MACROALGAE AND THEIR ASSOCIATED COMMUNITIES:

CHEMICAL, CONSERVATION, AND TROPHIC ECOLOGY

by

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A DISSERTATION

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DISSERTATION ABSTRACT

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Title: Macroalgae and their Associated Communities: Chemical, Conservation, and Trophic Ecology

Marine macroalgae are ubiquitous across coastal oceans worldwide and provide critical habitat and services for diverse assemblages of organisms as well as services including fisheries production, carbon sequestration, and nutrient cycling. The effects of climate change are altering multiple aspects of macroalgal community ecology including food web structure, organismal diversity, and community resilience. In this dissertation, I approach macroalgal community ecology on multiple scales, across broad geographic space, and through various analytical lenses to capture key insights into the functioning of macroalgal forests. In Chapter II I identify the unique fatty acid profiles and stable isotope content of 31 Antarctic macroalgae. While the phylogenetic differentiation driven by fatty acids has a stronger influence on distinguishing Antarctic macroalgae, the added dimension of stable isotopes can likely make the combination of the two approaches particularly powerful in the application of food web studies. In Chapter III I provide a summary of the effects of conservation areas on algal assemblages. I find that all targeted kelp species are observed across the marine reserves

but their presence varies among sites and years. In Chapter IV I describe the feeding preferences of the sunflower seastar through meta-analysis and a cafeteria experiment. A total of 114 prey taxa are reported across all studies, with bivalves and urchins tending to be the primary observed prey items. This agrees with our cafeteria experiments that find *Pycnopodia* tended to prefer green and purple urchins, and mussels, although the quantity of each prey type consumed is highly variable. In Chapter V I provide evidence for non-consumptive effects of the sunflower seastar on grazing sea urchins. I find that the presence of a waterborne *Pycnopodia* cue reduces the grazing rate of fed urchins by 50% over short (~24 h) time scales. In contrast, starved urchins consume kelp and do not exhibit an escape response in the presence of a *Pycnopodia* cue. This chapter highlights a trait-mediated indirect interaction between *Pycnopodia, S. purpuratus* and kelp, and how the urchin response to a predator cue may differ based on urchin metabolic conditions. This dissertation includes previously published and unpublished co-authored material.

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My PhD research was conducted on the traditional homelands of the original peoples of the Pacific Northwest who have lived here since time immemorial. The University of Oregon is located on Kalapuya Ilihi, the traditional indigenous homeland of the Kalapuya people whose descendants are now citizens of the Confederated Tribes of Grand Ronde Community of Oregon and the Confederated Tribes of Siletz Indians of Oregon. The Oregon Institute of Marine Biology is located on the traditional lands and waterways of the Confederated Tribes of the Coos, Lower Umpqua, and Siuslaw peoples. The field work for Chapter III of my dissertation was conducted within the Oregon Marine Reserve system on the ancestral lands and waterways of the Confederated Tribes of Grand Ronde, Confederated Tribes of Siletz Indians, Clatsop-Nehalem Confederated Tribes, Salmon River, Yakina, Tututni, and Tolowa Dee-ni' peoples. Field work for chapters IV-V was conducted at the Friday Harbor Laboratories on the ancestral lands and waterways of the Coast Salish, which touches the shared waters of all tribes and bands of the central Salish Sea including the Lhaq'temish (Lummi), Lekwungen (Songhees), Swinomish, Semiahmoo, Samish, T'sou-ke, WSÁNEĆ, and Jamestown S'Klallam. I recognize that my work in these places has only been possible because of the external and internal colonization of this region, forced removals, and efforts to render tribal and First Nations invisible, assimilated, and/or disappeared. I also recognize the continuing systemic harms from settler colonialism, nationalism, and White supremacy, that often play a role in educational and research endeavors. I grew up along the shores of the Salish Sea next to the original stewards of the land. For a long time I did not understand what it meant for me to be living and working on here. Now I understand, and I will use my science for collaboration, not extraction, for listening,

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* * *

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CHAPTER I

INTRODUCTION

Marine macroalgae are ubiquitous across coastal oceans worldwide and provide critical habitat and services for diverse assemblages of organisms (Dayton 1985, Wiencke & Amsler 2012). They are frequently defined as ‘foundation species’ (sensu Hughes 2010) that provide complex physical structure utilized by a host of organisms as nursery habitat, shelter, and sources of nutrition (Steneck et al. 2002). Typically found in nearshore environments and reaching depths of up to 50 m, these macroalgae form dense ‘forests’ that foster year-round and seasonal communities that can consist of both surface (canopy), mid-water or benthic (understory) components. Macroalgal forests composed of kelp (Laminariales) and other seaweeds (non-Laminariales) are marked by much higher primary productivity than adjacent habitat (Filbee-Dexter & Scheibling 2014), and can produce up to 1900 g C m-2 y-1 of which up to 35% can be released as dissolved organic matter into the water column (Mann 1973, Krumhansl & Scheibling 2012). These forests play a critical role in the delivery of nutrients in the form of macroalgal detritus to local consumers and adjacent habitats (Polis et al. 1997, Krumhansl & Scheibling 2012, Wiencke & Amsler 2012). They also provide additional services including fisheries production, carbon sequestration, and nutrient cycling that are estimated to have a value of $500 billion USD per year globally (Eger et al. 2023).

The effects of climate change are altering multiple aspects of macroalgal community ecology including food web structure, organismal diversity, and community resilience (Harley et

al. 2012). Removal of key players in coastal food webs by marine heat waves, disease exacerbated by climate change, and human use of marine habitat all have the potential to effect macroalgal assemblages in myriad ways resulting in changes in: distribution (Assis et al. 2017), community connectivity (Peña et al. 2017), biomass (O'Connor et al. 2017), food web complexity (Byrnes et al. 2011), detrital production (Krumhansl et al. 2014), carbon flow (Salomon et al. 2008), and physiology (Brown et al. 2014).

In this dissertation, I approach macroalgal community ecology on multiple scales, across broad geographic space, and through various analytical lenses to capture key insights into the functioning of macroalgal forests. The chapters are arranged by ecoregion and move from a broad (community-wide) to a narrow (species interactions) focus. Chapter II addresses the chemical ecology of Antarctic seaweeds, Chapter III describes the community-wide composition of macroalgae inside and outside of marine reserves on the West Coast of North America, and Chapters IV and V investigate the trophic interactions between important macroalgal forest-associated species, also along the West Coast of North America. Chapter VI summarizes my findings and suggests next-steps as research into the chemical, conservation, and trophic ecology of macroalgal forests continues into an uncertain future.

Chapter II, which contains previously unpublished co-authored material, concerns the chemical ecology of Antarctic seaweeds on the Western Antarctica Peninsula (WAP) and describes trophic biomarkers including fatty acids and stable isotopes found in algal tissues. The WAP supports extensive macroalgal forests that are home to a wide diversity of organisms (Wiencke & Amsler 2012). These macroalgal forests are important to the coastal food web (Iken et al. 1997, Dunton 2001, Aumack et al. 2017), however, the contribution of these macroalgal to Antarctic food webs on larger scales is not well understood. I used biomarker analysis to

quantify a suite of fatty acids and stable isotope values that can be used as a starting place to further investigate trophic pathways in Antarctic food webs (Budge et al. 2006, Boecklen et al. 2011). It summarizes the fatty acid content for 31 macroalgal species, 14 of which have no previously published values, and evaluates contributions of both types of biomarkers as taxonomic indicators. This chapter will be published with members of the Antarctic field team including: Chuck Amsler, Maggie Amsler, Katrin Iken, Julie Schram, Alex Lowe, Andrew Klein, Sabrina Heiser, and Aaron Galloway. All members will contribute to the final submitted of the manuscript.

Chapter III, which contains previously published co-authored material, uses long-term survey data from the Oregon Marine Reserve System (OMRS) to identify ecological community differences between marine reserves and nearby comparison areas, and provide guidance on best practices for reserve monitoring going forward. The OMRS was created in 2010 to address conservation concerns including human impacts on fish, invertebrate, and algal populations by restricting human access, the effects of which were assessed by long-term monitoring. This chapter summarises the findings of the macroalgal component of that monitoring effort between the reserves and nearby comparison areas for key species, and provides guidance on SCUBA survey methods including the use of permanent transects and shallower surveys, that can provide more consistent and informative data in the future. It was first published with coauthors Lindsay Alysworth and Stephanie Fields in the Oregon Department of Fish and Wildlife's OMRS report in 2022.

Chapter IV assesses the feeding preferences of the important macroalgal forest generalist predator, the sunflower sea star *Pycnopodia helianthoides*. *Pycnopodia* populations have been reduced drastically since 2012 along the West Coast of North America due to sea star wasting

disease, which has been implicated as a major contributing factor to the proliferation of their prey—grazing sea urchins—and the subsequent decline of macroalgal forests (Burt et al. 2018, Harvell et al. 2019, Hamilton et al. 2021, Galloway et al. 2023). Although *Pycnopodia* has been observed to consume sea urchins, the strength of their preference for this prey item is unknown. Understanding how desirable urchins are as prey to *Pycnopodia* is critical to understanding their role in recent macroalgal forest declines, particularly as macroalgal forests are full of potential prey items that *Pycnopodia* might want to eat. This chapter uses meta-analysis of existing observations about *Pycnopodia* diet in the wild, coupled with a cafeteria feeding experiment to determine the relative preferences that *Pycnopodia* display toward common macroalgal forest-associated prey. It will be published with coauthors Kindall Murie, who provided field and lab support, and Aaron Galloway, who provided conceptual support. Both authors will provide editorial contributions to the final submitted manuscript.

