E., & Webster, N. (2024). *2024 Oregon Kelp Status Report*. Oregon Kelp Alliance.

Hamilton, S. L., Saccomanno, V. R., Heady, W. N., Gehman, A. L., Lonhart, S. I., Beas-Luna, R., Francis, F. T., Lee, L., Rogers-Bennett, L., Salomon, A. K., & Gravem, S. A. (2021). Disease-driven mass mortality event leads to widespread extirpation and variable recovery potential of a marine predator across the eastern Pacific. *Proceedings of the Royal Society B: Biological Sciences*, *288*(1957), 20211195. https://doi.org/10.1098/rspb.2021.1195

Happel, A., Stratton, L., Kolb, C., Hays, C., Rinchard, J., & Czesny, S. (2016). Evaluating quantitative fatty acid signature analysis (QFASA) in fish using controlled feeding experiments. *Canadian Journal of Fisheries and Aquatic Sciences*, *73*(8), 1222–1229. https://doi.org/10.1139/cjfas-2015-0328

Harvell, C. D., Montecino-Latorre, D., Caldwell, J. M., Burt, J. M., Bosley, K., Keller, A., Heron, S. F., Salomon, A. K., Lee, L., Pontier, O., Pattengill-Semmens, C., & Gaydos, J. K. (2019). Disease epidemic and a marine heat wave are associated with the continental-scale collapse of a pivotal predator (*Pycnopodia helianthoides*). *Science Advances*, *5*(1), eaau7042. https://doi.org/10.1126/sciadv.aau7042

Heithaus, M. R., Frid, A., Wirsing, A. J., & Worm, B. (2008). Predicting ecological consequences of marine top predator declines. *Trends in Ecology & Evolution*, *23*(4), 202–210. https://doi.org/10.1016/j.tree.2008.01.003

Herrlinger, T. J. (1983). *The diet and predator-prey relationships of the sea star* Pycnopodia helianthoides *(Brandt) from a central California kelp forest* [MA thesis, San Jose State University].

Hillgartner, F. B., Salati, L. M., & Goodridge, A. G. (1995). Physiological and molecular mechanisms involved in nutritional regulation of fatty acid synthesis. *Physiological Reviews*, *75*(1), 47–76. https://doi.org/10.1152/physrev.1995.75.1.47

Hodin, J., Pearson-Lund, A., Anteau, F. P., Kitaeff, P., & Cefalu, S. (2021). Progress toward complete life-cycle culturing of the endangered sunflower star, *Pycnopodia helianthoides*. *The Biological Bulletin*, *241*(3), 243–258. https://doi.org/10.1086/716552

Howell, K., Pond, D., Billett, D., & Tyler, P. (2003). Feeding ecology of deep-sea seastars (Echinodermata: Asteroidea): a fatty-acid biomarker approach. *Marine Ecology Progress Series*, *255*, 193–206. https://doi.org/10.3354/meps255193

Howells, R. J., Burthe, S. J., Green, J. A., Harris, M. P., Newell, M. A., Butler, A., Johns, D. G., Carnell, E. J., Wanless, S., & Daunt, F. (2017). From days to decades: Short- and long-term variation in environmental conditions affect offspring diet composition of a marine top predator. *Marine Ecology Progress Series*, *583*, 227–242. https://doi.org/10.3354/meps12343

Iverson, S. J. (2009). Tracing aquatic food webs using fatty acids: From qualitative indicators to quantitative determination. In M. Kainz, M. T. Brett, & M. T. Arts (Eds.), *Lipids in Aquatic Ecosystems* (pp. 281–308). Springer. https://doi.org/10.1007/978-0-387-89366-2_12

Iverson, S. J., Field, C., Don Bowen, W., & Blanchard, W. (2004). Quantitative fatty acid signature analysis: A new method of estimating predator diets. *Ecological Monographs*, *74*(2), 211–235. https://doi.org/10.1890/02-4105

Jardine, T. D., Galloway, A. W. E., & Kainz, M. J. (2020). Unlocking the power of fatty acids as dietary tracers and metabolic signals in fishes and aquatic invertebrates. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *375*(1804), 20190639. https://doi.org/10.1098/rstb.2019.0639