Chapter V, which contains unpublished co-authored material, quantifies the non-consumptive effect *Pycnopodia helianthoides* on grazing urchin behavior. The interactions between predator and prey can have far reaching consequences for the stability and resilience of ecological communities (Paine 1966, Kissling & Schleuning 2015). Although the direct consumptive effect of *Pycnopodia* on sea urchins has been quantified (Galloway et al. 2023), the non-consumptive effects (i.e., behavioral responses) of urchins to *Pycnopodia* is not well understood. In this chapter I quantified the non-consumptive effect of *Pycnopodia* on the purple sea urchin *Strongylocentrotus purpuratus* in two experiments to determine the presence and strength of these effects on grazing rates and foraging behavior of the urchins. This chapter will be published with coauthors Sarah Gravem who provided conceptual and statistical support, Ethan Porter-Hughes who conducted video analyses, and Aaron Galloway who provided

conceptual support. All authors will provide editorial contributions to the final submitted manuscript.

Taken together, these chapters describe important aspects of macroalgal forest ecology from Antarctica to the Western North American coast, and are summarized with main conclusions in Chapter VI.

CHAPTER II

FATTY ACID PROFILES AND STABLE ISOTOPE COMPOSITION OF ANTARCTIC MACROALGAE: A BASELINE FOR A COMBINED BIOMARKER APPROACH IN FOOD WEB STUDIES

This study contains unpublished coauthored material that was conceived by me as part of a larger project led by coauthors Charles Amsler, Margaret Amsler, Katrin Iken, James McClintock, Andrew Klein, and Aaron Galloway. Field collections were made by myself and coauthors Charles Amsler, Margaret Amsler, Katrin Iken, Alex Lowe, Julie Schram, Sabrina Heiser, Aaron Galloway. I was responsible for all fatty acid biomarker extraction and lab work, and stable isotope data was prepared by Katrin Iken. All statistical analyses were done by myself, and I also wrote the manuscript with editorial input from all authors.

1. INTRODUCTION

Along the coastal Western Antarctic Peninsula (WAP) dense nearshore macroalgal forests provide habitat and carbon to a diverse assemblage of marine organisms (Wiencke et al. 2014, Valdivia et al. 2015, Oliveira et al. 2020). Nearshore marine habitats of the WAP (0-50 m depth) contain the greatest macroalgal biomass found on the continent, with approximately 110 species of an overall diversity of 151 described macroalgal species in Antarctica (Mystikou et al. 2014, Pellizzari et al. 2017, Oliveira et al. 2020). These macroalgal species make up the majority of benthic primary production along the WAP (Wiencke et al. 2014, Barnes 2017). It is estimated that much of the total macroalgal biomass production is channeled annually into the coastal system (Quartino & Boraso de Zaixso 2008) or transported to adjacent, deeper habitats as a

spatial subsidy (Fischer & Wiencke 1992). Multiple studies along the WAP have identified the overall importance of macroalgae in supporting the coastal food web (e.g., Iken et al. 1997, Dunton 2001, Huang et al. 2006, Aumack et al. 2017, Zenteno et al. 2019, Cardona et al. 2021); however, the contributions of individual macroalgal species to the Antarctic food web on larger scales are less well understood.

The role that macroalgae play in the WAP coastal food web is complex, and is controlled by various factors, including physiological differences in macroalgae marked by the presence of chemical defense compounds (Amsler et al. 2005, 2009), and environmental factors such as sea ice cover (Quartino et al. 2013, Amsler et al. in revision), temperature (Becker et al. 2010, Cordone et al. 2018), and light (Deregibus et al. 2016). Amphipods and gastropods are some of the most abundant mesograzers in the Antarctic subtidal and mostly consume small epiphytes growing on the larger, often chemically defended macroalgae (Iken 1999, Aumack et al. 2017). These small mesograzers are important prey for other predators, and may serve as a link between benthic primary production and higher trophic levels (e.g., Dauby et al. 2003, Zamzow et al. 2011, Casaux & Barrera-Oro 2013). However, some consumers prefer chemically-defended species to reduce competition for food resources or capitalize on the chemicals for their own defenses (Amsler et al. 2013, Heiser et al. 2022). In addition, chemical defenses of Antarctic macroalgae can dissipate once algae senesce or die, increasing the palatability to consumers once the algal material enters the detrital pathway (Reichardt & Dieckmann 1985, Amsler et al. 2012, Schram et al. 2019).

Antarctic macroalgae also differ in other attributes important to consumers, such as morphological or biochemical characteristics. For example, thallus toughness mediates

palatability for some consumers (Amsler et al. 2005). Also, while nutritional quality of Antarctic macroalgae is typically high (Weykam et al. 1996, Amsler et al. 2005), there are differences in biochemical content that increase the nutritional quality of some algal species over others (Peters et al. 2005). One of the nutritional sources that are especially important to consumers are fatty acids (FA). The FAs of both Arctic and Antarctic seaweeds are known to differ among taxonomic groups (e.g., Graeve et al. 2002, Aumack et al. 2017, Berneira et al. 2020). This aligns with well-established FA patterns in macroalgae elsewhere: the FA composition is strongly differentiated by the phylogenetic relationships of the groups, with closely related taxa having high similarity in FA profiles (Kelly & Scheibling 2012, Galloway et al. 2012). FAs are vital to macroalgal metabolic functions, such as maintaining membrane fluidity (Santos et al. 2017), in addition to contributing critical nutrients to the metabolic processes of most consumers (Dalsgaard et al. 2003, Budge et al. 2006).

Some of the more comprehensive studies on FAs in Antarctic macroalgae established that they contain significant amounts of polyunsaturated fatty acids (PUFAs) (Graeve et al. 2002, Santos et al. 2017, Teixeira et al. 2019, Schram et al. 2019, Berneira et al. 2020), especially long-chained (C_{18} and C_{20}) PUFAs, which have been identified as key nutritional components in aquatic food webs (Ruess & Müller-Navarra 2019). PUFAs are important for growth, production and fecundity in marine consumers (e.g., Parrish 2009) and enhance the energy transfer efficiency among trophic levels in marine systems (Müller-Navarra et al. 2000, Troch et al. 2012, Eddy et al. 2021). In Antarctic coastal food webs, PUFAs and other FAs from macroalgae are effectively assimilated by consumers (Aumack et al. 2017, Schram et al. 2019), likely contributing to their nutrition and performance.

In addition to providing important nutrition to consumers, FAs can also be used in food web studies to trace the consumption of various macroalgal species into consumers (Kelly & Scheibling 2012). This applies especially to essential fatty acids (EFAs), which are organic molecules required for biological processes that are only synthesized by primary producers and must be assimilated into consumer tissues through consumption (Arts et al. 2001, Galloway & Winder 2015). These EFAs, along with other FAs, can be used as biomarkers to trace the flow of trophic resources though a food web and clarify relationships between specific producers and consumers (Dalsgaard et al. 2003, Budge et al. 2006). This is enhanced by the fact that FA profiles of macroalgal species seem mostly conserved across large geographical scales, making them conservative markers for a source species (Khotimchenko et al. 2002). EFAs, in particular, can provide important information on what resources a consumer has been assimilating, generating quantitative estimates of the consumers diet (Iverson et al. 2004, Galloway & Winder 2015, Bromaghin 2017, Guerrero & Rogers 2020).

At the same time, a combination of different biomarkers, such as fatty acids with stable isotope (SI) information, can be especially powerful in distinguishing different primary producer sources in benthic food webs (Hanson et al. 2010, Kelly & Scheibling 2012, Dethier et al. 2013, Aumack et al. 2017). SI are typically used to provide information about food resources ($^{13}C/^{12}C$; $\delta^{13}C$) and trophic position ($^{15}N/^{14}N$; $\delta^{15}N$) in food web analyses, where the $\delta^{13}C$ values of different primary producer sources are preserved with minimal fractionation (~1 ‰) between consumer trophic levels (DeNiro & Epstein 1978, Caut et al. 2009), and can, thus, be traced through the food web (Boecklen et al. 2011). This applies, however, mostly to differences among larger groupings of primary producer sources, such as phytoplankton and macroalgal production

or terrestrial organic matter, in coastal systems (France 1995, Peterson 1999, Raven et al. 2002). However, the ability of SI to distinguish high-resolution (e.g., species-level) taxonomy is limited. Using SI to distinguish trophic levels in food webs is particularly valuable as FAs typically cannot determine trophic level (Kelly & Scheibling 2012). Data collected during SI analyses also allow for the calculation of the C:N ratio, which is a useful measure of the nutritional value of macroalgae as a food source (e.g., Weykam et al. 1996, Peters et al. 2005), and can provide additional information about trophic resource use.

Here, we provide FA profiles of 31 species of Antarctic macroalgae across the three major macroalgal divisions (Chlorophyta, Ochrophyta, Rhodophyta), significantly expanding the published profiles of 18 species (Graeve et al. 2002, Santos et al. 2017, Aumack et al. 2017, Teixeira et al. 2019, Schram et al. 2019, Berneira et al. 2020). In addition, we provide the carbon and nitrogen SI values and C:N ratios for most of the same species. Our expanded biomarker baselines, especially for FA profiles, is obtained from field-collected specimens, which may differ from profiles of some specimens previously analyzed from long-term lab cultures (Graeve et al. 2002). We also assess the ability of the two biomarkers types, individually and combined, to distinguish macroalgal species and the value of a multiple biomarker (FA and SI) approach in Antarctic coastal food web studies. Specifically, we used the various biomarker data sets to test how well they could differentiate some taxonomically close and morphologically similar macroalgal species, which may contribute differently to coastal food webs.

2. METHODS

2.1 Site selection

Macroalgae were collected in 2019 at 15 sites spanning the WAP from the Joubin Islands (64°S) in the north to Millerand Island (68°S) in the south (Figure 2.1, Table 2.1), which were accessed using small boats from the Antarctic Research and Support Vessel *Laurence M. Gould*. Collections were made opportunistically as part of a larger project assessing the macroalgal distribution and their role in the coastal food web along a gradient of sea ice cover (Amsler et al. in revision); collections for the present study were aimed to capture the diversity of macroalgal species across all sites.

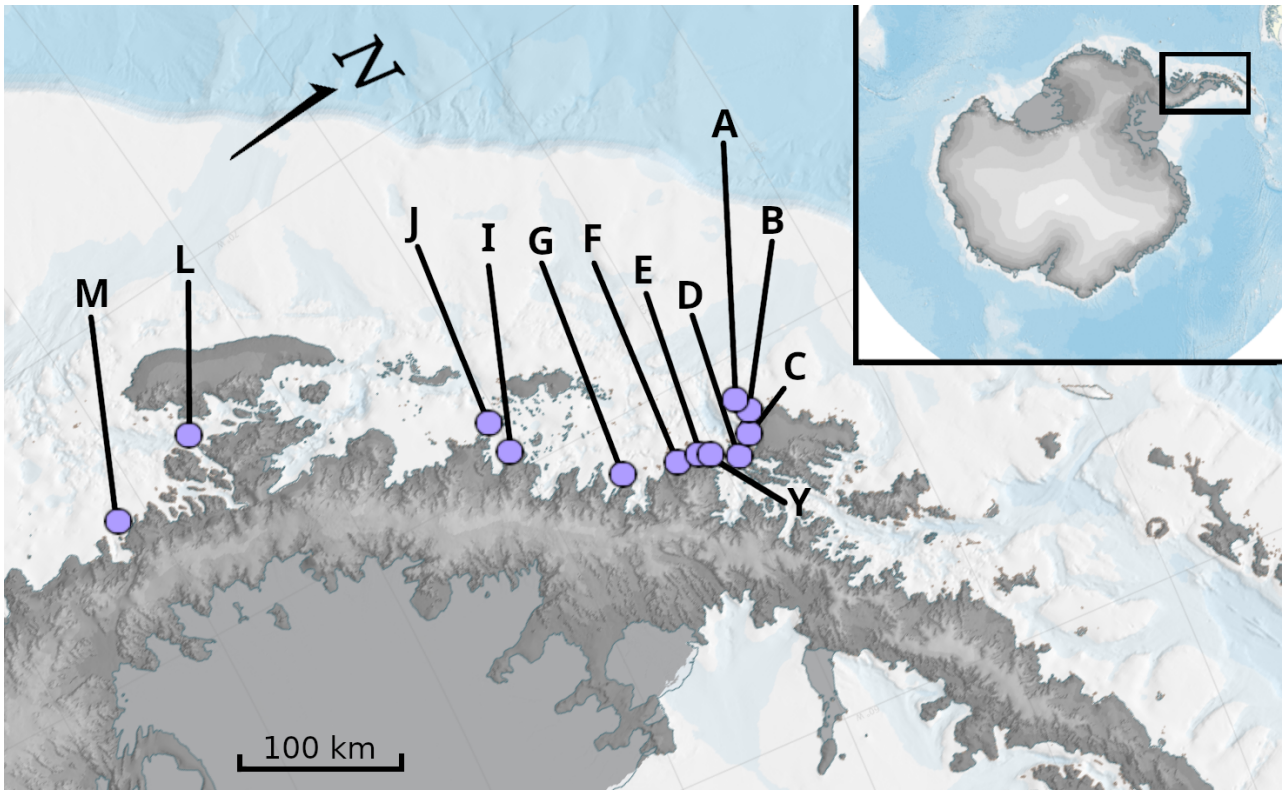


Figure 2.1: Map of study locations along the Western Antarctic Peninsula. Land masses are in gray and ocean is in white with off-shelf portions in blue. Study locations are indicated by letters according to Table 2.1.

Table 2.1: Field collection sites

Site ID	Latitude (dd)	Longitude (dd)	Description
A	-64.7720	-64.3699	Joubin Islands
B	-64.7793	-64.0441	Southeast corner of Bonaparte Point, Anvers Island
C	-64.7932	-64.0072	Limitrophe Island
D	-64.9002	-63.8531	Wauwermans Islands
E	-65.1043	-64.0471	Pleneau Island (off penguin colony on SE corner)
F	-65.2402	-64.2309	Argentine Islands near Vernadsky
G	-65.5131	-64.4203	Island north of Lahille Island and south of Lippmann Island
I	-66.0251	-65.3533	Minnow Islands (part of Fish Islands South of Prospect Point)
J	-66.0894	-65.8386	Southern Saffery Islands
L	-67.5488	-67.7714	Small island off a small island to the north of Pinero Island
M	-68.1758	-67.2682	Randall Rocks off Millerand Island
Y	-65.0995	-63.9874	Booth Island

2.2 Field collections

Algal samples were collected between April 28 and May 18 of 2019 using SCUBA, between 5 m and 40 m depth. Algal samples were returned to the lab within 1 h of collection where they were sorted and identified. Team phycologists verified the identities of the samples from morphological and anatomical features, as well as later confirmation from pressed voucher specimens, described in Amsler et al. (in revision). A maximum of seven replicates and a minimum of one algal tissue sample per species were taken (>20 mg per sample when possible) for FA analysis and frozen at -80 °C in microcentrifuge tubes. In most cases, up to three replicates per species were collected for bulk SI analysis. Mostly, these samples were from the same thalli as for FA analysis but on occasion, specimens were too small and different thalli were sampled for the two biomarker approaches. Algal tissues for SI analysis were dried at 60 °C until constant weight, at least 24 h.

2.3 Fatty acid analysis

Collections of each macroalgal species were freeze-dried in a lyophilizer for 48 h (or until completely dry) within 8 months of collection. Homogenized tissue was then processed for FA extraction at the Oregon Institute of Marine Biology lab (following Taipale et al. 2016).

2.3.1 Extraction Approximately 10-20 mg of dried macroalgal tissue was homogenized from each sample with a mortar and pestle, suspended in 2 ml of chloroform in a 10 ml graduated centrifuge tube under N_2, and held at -20 °C overnight. Tissue samples were sufficiently large for each replicate that samples did not need to be pooled. After 24 h extraction, 70 µl C19 standard (GLC Reference Standard 566 C, Nu-Check-Prep, Elysian, MN), 1 ml methanol, and 0.75 ml 0.9% NaCl water solution were added to each tube containing the tissues. Between each step throughout the extraction and transesterification process (see below), sample tubes were topped off with N_2 to avoid FA oxidation. Samples were then sonicated in an ice water bath for 10 min. Each tube was vortexed for 10 sec and centrifuged for 5 min at 3000 rpm at 4 °C. The separated chloroform solution was transferred to an 8-ml scintillation vial, and the remaining material including algal tissues in the centrifuge tube was topped off with 1 ml chloroform and re-run through the vortexing, centrifuging, and chloroform removal process. All chloroform fractions from one sample were combined in the same scintillation vial. The chloroform solution was then evaporated under N_2 to approximately 1.5 ml, and 1 ml of this remaining extract was moved to a 10-ml graduated centrifuge tube for FA transesterification. This 1-ml chloroform subsample of each extract in the centrifuge tubes was evaporated to dryness under N_2 and 1 ml toluene added to each sample, and the remaining sample volume was dried and total lipid content gravimetrically determined (data not reported here).

2.3.2 Transesterification To transesterify each sample, 2 ml of 1% H_2SO_4 in methanol was added to each toluene extract and vortexed for 10 sec. Centrifuge tubes were held at 90 °C for 90 min in a water bath. After the incubation period, 1.5 ml 2% $KHCO_3$ and 2 ml hexane were added to each tube and vortexed for 10 sec. Tubes were then centrifuged for 2 min at 1500 rpm at 4 °C.

Fatty acid methyl esters (FAME) were transferred from the top phase into a new tube for evaporation under N_2 in a ~30 °C water bath. An additional 2 ml hexane was added to the remaining lower phase in the centrifuge tube, vortexed, and centrifuged again as above. The FAME were then combined into each respective sample's tube in the water bath. Samples were evaporated to dryness, then 1.5 ml hexane was added to each, and the resulting FAME transferred to a GC vial and held at -20 °C.

2.3.3 Mass spectrometry The FA content of each sample was quantified in a gas chromatograph equipped with a mass spectrometer (GC-MS, Model QP2020, Shimadzu), with a DB-23 column 29.6 m long, 0.15 μm thick, and a diameter of 0.25 mm, using helium as a carrier gas. For each sample, 1 μl of FAME was run through the following heating protocol: 50 °C for 1 min, increased by 20 °C per min to 240 °C, and held for 10 min. Retention time and ion content were used to identify specific FA in the sample. A calibration curve using four serial dilutions (15 ng/ml, 50 ng/ml, 100 ng/ml, 250 ng/ml) of known FAs (GLC Reference Standard 566 C, Nu-Check-Prep, Elysian, MN; Pearson correlation coefficient >0.99) and the major ions for each identified sample peak were used to quantify FA concentrations. Total concentrations of FA in each sample were extracted using GCMS Postrun Analysis software (v4.41, Shimadzu Corporation, Kyoto, Japan). FA quantified were those included in the GLC Reference Standard 566 C, Nu-Check-Prep, Elysian, MN (Table 2.2) to ensure confident identification. The PUFA 18:4ω3, which is a diagnostic biomarker for the brown algae, while not part of the standard mix, was also included in our analyses as it can be identified with high confidence in chromatogram outputs. FAs are presented here according to the omega nomenclature including the number of carbons in the FA, number of double-bonds, and the number of carbons from the methyl end to

the first carbon in the double bond closest to the methyl end.