Kabeya, N., Fonseca, M. M., Ferrier, D. E. K., Navarro, J. C., Bay, L. K., Francis, D. S., Tocher, D. R., Castro, L. F. C., & Monroig, Ó. (2018). Genes for de novo biosynthesis of omega-3 polyunsaturated fatty acids are widespread in animals. *Science Advances*, *4*(5), eaar6849. https://doi.org/10.1126/sciadv.aar6849

Kainz, M., Brett, M. T., & Arts, M. T. (Eds.). (2009). *Lipids in Aquatic Ecosystems*. Springer. https://doi.org/10.1007/978-0-387-89366-2

Kelly, J., & Scheibling, R. (2012). Fatty acids as dietary tracers in benthic food webs. *Marine Ecology Progress Series*, *446*, 1–22. https://doi.org/10.3354/meps09559

Kimmerer, W. J., & Lougee, L. (2015). Bivalve grazing causes substantial mortality to an estuarine copepod population. *Journal of Experimental Marine Biology and Ecology*, *473*, 53–63. https://doi.org/10.1016/j.jembe.2015.08.005

Konar, B., & Estes, J. A. (2003). The stability of boundary regions between kelp beds and deforested areas. *Ecology*, *84*(1), 174–185. https://doi.org/10.1890/0012-9658(2003)084[0174:TSOBRB]2.0.CO;2

Lambert, P. (2000). *Sea Stars of British Columbia, Southeast Alaska, and Puget Sound*. UBC Press.

Latyshev, N., Khardin, A., & Kiyashko, S. (2001). Fatty acids as markers of starfish food sources. *Doklady Biological Sciences: Proceedings of the Academy of Sciences of the USSR, Biological Sciences Sections / Translated from Russian*, *380*, 489–491. https://doi.org/10.1023/A:1012391909338

Lee, L. C., Watson, J. C., Trebilco, R., & Salomon, A. K. (2016). Indirect effects and prey behavior mediate interactions between an endangered prey and recovering predator. *Ecosphere*, *7*(12), e01604. https://doi.org/10.1002/ecs2.1604

Mauzey, K. P., Birkeland, C., & Dayton, P. K. (1968). Feeding behavior of asteroids and escape responses of their prey in the Puget Sound region. *Ecology*, *49*(4), 603–619. https://doi.org/10.2307/1935526

McPherson, M. L., Finger, D. J. I., Houskeeper, H. F., Bell, T. W., Carr, M. H., Rogers-Bennett, L., & Kudela, R. M. (2021). Large-scale shift in the structure of a kelp forest ecosystem co-occurs with an epizootic and marine heatwave. *Communications Biology*, *4*(1), 1–9. https://doi.org/10.1038/s42003-021-01827-6

Menge, B. A., & Sanford, E. (2013). Ecological Role of Sea Stars from Populations to Meta-Ecosystems. In *Starfish: Biology and Ecology of the Asteroidea*. JHU Press.

Meunier, Z. D., Hacker, S. D., & Menge, B. A. (2024). Regime shifts in rocky intertidal communities associated with a marine heatwave and disease outbreak. *Nature Ecology & Evolution*, *8*(7), 1285–1297. https://doi.org/10.1038/s41559-024-02425-5

Moitoza, D. J., & Phillips, D. W. (1979). Prey defense, predator preference, and nonrandom diet: The interactions between *Pycnopodia helianthoides* and two species of sea urchins. *Marine Biology*, *53*(4), 299–304. https://doi.org/10.1007/BF00391611

Monroig, Ó., Tocher, D. R., & Navarro, J. C. (2013). Biosynthesis of polyunsaturated fatty acids in marine invertebrates: Recent advances in molecular mechanisms. *Marine Drugs*, *11*(10), 3998–4018. https://doi.org/10.3390/md11103998

Morais, S., Mourente, G., Martínez, A., Gras, N., & Tocher, D. R. (2015). Docosahexaenoic acid biosynthesis via fatty acyl elongase and Δ4-desaturase and its modulation by dietary lipid level and fatty acid composition in a marine vertebrate. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1851*(5), 588–597. https://doi.org/10.1016/j.bbalip.2015.01.014