Table 2.2: List of FA targeted in analysis as part of Nu-check Prep standards

Chain	Standard
c8:0	Methyl Octanoate
c9:0	Methyl Nonanoate
c10:0	Methyl Decanoate
c11:0	Methyl Undecanoate
c11:1	Methyl Undecanoate
c12:0	Methyl Laurate
c13:0	Methyl Tridecanoate
c13:1	Methyl Tridecanoate
c14:0	Methyl Myristate
c14:1ω5	Methyl Myristoleate
c15:0	Methyl Pentadecanoate
c16:0	Methyl Palmitate
c16:1	Methyl Palmitoleate
c17:0	Methyl Heptadecanoate
c18:0	Methyl Stearate
c18:1ω9	Methyl Oleate
c18:1ω7	Methyl Vaccenate
c18:2	Methyl Linoleate
c18:3ω6	Methyl Gamma Linolenate
c19:0	Methyl Nonadecanoate
c18:3ω3	Methyl Linolenate
c20:0	Methyl Arachidate
c20:1	Methyl 11-Eicosenoate
c20:2	Methyl 11-14 Eicosadienoate
c20:3ω6	Methyl Homogamma Linolenate
c20:4	Methyl Arachidonate
c20:3ω3	Methyl Eicosatrienoate
c22:0	Methyl Behenate
c22:1	Methyl Erucate
c20:5	Methyl Eicosapentaenoate
c22:2	Methyl Docosadienoate
c22:3	Methyl Docosatrienoate
c22:4	Methyl Docosatetraenoate
c22:5ω6	Methyl Docosapentaenoate
c22:5ω3	Methyl Docosapentaenoate
c22:6	Methyl Docosahexaenoate
c23:0	Methyl Tricosanoate
c24:0	Methyl Lignocerate
c24:1	Methyl Nervonate

2.4 Stable isotope analysis

Dried macroalgal samples were homogenized to a powder and the carbon and nitrogen SI composition determined at the Alaska Stable Isotope Facility (ASIF) at the University of Alaska Fairbanks. Analyses were conducted on a continuous-flow isotope ratio mass spectrometry

(CFIRMS) using a Thermo Scientific Flash 2000 elemental analyzer and Thermo Scientific Conflo IV interfaced with a Thermo Scientific DeltaV^{Plus} Mass Spectrometer. Approximately 0.8-1.2 mg macroalgal material were weighed into tin capsules for analysis. Results are expressed as conventional δ notation in parts per thousand (‰) according to the following equation: $\delta X\ (‰) = ([R_{sample}/R_{standard}] - 1) \cdot 1000$, where X is ^{13}C or ^{15}N of the sample and R is the corresponding ^{13}C:^{12}C or ^{15}N:^{14}N ratio. Pee Dee Belemnite and atmospheric N_2 served as standards for carbon and nitrogen, respectively. Instrument error at ASIF was <0.2 ‰ for both $\delta^{13}C$ and $\delta^{15}N$ values. The molar ratio of carbon to nitrogen (C:N ratio) was also calculated from the outputs of the SI analysis.

2.5 Statistical analysis

Descriptive patterns of mean FA compositional and SI data for each species were visualized in R using the tidyverse and viridis packages (Wickham et al. 2019, Garnier et al. 2021, R Core Team 2022). Additionally, a SIMPER analysis was used to identify FAs that had the strongest effect (up to 80% cumulative) on sample differences, which were then used in reduced-biomarker PCA and PERMANOVA analyses using the vegan package (Oksanen et al. 2018). Both sets of biomarkers were visualized using cluster analysis and non-metric multidimensional scaling (nMDS). To determine how FA and SI biomarkers drive differences among individual samples and taxonomic groupings, a combination of PCA analysis, cluster analysis, and PERMANOVA were run for: all samples with FA data; all samples with SI data;

samples for which both types of data were available; select sample pairs of species that are morphologically and taxonomically similar, using the vegan and factoextra packages (Oksanen et al. 2018, Kassambara & Mundt 2020). Differences in C:N ratios (log transformed to meet assumptions of normality) among taxonomic divisions were tested with one-way ANOVA and a post-hoc Tukey test.

3. RESULTS

We quantified 40 FAs across all samples, 11 of which constituted at least 5% of the mean proportion of FA content across species (Figure 2.2, Table 2.3). These 11 ‘common’ FAs included saturated and unsaturated FAs, including EFAs such as 20:5ω3 (Eicosapentaenoic acid), 18:3ω3 (Alpha-linolenic acid), 22:5ω3 (Docosapentaenoic acid), 18:4ω3 (Stearidonic acid), and 20:4ω6 (Arachidonic acid). Among the other most common FA was the saturated FA (SAFA) 16:0 (Palmitic acid). Macroalgal samples contained between 20.5-44.4% SAFA and 43.5-70.6% PUFAs. *Lambia antarctica*, the only green algal representative in our collection, was particularly rich in 18:3ω3 compared to other taxa (Figure 2). Most brown algal species contained greater proportions of 18:1ω7 (Vaccenic acid), 18:1ω9 (Oleic acid), and 18:2ω6 (Linoleic acid) compared to red algae, which had overall higher proportions of 20:5ω3.

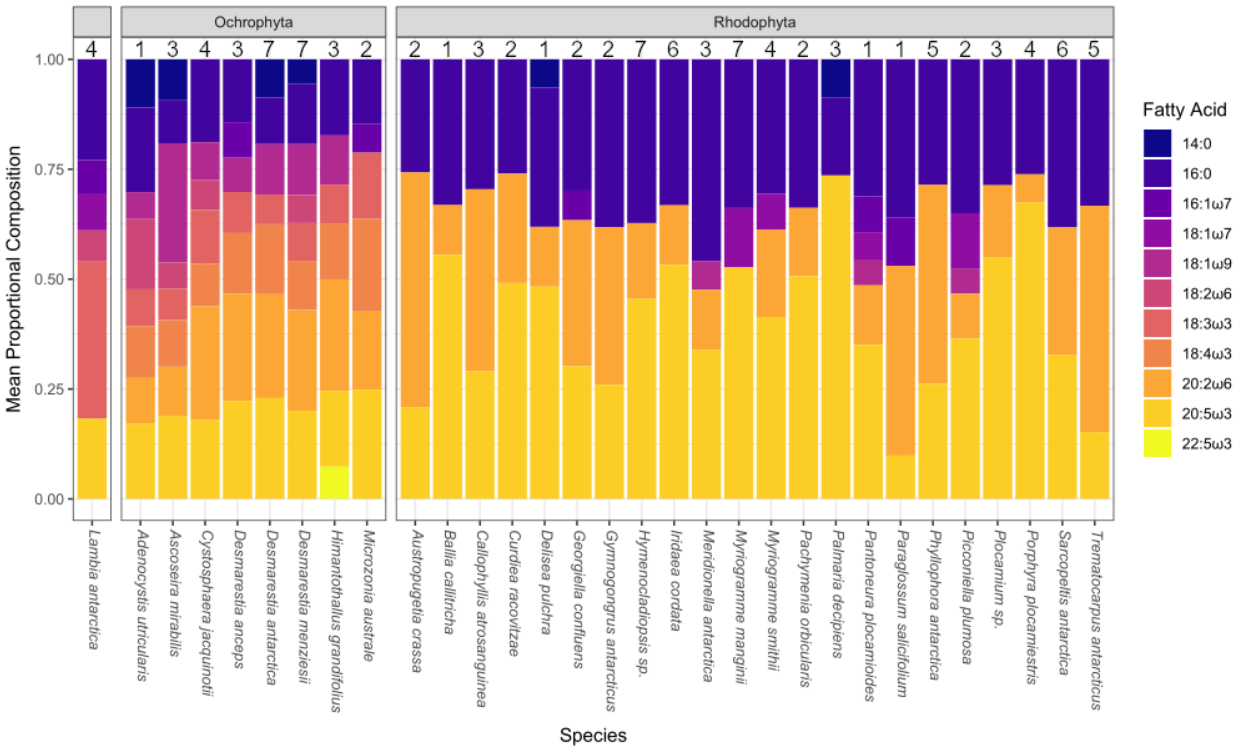


Figure 2.2: Mean proportional composition of individual fatty acids that contributed at least 5% of total fatty acid content to each macroalgal species. Species are organized in groups by division, with the first column being Chlorophyta, followed by Ochrophyta, and lastly Rhodophyta. Fatty acids are listed in the legend according to the omega nomenclature, and number or replicates of each species are listed above columns.

Table 2.3: Fatty acid composition (mass %), stable isotope values, and C:N ratios of all algae. SAFA: sum of saturated fatty acids; MUFA: sum of monounsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids.