Novak, M., Wolf, C., Coblentz, K. E., & Shepard, I. D. (2017). Quantifying predator dependence in the functional response of generalist predators. *Ecology Letters*, *20*(6), 761–769. https://doi.org/10.1111/ele.12777

Olson, R. J., Duffy, L. M., Kuhnert, P. M., Galván-Magaña, F., Bocanegra-Castillo, N., & Alatorre-Ramírez, V. (2014). Decadal diet shift in yellowfin tuna *Thunnus albacares* suggests broad-scale food web changes in the eastern tropical Pacific Ocean. *Marine Ecology Progress Series*, *497*, 157–178. https://doi.org/10.3354/meps10609

Paine, R. T. (1980). Food Webs: Linkage, interaction strength and community infrastructure. *Journal of Animal Ecology*, *49*(3), 667–685. https://doi.org/10.2307/4220

Parrish, C. C. (2013). Lipids in Marine Ecosystems. *ISRN Oceanography*, *2013*, 1–16. https://doi.org/10.5402/2013/604045

Parrish, C. C., Abrajano, T. A., Budge, S. M., Helleur, R. J., Hudson, E. D., Pulchan, K., & Ramos, C. (2000). Lipid and Phenolic Biomarkers in Marine Ecosystems: Analysis and Applications. In P. J. Wangersky (Ed.), *Marine Chemistry* (pp. 193–223). Springer. https://doi.org/10.1007/10683826_8

Pearse, J. S. (2006). Ecological role of purple sea urchins. *Science*, *314*(5801), 940–941. https://doi.org/10.1126/science.1131888

Pearse, J. S., & Hines, A. H. (1987). Long-term population dynamics of sea urchins in a central California kelp forest: Rare recruitment and rapid decline. *Marine Ecology Progress Series*, *39*, 275–283.

Pitt, K. A., & Purcell, J. E. (Eds.). (2009). *Jellyfish Blooms: Causes, Consequences, and Recent Advances*. Springer Netherlands. https://doi.org/10.1007/978-1-4020-9749-2

Provencher, J. F., Gaston, A. J., O'Hara, P. D., & Gilchrist, H. G. (2012). Seabird diet indicates changing Arctic marine communities in eastern Canada. *Marine Ecology Progress Series*, *454*, 171–182. https://doi.org/10.3354/meps09299

Read, K. D., Lessard, J., & Boulding, E. G. (2013). Improving outplanting designs for northern abalone (*Haliotis kamtschatkana*): The addition of complex substrate increases survival. *Journal of Shellfish Research*, *32*(1), 171–180. https://doi.org/10.2983/035.032.0123

Rogers-Bennett, L., & Catton, C. A. (2019). Marine heat wave and multiple stressors tip bull kelp forest to sea urchin barrens. *Scientific Reports*, *9*(1), 15050. https://doi.org/10.1038/s41598-019-51114-y

Sargent, J. R., Falk-Petersen, I.-B., & Calder, A. G. (1983). Fatty acid compositions of neutral glycerides from the ovaries of the asteroids *Ctenodiscus crispatus*, *Asterias lincki* and *Pteraster militaris* from Balsfjorden, northern Norway. *Marine Biology*, *72*(3), 257–264. https://doi.org/10.1007/BF00396831

Schram, J. B., Amsler, M. O., Galloway, A. W. E., Amsler, C. D., & McClintock, J. B. (2019). Fatty acid trophic transfer of Antarctic algae to a sympatric amphipod consumer. *Antarctic Science*, *31*(6), 315–316. https://doi.org/10.1017/S0954102019000397

Schram, J. B., Kobelt, J. N., Dethier, M. N., & Galloway, A. W. E. (2018). Trophic transfer of macroalgal fatty acids in two urchin species: digestion, egestion, and tissue building. *Frontiers in Ecology and Evolution*, *6*. https://doi.org/10.3389/fevo.2018.00083

Shivji, M., Parker, D., Hartwick, B., & Smith, M. J. (1983). Feeding and distribution study of the sunflower sea star *Pycnopodia helianthoides* (Brandt, 1835). *Pacific Science*, *37*(2), 133–140.