	Chlorophyta	Ochrophyta																	
	Lambia antarctica	*Adenocystis utricularis*	*Ascoseira mirabilis*	*Cystosphaera jacquinotii*	*Desmarestia anceps*	*Desmarestia antarctica*	*Desmarestia menziesii*	*Himantothallus grandifolius*	*Microzonia australe*	*Austropugetia crassa*	*Ballia callitricha*	*Callophyllis atrosanguinea*	*Curdiea racovitzae*	*Delisea pulchra*	*Georgiella confluens*	*Gymnogongrus antarcticus*	*Hymenocladiopsis sp.*	*Iridaea cordata*	
N (FA/SI/Shared)	4/3/3	1/0/-	3/0/-	4/4/4	3/3/3	7/5/5	7/7/7	3/3/3	2/0/-	2/0/-	1/1/1	3/3/1	2/0/-	1/1/1	2/2/1	2/0/-	7/6/6	6/6/3	
8:0	0.009 ± 0.006	0.025	0.002 ± 0.001	0.007 ± 0.002	0.002 ± 0.002	0.003 ± 0.003	0.004 ± 0.002	0.006 ± 0.004	0.001 ± 0.001	0.004 ± 0.000	0.003	0.004 ± 0.002	0.001 ± 0.002	0.014	0.007 ± 0.010	0.013 ± 0.006	0.011 ± 0.009	0.026 ± 0.016	
9:0	0.193 ± 0.087	0.619	0.044 ± 0.023	0.121 ± 0.086	0.130 ± 0.092	0.077 ± 0.075	0.048 ± 0.050	0.035 ± 0.030	0.036 ± 0.017	0.318 ± 0.131	0.359	0.024 ± 0.023	0.124 ± 0.136	0.524	0.253 ± 0.346	0.319 ± 0.397	0.302 ± 0.151	0.134 ± 0.099	
10:0	0.016 ± 0.007	0.058	0.005 ± 0.001	0.011 ± 0.006	0.005 ± 0.002	0.005 ± 0.002	0.005 ± 0.001	0.005 ± 0.001	0.004 ± 0.002	0.013 ± 0.001	0.008	0.006 ± 0.003	0.012 ± 0.006	0.012	0.019 ± 0.026	0.021 ± 0.011	0.019 ± 0.010	0.022 ± 0.009	
11:0	0.004 ± 0.002	0.015	0.003 ± 0.001	0.005 ± 0.003	0.024 ± 0.023	0.009 ± 0.003	0.005 ± 0.002	0.007 ± 0.001	0.002 ± 0.000	0.063 ± 0.015	0.005	0.013 ± 0.003	0.007 ± 0.000	0.012	0.021 ± 0.016	0.012 ± 0.004	0.013 ± 0.005	0.014 ± 0.006	
11:1	0.029 ± 0.025	0.107	0.004 ± 0.002	0.018 ± 0.013	0.007 ± 0.006	0.009 ± 0.008	0.003 ± 0.002	0.007 ± 0.003	0.006 ± 0.003	0.021 ± 0.008	0.017	0.023 ± 0.009	0.004 ± 0.006	0.016	0.057 ± 0.081	0.037 ± 0.024	0.033 ± 0.026	0.017 ± 0.013	
12:0	0.109 ± 0.027	0.105	0.031 ± 0.012	0.027 ± 0.014	0.022 ± 0.016	0.022 ± 0.007	0.023 ± 0.008	0.018 ± 0.004	0.019 ± 0.006	0.091 ± 0.021	0.074	0.063 ± 0.017	1.164 ± 0.031	1.310	0.150 ± 0.069	0.106 ± 0.058	0.099 ± 0.056	0.823 ± 1.097	
13:0	0.005 ± 0.007	0.015	0.004 ± 0.004	0.004 ± 0.004	0.006 ± 0.002	0.010 ± 0.010	0.003 ± 0.003	0.001 ± 0.003	0.000 ± 0.000	0.039 ± 0.003	0.034	0.000 ± 0.000	0.039 ± 0.008	0.114	0.022 ± 0.008	0.029 ± 0.011	0.019 ± 0.006	0.027 ± 0.024	
13:1	0.013 ± 0.026	0.019	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.003 ± 0.007	0.001 ± 0.002	0.000 ± 0.000	0.000 ± 0.000	0.041 ± 0.058	0.000	0.015 ± 0.025	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.008 ± 0.022	0.023 ± 0.021	
14:0	2.109 ± 0.784	10.306	8.862 ± 0.563	3.081 ± 0.445	3.597 ± 0.461	7.921 ± 0.490	5.245 ± 1.129	4.100 ± 0.522	4.163 ± 0.253	4.286 ± 1.108	4.629	1.882 ± 0.363	1.899 ± 0.238	5.374	3.033 ± 0.467	2.715 ± 0.209	2.068 ± 0.260	1.981 ± 0.312	
14:1	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.014 ± 0.024	0.113 ± 0.195	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	
15:0	0.107 ± 0.037	0.389	0.279 ± 0.052	0.227 ± 0.047	0.177 ± 0.086	0.150 ± 0.028	0.155 ± 0.032	0.286 ± 0.046	0.294 ± 0.070	0.497 ± 0.031	0.481	0.558 ± 0.038	0.239 ± 0.004	0.570	0.292 ± 0.095	0.699 ± 0.102	0.651 ± 0.189	0.280 ± 0.049	
16:0	20.289 ± 1.324	18.043	9.554 ± 0.334	17.639 ± 0.636	12.218 ± 1.754	9.600 ± 2.281	12.737 ± 1.987	14.519 ± 1.363	11.918 ± 1.051	22.524 ± 0.961	25.495	27.055 ± 3.224	22.837 ± 0.483	26.452	25.940 ± 0.113	31.933 ± 1.157	31.267 ± 2.239	28.969 ± 3.449	
16:1w7c	6.879 ± 4.065	0.479	0.643 ± 0.149	0.148 ± 0.188	6.931 ± 3.619	1.361 ± 1.216	2.150 ± 2.543	3.036 ± 0.676	5.252 ± 2.347	3.736 ± 0.441	3.844	1.867 ± 0.970	0.287 ± 0.035	2.379	5.830 ± 0.394	3.618 ± 0.510	1.156 ± 0.720	0.980 ± 0.715	
17:0	0.045 ± 0.008	0.104	0.126 ± 0.011	0.179 ± 0.021	0.103 ± 0.030	0.057 ± 0.009	0.108 ± 0.018	0.129 ± 0.021	0.217 ± 0.045	0.115 ± 0.025	0.274	0.176 ± 0.008	0.100 ± 0.002	0.148	0.069 ± 0.014	0.131 ± 0.058	0.247 ± 0.058	0.109 ± 0.021	
17:1w7c	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	
18:0	1.352 ± 0.338	3.598	0.623 ± 0.143	1.432 ± 0.705	1.505 ± 0.575	1.171 ± 0.471	2.024 ± 1.019	1.301 ± 0.156	1.573 ± 0.114	0.839 ± 0.091	3.303	0.980 ± 0.234	0.817 ± 0.211	1.633	2.117 ± 0.843	2.068 ± 1.542	1.800 ± 0.488	1.685 ± 0.450	
18:1w9c	1.497 ± 0.332	5.762	26.168 ± 1.212	8.071 ± 0.611	6.654 ± 0.839	10.420 ± 3.835	10.797 ± 2.943	9.375 ± 0.884	3.797 ± 0.093	1.792 ± 0.619	2.411	1.128 ± 0.308	1.449 ± 0.347	4.173	2.365 ± 0.803	4.692 ± 0.569	2.953 ± 0.518	2.998 ± 1.486	
18:1w7c	7.260 ± 1.992	0.133	0.000 ± 0.000	0.014 ± 0.028	0.241 ± 0.194	0.023 ± 0.041	0.093 ± 0.104	0.043 ± 0.038	0.613 ± 0.020	0.241 ± 0.051	1.345	0.294 ± 0.117	1.508 ± 0.254	1.460	1.469 ± 0.193	0.502 ± 0.081	1.402 ± 0.439	0.906 ± 0.365	
18:2w6c	6.280 ± 1.141	14.998	5.735 ± 0.547	6.340 ± 0.900	2.479 ± 1.943	4.857 ± 1.735	5.942 ± 1.268	3.660 ± 1.364	4.178 ± 0.852	0.165 ± 0.075	0.631	0.392 ± 0.142	0.978 ± 0.257	2.873	1.980 ± 0.381	1.110 ± 0.049	4.228 ± 0.515	1.199 ± 0.494	
18:3w6	0.463 ± 0.118	0.204	0.612 ± 0.104	0.470 ± 0.097	0.421 ± 0.179	0.689 ± 0.264	0.622 ± 0.184	0.452 ± 0.033	0.591 ± 0.147	0.000 ± 0.000	0.148	0.196 ± 0.021	0.546 ± 0.233	0.249	0.153 ± 0.017	0.029 ± 0.041	0.391 ± 0.216	0.273 ± 0.157	
18:3w3	31.597 ± 9.439	7.830	6.897 ± 0.707	11.391 ± 1.574	8.000 ± 1.470	6.142 ± 1.192	8.006 ± 1.608	7.357 ± 0.415	12.183 ± 0.500	0.048 ± 0.067	0.462	0.085 ± 0.147	0.013 ± 0.018	0.000	0.290 ± 0.410	0.000 ± 0.000	0.035 ± 0.049	0.157 ± 0.154	
18:4w3	1.201 ± 0.395	11.087	10.251 ± 1.213	9.088 ± 2.233	11.799 ± 2.963	14.448 ± 4.173	10.414 ± 3.030	10.688 ± 2.049	17.063 ± 2.033	0.014 ± 0.020	0.712	0.128 ± 0.149	0.003 ± 0.004	0.000	0.236 ± 0.199	0.099 ± 0.140	0.007 ± 0.013	0.027 ± 0.043	
20:0	0.007 ± 0.015	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.067 ± 0.177	0.574 ± 0.838	0.899 ± 0.851	0.000 ± 0.000	0.000 ± 0.000	0.000	0.006 ± 0.010	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.039 ± 0.025	
20:1w9	0.375 ± 0.114	0.000	0.015 ± 0.013	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.001	0.000 ± 0.000	0.413 ± 0.374	0.042 ± 0.059	0.697	0.033 ± 0.001	0.100 ± 0.010	0.000	0.041 ± 0.057	0.000 ± 0.000	0.000 ± 0.000	0.012 ± 0.031	
20:2w6	0.116 ± 0.024	0.097	0.129 ± 0.007	0.041 ± 0.049	0.097 ± 0.076	0.018 ± 0.018	0.208 ± 0.043	0.175 ± 0.016	0.303 ± 0.159	0.000 ± 0.000	0.216	0.009 ± 0.016	0.647 ± 0.103	0.185	0.124 ± 0.011	0.024 ± 0.034	0.081 ± 0.021	0.145 ± 0.226	
20:3w6	0.051 ± 0.034	0.000	0.809 ± 0.156	0.193 ± 0.086	0.251 ± 0.100	0.622 ± 0.374	0.511 ± 0.209	0.416 ± 0.217	1.082 ± 0.074	0.027 ± 0.039	0.144	0.078 ± 0.001	2.103 ± 0.193	0.738	0.139 ± 0.020	0.103 ± 0.145	0.438 ± 0.097	0.669 ± 0.814	
20:4w6	2.793 ± 0.444	9.729	10.769 ± 0.223	24.093 ± 2.439	20.896 ± 0.777	21.458 ± 5.326	21.385 ± 2.670	21.305 ± 4.021	14.358 ± 1.076	46.840 ± 0.607	8.720	37.906 ± 2.640	21.884 ± 4.809	11.364	28.846 ± 0.942	30.005 ± 0.256	14.494 ± 1.835	11.778 ± 6.119	
20:3w3	0.196 ± 0.055	0.000	0.059 ± 0.102	0.029 ± 0.057	0.000 ± 0.000	0.021 ± 0.026	0.119 ± 0.089	0.126 ± 0.109	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	
20:5w3	16.133 ± 8.676	16.084	18.231 ± 1.107	16.853 ± 1.801	19.005 ± 3.282	20.829 ± 1.712	18.673 ± 3.494	14.376 ± 1.002	20.083 ± 1.362	18.199 ± 1.115	42.645	26.555 ± 2.016	43.160 ± 3.265	40.330	26.183 ± 3.090	21.701 ± 0.873	38.163 ± 2.611	46.466 ± 4.741	
22:0	0.000 ± 0.000	0.195	0.137 ± 0.123	0.173 ± 0.203	0.018 ± 0.016	0.002 ± 0.005	0.019 ± 0.015	0.010 ± 0.018	0.038 ± 0.001	0.026 ± 0.036	0.000	0.004 ± 0.007	0.044 ± 0.062	0.000	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.004	0.011 ± 0.027	
22:1w9c	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.029 ± 0.050	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.035 ± 0.087	
22:2w6	0.000 ± 0.000	0.000	0.002 ± 0.003	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.014 ± 0.038	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.001	
23:0	0.000 ± 0.000	0.000	0.001 ± 0.001	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.006 ± 0.014	
22:4w6	0.036 ± 0.072	0.000	0.000 ± 0.000	0.000 ± 0.000	1.094 ± 1.894	0.000 ± 0.000	0.000 ± 0.000	1.394 ± 2.415	0.000 ± 0.000	0.019 ± 0.027	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	
22:5w6	0.043 ± 0.050	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.041 ± 0.000	0.000 ± 0.000	0.000	0.010 ± 0.018	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	
22:3w6	0.012 ± 0.024	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.001	
22:5w3	0.218 ± 0.062	0.000	0.007 ± 0.007	0.292 ± 0.079	4.294 ± 3.721	0.001 ± 0.004	0.018 ± 0.032	6.145 ± 1.311	0.191 ± 0.025	0.000 ± 0.000	0.359	0.052 ± 0.031	0.034 ± 0.048	0.000	0.103 ± 0.146	0.000 ± 0.000	0.020 ± 0.019	0.026 ± 0.064	
22:6w3	0.447 ± 0.231	0.000	0.000 ± 0.000	0.000 ± 0.000	0.026 ± 0.044	0.000 ± 0.000	0.053 ± 0.067	0.000 ± 0.000	1.581 ± 0.713	0.000 ± 0.000	2.986	0.297 ± 0.114	0.000 ± 0.000	0.070	0.259 ± 0.090	0.035 ± 0.049	0.092 ± 0.077	0.054 ± 0.132	
24:0	0.116 ± 0.135	0.000	0.000 ± 0.000	0.055 ± 0.039	0.000 ± 0.000	0.004 ± 0.006	0.026 ± 0.020	0.016 ± 0.028	0.000 ± 0.000	0.000 ± 0.000	0.000	0.015 ± 0.007	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.023 ± 0.057	
24:1w9	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.120 ± 0.104	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.084 ± 0.206	
CN ratio	11.43 ± 6.11	-	-	22.15 ± 1.41	12.46 ± 0.33	12.35 ± 0.96	14.41 ± 1.85	18.77 ± 1.51	-	-	9.36	6.21 ± 0.51	-	7.46	7.83 ± 0.79	6.93	6.62 ± 0.63	12.59 ± 1.11	
d15N	2.16 ± 0.48	-	-	1.25 ± 0.61	2.60 ± 2.94	2.82 ± 0.55	4.78 ± 1.60	2.33 ± 0.50	-	-	2.33	3.52 ± 0.41	-	2.20	2.22 ± 0.47	2.29	1.57 ± 1.26	4.39 ± 1.02	
d13C	-31.02 ± 1.37	-	-	-28.28 ± 1.06	-31.54 ± 1.90	-25.72 ± 1.27	-27.62 ± 1.96	-22.85 ± 0.10	-	-	-33.23	-22.66 ± 2.85	-	-34.89	-36.08 ± 0.67	-35.32	-33.56 ± 0.96	-20.00 ± 4.49	
ΣSAFA	24.361	33.472	19.671	22.961	17.807	19.098	20.976	21.332	18.265	28.815	34.665	30.786	27.283	36.163	31.923	38.046	36.497	34.149	
ΣMUFA	16.053	6.500	26.830	8.251	13.833	11.816	13.058	12.574	10.081	5.873	8.314	3.509	3.348	8.028	9.762	8.849	5.552	5.055	
ΣPUFA	59.586	60.029	53.501	68.790	68.362	69.085	65.965	66.094	71.654	65.312	57.023	65.708	69.368	55.809	58.313	53.106	57.949	60.794	
Σ*n3*	49.792	35.001	35.445	37.653	43.124	41.441	37.283	38.692	51.101	18.261	47.164	27.117	43.210	40.400	27.071	21.835	38.317	46.730	
Σ*n6*	9.794	25.028	18.056	31.137	25.238	27.644	28.682	27.402	20.553	47.051	9.859	38.591	26.158	15.409	31.242	31.271	19.632	14.064	
Σ*n3*/Σ*n6*	5.084	1.398	1.963	1.209	1.709	1.499	1.300	1.412	2.486	0.388	4.784	0.703	1.652	2.622	0.866	0.698	1.952	3.323	
ΣSAFA/ΣPUFA	0.409	0.558	0.368	0.334	0.260	0.276	0.318	0.323	0.255	0.441	0.608	0.469	0.393	0.648	0.547	0.716	0.630	0.562	