Smale, D. A., Wernberg, T., & Vanderklift, M. A. (2017). Regional-scale variability in the response of benthic macroinvertebrate assemblages to a marine heatwave. *Marine Ecology Progress Series*, *568*, 17–30. https://doi.org/10.3354/meps12080

Takagi, T., Eaton, C., & Ackman, R. (1980). Distribution of fatty acids in lipids of the common Atlantic sea urchin *Strongylocentrotus droebachiensis*. *Canadian Journal of Fisheries and Aquatic Sciences*, *37*, 195–202. https://doi.org/10.1139/f80-025

Taradash, A. (2022). *Examining variations in fatty acid profiles of the sea star* Pycnopodia helianthoides *on the coast of British Columbia*. [BS thesis, University of Oregon].

Thomas, M. D., Schram, J. B., Clark-Henry, Z. F., Yednock, B. K., Shanks, A. L., & Galloway, A. W. E. (2020). Juvenile Dungeness crabs (*Metacarcinus magister*) selectively integrate and modify the fatty acids of their experimental diets. *Philosophical Transactions of the*

Royal Society B: Biological Sciences, *375*(1804), 20200038. https://doi.org/10.1098/rstb.2020.0038

Traiger, S., Konar, B., Doroff, A., & McCaslin, L. (2016). Sea otters versus sea stars as major clam predators: Evidence from foraging pits and shell litter. *Marine Ecology Progress Series*, *560*, 73–86. https://doi.org/10.3354/meps11871

Vitense, K., Wirsing, A. J., Tyson, R. C., & Anderson, J. J. (2016). Theoretical impacts of habitat loss and generalist predation on predator–prey cycles. *Ecological Modelling*, *327*, 85–94. https://doi.org/10.1016/j.ecolmodel.2016.02.002

Watanabe, J. M. (1984). The influence of recruitment, competition, and benthic predation on spatial distributions of three species of kelp forest gastropods (Trochidae: *Tegula*). *Ecology*, *65*(3), 920–936. https://doi.org/10.2307/1938065

Watson, J., & Estes, J. A. (2011). Stability, resilience, and phase shifts in rocky subtidal communities along the west coast of Vancouver Island, Canada. *Ecological Monographs*, *81*(2), 215–239. https://doi.org/10.1890/10-0262.1

Wei, Z., Liu, X., Zhou, Z., & Xu, J. (2019). De novo transcriptomic analysis of gonad of *Strongylocentrotus nudus* and gene discovery for biosynthesis of polyunsaturated fatty acids. *Genes & Genomics*, *41*(5), 583–597. https://doi.org/10.1007/s13258-019-00799-6

Wetmore, L. (2022). *Predatory regulation of tegula grazing activity in giant kelp forests* [doctoral dissertation, San Diego State University]. https://www.proquest.com/docview/2778205551/abstract/E1A035971BD14BB0PQ/1

Whippo, R., Gravem, S., Porter-Hughes, E., & Galloway, A. W. E. (2024). The sunflower sea star reduces grazing rates of purple sea urchins dependent upon urchin starvation state. *Ecosphere*, *15*(7), e4948. https://doi.org/10.1002/ecs2.4948

Xu, J., Wen, Z., Gong, Z., Zhang, M., Xie, P., & Hansson, L.-A. (2012). Seasonal trophic niche shift and cascading effect of a generalist predator fish. *PLoS ONE*, *7*(12), e49691. https://doi.org/10.1371/journal.pone.0049691

Zhang, J., Ren, C., Zhang, H., Yin, F., Zhang, S., Wan, R., & Kitazawa, D. (2020). Review of estimating trophic relationships by quantitative fatty acid signature analysis. *Journal of Marine Science and Engineering*, *8*(12), 1030. https://doi.org/10.3390/jmse8121030

Zhukova, N. V. (2019). Fatty acids of marine mollusks: Impact of diet, bacterial symbiosis and biosynthetic potential. *Biomolecules*, *9*(12), 857. https://doi.org/10.3390/biom9120857