Table 2.3 (cont.)

Rhodophyta												
Meridionella antarctica	*Myriogramme marginii*	*Myriogramme smithii*	*Pachymenia orbicularis*	*Palmaria decipiens*	*Pantoneura plocamioides*	*Paraglossum salicifolium*	*Phyllophora antarctica*	*Picconiella plumosa*	*Plocamium sp.*	*Porphyra plocaniestris*	*Sarcopeltis antarctica*	*Trematocarpus antarcticus*
3/4/1	7/6/6	4/3/2	2/3/2	3/3/3	1/3/1	1/0/-	5/5/5	2/2/2	3/3/3	3/3/3	6/7/4	5/5/5
0.004 ± 0.004	0.014 ± 0.004	0.011 ± 0.008	0.003 ± 0.004	0.007 ± 0.005	0.011	0.011	0.008 ± 0.008	0.001 ± 0.002	0.006 ± 0.002	0.010 ± 0.007	0.022 ± 0.015	0.010 ± 0.005
0.323 ± 0.331	0.198 ± 0.062	0.252 ± 0.205	0.915 ± 0.661	0.091 ± 0.022	0.233	0.214	0.050 ± 0.039	0.066 ± 0.091	0.042 ± 0.024	0.358 ± 0.490	0.640 ± 0.554	0.258 ± 0.214
0.008 ± 0.004	0.021 ± 0.017	0.020 ± 0.014	0.022 ± 0.015	0.014 ± 0.010	0.067	0.018	0.007 ± 0.004	0.008 ± 0.002	0.015 ± 0.003	0.018 ± 0.015	0.038 ± 0.030	0.018 ± 0.016
0.005 ± 0.001	0.015 ± 0.009	0.060 ± 0.034	0.014 ± 0.007	0.008 ± 0.005	0.031	0.028	0.005 ± 0.002	0.008 ± 0.005	0.007 ± 0.001	0.007 ± 0.004	0.016 ± 0.008	0.020 ± 0.007
0.017 ± 0.006	0.037 ± 0.019	0.035 ± 0.015	0.013 ± 0.019	0.022 ± 0.018	0.082	0.012	0.015 ± 0.007	0.006 ± 0.008	0.034 ± 0.052	0.029 ± 0.025	0.094 ± 0.131	0.063 ± 0.067
0.056 ± 0.025	0.064 ± 0.036	0.099 ± 0.069	0.159 ± 0.053	0.075 ± 0.047	1.872	0.210	0.062 ± 0.018	0.104 ± 0.047	0.713 ± 0.053	0.040 ± 0.019	0.169 ± 0.054	0.097 ± 0.056
0.006 ± 0.005	0.004 ± 0.008	0.008 ± 0.010	0.019 ± 0.027	0.008 ± 0.009	0.053	0.026	0.005 ± 0.012	0.015 ± 0.007	0.026 ± 0.012	0.000 ± 0.000	0.017 ± 0.014	0.016 ± 0.018
0.000 ± 0.000	0.000 ± 0.000	0.015 ± 0.031	0.000 ± 0.000	0.023 ± 0.040	0.140	0.000	0.005 ± 0.011	0.000 ± 0.000	0.006 ± 0.010	0.000 ± 0.000	0.000 ± 0.000	0.011 ± 0.025
0.871 ± 0.209	2.119 ± 0.552	1.926 ± 0.603	1.104 ± 0.092	7.480 ± 0.720	1.205	2.889	1.771 ± 0.190	1.989 ± 0.235	4.562 ± 0.230	0.203 ± 0.078	2.091 ± 0.833	2.374 ± 0.848
0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.161 ± 0.279	0.000	0.000	0.019 ± 0.042	0.000 ± 0.000	0.000 ± 0.000	0.019 ± 0.038	0.000 ± 0.000	0.000 ± 0.000
0.863 ± 0.117	0.314 ± 0.078	0.272 ± 0.093	0.357 ± 0.053	0.577 ± 0.144	0.324	0.500	0.400 ± 0.048	0.440 ± 0.018	0.282 ± 0.073	0.077 ± 0.022	0.456 ± 0.050	0.391 ± 0.120
39.511 ± 1.414	27.836 ± 4.852	27.505 ± 5.379	29.292 ± 0.991	15.067 ± 1.191	28.247	31.036	25.158 ± 1.881	30.954 ± 0.662	22.695 ± 0.141	21.849 ± 0.481	32.667 ± 4.014	30.630 ± 3.008
3.475 ± 0.770	1.314 ± 0.619	0.908 ± 0.597	2.518 ± 0.021	3.943 ± 2.525	7.353	9.415	3.409 ± 5.062	4.648 ± 0.103	3.996 ± 4.935	0.190 ± 0.170	1.751 ± 0.610	0.764 ± 0.398
0.199 ± 0.033	0.127 ± 0.028	0.097 ± 0.034	0.199 ± 0.032	0.074 ± 0.027	0.125	0.154	0.190 ± 0.061	0.100 ± 0.011	0.102 ± 0.021	0.038 ± 0.013	0.192 ± 0.029	0.159 ± 0.039
0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.018 ± 0.039
1.995 ± 1.495	2.377 ± 1.348	1.458 ± 0.382	2.811 ± 1.461	1.396 ± 0.838	2.648	1.720	0.896 ± 0.126	1.638 ± 0.195	1.424 ± 0.649	1.539 ± 0.777	3.477 ± 1.935	1.728 ± 1.307
5.548 ± 0.321	1.917 ± 0.769	1.312 ± 0.255	4.366 ± 0.715	3.242 ± 0.593	5.339	4.378	2.024 ± 0.396	5.079 ± 0.563	0.654 ± 0.263	1.201 ± 0.382	4.686 ± 1.543	1.367 ± 0.357
2.809 ± 0.279	11.100 ± 1.628	7.274 ± 2.405	0.302 ± 0.062	1.959 ± 0.389	5.632	2.113	0.509 ± 0.496	11.107 ± 0.494	0.391 ± 0.077	0.928 ± 0.165	0.443 ± 0.328	0.157 ± 0.091
0.472 ± 0.027	2.218 ± 0.229	1.562 ± 0.406	0.507 ± 0.096	0.231 ± 0.127	1.575	0.333	1.602 ± 0.213	0.788 ± 0.011	0.647 ± 0.340	2.945 ± 0.655	0.352 ± 0.050	0.374 ± 0.076
1.670 ± 0.203	0.659 ± 0.045	0.431 ± 0.099	0.000 ± 0.000	0.174 ± 0.066	0.066	0.277	0.132 ± 0.062	0.296 ± 0.034	0.345 ± 0.130	0.185 ± 0.065	0.086 ± 0.079	0.132 ± 0.036
0.112 ± 0.179	0.143 ± 0.110	0.305 ± 0.395	0.000 ± 0.000	0.294 ± 0.207	0.123	0.162	0.084 ± 0.089	0.163 ± 0.109	0.418 ± 0.276	0.147 ± 0.117	0.000 ± 0.000	0.015 ± 0.032
0.509 ± 0.516	0.061 ± 0.082	0.657 ± 0.908	0.000 ± 0.000	0.657 ± 0.836	0.104	0.146	0.165 ± 0.302	0.199 ± 0.089	0.892 ± 0.644	0.084 ± 0.084	0.000 ± 0.000	0.026 ± 0.049
0.000 ± 0.000	0.004 ± 0.011	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000	0.004 ± 0.008	0.000 ± 0.000	0.250 ± 0.047	0.000 ± 0.000	0.000 ± 0.000	0.003 ± 0.006
0.041 ± 0.071	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.336 ± 0.123	0.000	0.000	0.007 ± 0.016	0.144 ± 0.038	0.022 ± 0.019	2.868 ± 0.729	0.000 ± 0.000	0.000 ± 0.000
0.019 ± 0.034	0.207 ± 0.033	0.222 ± 0.072	0.000 ± 0.000	0.000 ± 0.000	0.194	0.000	0.034 ± 0.020	0.041 ± 0.058	0.145 ± 0.016	3.649 ± 0.496	0.000 ± 0.000	0.004 ± 0.009
0.542 ± 0.188	0.254 ± 0.031	0.395 ± 0.038	0.014 ± 0.020	0.048 ± 0.056	0.075	0.050	0.091 ± 0.032	0.521 ± 0.102	4.953 ± 0.728	1.507 ± 0.266	0.000 ± 0.000	0.052 ± 0.048
11.711 ± 0.094	4.635 ± 0.905	17.866 ± 1.444	13.460 ± 1.265	0.649 ± 0.261	12.273	37.177	39.810 ± 19.079	9.069 ± 0.309	13.061 ± 0.572	5.362 ± 1.108	24.865 ± 9.380	47.301 ± 5.332
0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.166 ± 0.194	0.000 ± 0.000	0.000 ± 0.000
29.164 ± 1.521	43.312 ± 9.066	37.149 ± 10.768	43.914 ± 1.209	62.783 ± 6.249	31.707	8.481	23.093 ± 13.235	32.212 ± 0.345	43.436 ± 4.584	56.361 ± 4.398	27.937 ± 10.537	13.959 ± 4.987
0.000 ± 0.000	0.044 ± 0.042	0.033 ± 0.024	0.009 ± 0.013	0.049 ± 0.047	0.000	0.000	0.002 ± 0.005	0.011 ± 0.015	0.455 ± 0.296	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000	0.010 ± 0.022	0.000 ± 0.000	0.020 ± 0.034	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.454 ± 0.786	0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.136 ± 0.015	0.000 ± 0.000	0.000 ± 0.000
0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000	0.017 ± 0.024	0.000 ± 0.000	0.001 ± 0.002	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.093	0.000 ± 0.000	0.000 ± 0.000	0.002 ± 0.004	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.001	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
0.016 ± 0.029	0.009 ± 0.024	0.046 ± 0.054	0.000 ± 0.000	0.078 ± 0.075	0.165	0.171	0.103 ± 0.231	0.142 ± 0.134	0.026 ± 0.046	0.085 ± 0.061	0.000 ± 0.000	0.002 ± 0.004
0.054 ± 0.064	0.090 ± 0.194	0.059 ± 0.119	0.000 ± 0.000	0.061 ± 0.106	0.356	0.385	0.218 ± 0.374	0.254 ± 0.041	0.369 ± 0.279	0.000 ± 0.000	0.000 ± 0.000	0.023 ± 0.033
0.000 ± 0.000	0.111 ± 0.082	0.023 ± 0.026	0.000 ± 0.000	0.036 ± 0.062	0.000	0.000	0.008 ± 0.017	0.000 ± 0.000	0.004 ± 0.007	0.000 ± 0.000	0.000 ± 0.000	0.002 ± 0.004
0.000 ± 0.000	0.796 ± 0.996	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	0.000	0.089 ± 0.199	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.030 ± 0.068
11.47 ± 8.22	7.61 ± 0.68	5.27 ± 1.02	9.39 ± 1.10	8.81 ± 2.22	7.68 ± 0.85	-	7.07 ± 0.49	9.10 ± 0.58	7.56 ± 1.21	7.49 ± 0.30	14.42 ± 1.17	6.40 ± 1.01
1.39 ± 1.02	3.09 ± 0.48	4.49 ± 0.83	3.16 ± 1.37	3.26 ± 0.34	2.54 ± 1.33	-	2.37 ± 0.68	2.71 ± 1.44	2.96 ± 0.67	6.13 ± 0.37	2.30 ± 0.51	3.72 ± 0.65
-33.46 ± 4.06	-34.35 ± 0.79	-37.28 ± 0.32	-33.98 ± 1.16	-23.70 ± 0.52	-33.72 ± 5.38	-	-35.44 ± 2.03	-34.77 ± 0.10	-33.54 ± 1.12	-29.48 ± 0.84	-24.90 ± 1.79	-22.56 ± 3.01
43.841	33.248	31.764	34.904	24.882	34.816	36.806	28.566	35.334	30.583	24.139	39.785	35.706
11.890	15.164	9.544	7.199	9.686	18.546	15.918	6.087	20.984	5.123	5.235	6.974	2.410
44.269	51.588	58.692	57.895	65.429	46.638	47.275	65.349	43.685	64.296	70.627	53.240	61.888
29.855	43.615	38.216	43.914	63.873	32.455	9.345	23.663	32.970	45.141	56.843	27.937	14.025
14.414	7.973	20.476	13.981	1.556	14.183	37.930	41.686	10.715	19.155	13.784	25.303	47.863
2.071	5.470	1.866	3.141	41.049	2.288	0.246	0.568	3.077	2.357	4.124	1.104	0.293
0.990	0.644	0.541	0.603	0.380	0.747	0.779	0.437	0.809	0.476	0.342	0.747	0.577

Cluster analysis identified four primary groupings of all algal species based on the full suite of FAs quantified (Figure 2.3). These groupings matched division-level differences, with the Chlorophyta and Ochrophyta separating out into individual groupings, while the Rhodophyta were further subdivided into two primary groupings.

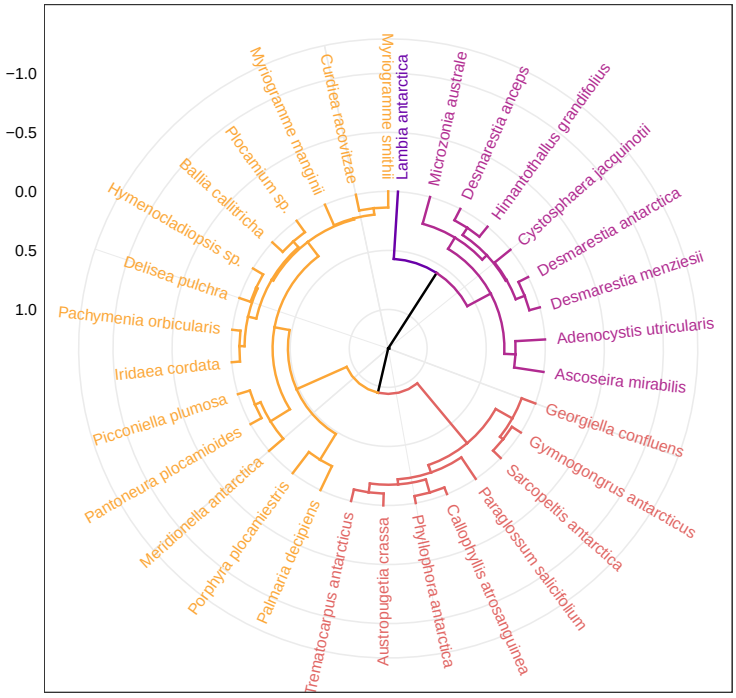


Figure 2.3: Cluster diagram of macroalgal species' average fatty acid profiles based on 40 fatty acids analyzed in this study. Colors represent major groupings of algal species by fatty acid profiles. Algal species group phylogenetically by Division, with Ochrophyta as one group (pink), followed by a single species group for Chlorophyta (purple), and two Rhodophyta groups (yellow and orange).

The phylogenetic resolution of these division-level cluster groupings was confirmed by nMDS and extended to the levels of order and even family (Figure 2.4; see Table 2.4 for taxonomic information). Additionally, the full suite of FAs detected supported this division-level separation

of the macroalgal species in PCA analysis, with PC1 and PC2 explaining 15.5% and 8.9% of variation, respectively (Figure 2.5).

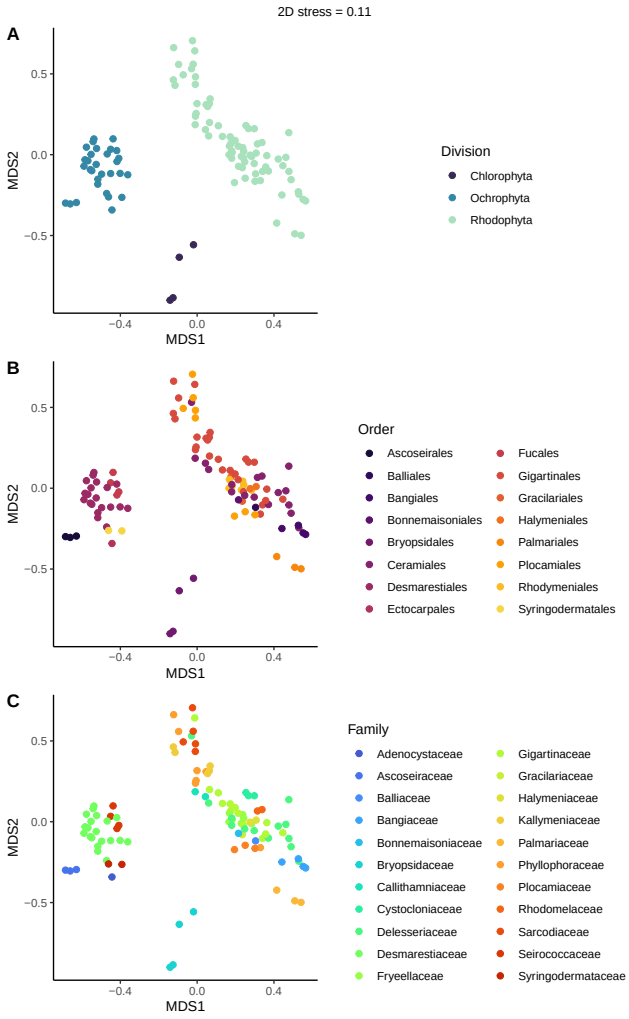


Figure 2.4: nMDS of all macroalgal FA profiles coded by Division (A), Order (B), and Family (C).

Table 2.4: Macroalgal species collected during the 2019 Western Antarctic Gradients Project cruise for FA analysis. Species highlighted in gray do not have a published FA profile.

Phylum	Order	Family	Species	Reference
Chlorophyta	Bryopsidales	Bryopsidaceae	*Lambia antarctica*	(Graeve et al. 2002)
Ochrophyta	Ascoseirales	Ascoseiraceae	*Ascoseira mirabilis*	(Teixeira et al. 2019)
	Desmarestiales	Desmarestiaceae	*Desmarestia anceps*	(Aumack et al. 2017, Teixeira et al. 2019, Schram et al. 2019)
			Desmarestia antarctica	(Graeve et al. 2002)
			Desmarestia menziesii	(Aumack et al. 2017)
			Himantothallus grandifolius	(Schram et al. 2019)
	Ectocarpales	Adenocystaceae	*Adenocystis utricularis*	(Teixeira et al. 2019, Berneira et al. 2020)
	Fucales	Seirococcaceae	*Cystosphaera jacquinotii*	
	Syringodermatales	Syringodermataceae	*Microzonia australe*	
Rhodophyta	Balliales	Balliaceae	*Ballia callitricha*	
	Bangiales	Bangiaceae	*Porphyra plocamiestris*	
	Bonnemaisoniales	Bonnemaisoniaceae	*Delisea pulchra*	(Schmid et al. 2018)
	Ceramiales	Callithamniaceae	*Georgiella confluens*	(Graeve et al. 2002)
		Delesseriaceae	*Myriogramme smithii*	(Graeve et al. 2002)
			Myriogramme manginii	(Berneira et al. 2020)
			Pantoneura plocamioides	(Graeve et al. 2002)
			Paraglossum salicifolium	
		Rhodomelaceae	*Picconiella plumosa*	
	Gigartinales	Cystocloniaceae	*Meridionella antarctica*	
		Gigartinaceae	*Sarcopeltis antarctica*	(Graeve et al. 2002, Aumack et al. 2017, Berneira et al. 2020)*
			Iridaea cordata	(Santos et al. 2017)
		Kallymeniaceae	*Austropugetia crassa*	
			Callophyllis atrosanguinea	
		Phyllophoraceae	*Gymnogongrus antarcticus*	
			Phyllophora antarctica	
	Gracilariales	Gracilariaceae	*Curdiea racovitzae*	(Berneira et al. 2020)
	Halymeniales	Halymeniaceae	*Pachymenia orbicularis*	
	Palmariales	Palmariaceae	*Palmaria decipiens*	(Graeve et al. 2002, Santos et al. 2017, Schram et al. 2019)
	Plocamiales	Plocamiaceae	*Plocamium sp.*	(Santos et al. 2017, Aumack et al. 2017)†
		Sarcodiaceae	*Trematocarpus antarcticus*	
	Rhodymeniales	Fryeellaceae	*Hymenocladiopsis sp.*	(Graeve et al. 2002)‡

* as *Gigartina skottsbergii*
† *Plocamium cartilagineum*
‡ *Hymenocladiopsis prolifera (crustigena)*

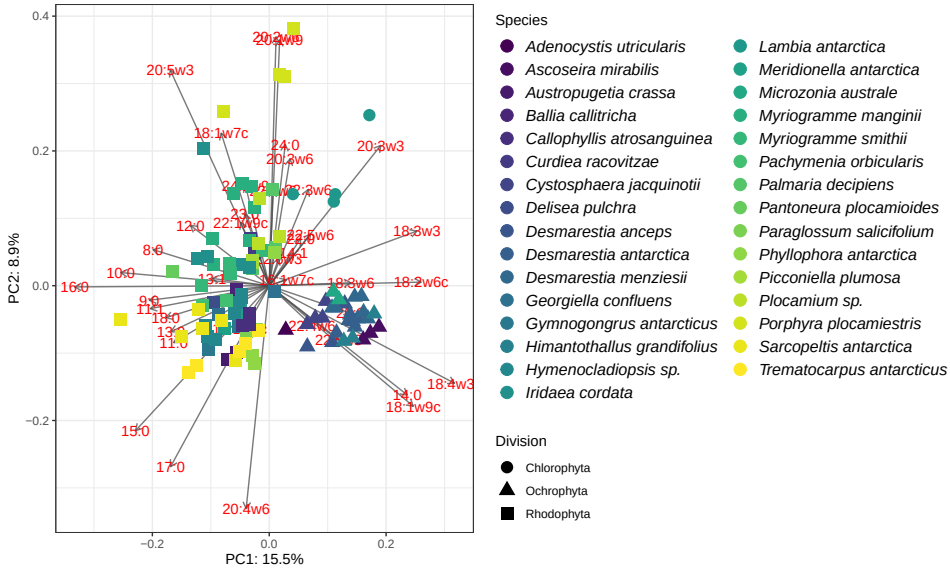


Figure 2.5: Principle Component Analysis (PCA) of all macroalgal samples based on the full suite of 40 fatty acids. Algal species are color-coded in the legend, with phylum indicated by shapes. All individual replicates of the algal species are represented. Vectors represent individual fatty acids, with the length and direction indicating the amount of variability and the direction

associated with each fatty acid according to the principle component axes. Variance explained by each axis is given as percent along each axis.

Ochrophyta grouped distinctly and tightly along PC1, while Rhodophyta were separated along PC1 from the Ochrophyta but had a larger spread along PC2. Species-level differences in full FA profiles were also detected (PERMANOVA Table 2.5), though there was much overlap among species (Figure 2.5). A subset of FA driving differences among samples was determined with SIMPER analysis and used in additional PCA analyses to confirm that major division-level groupings were still apparent (Figure 2.6, Table 2.5, 2.6). This reduced PCA included primarily FAs that were also part of the suite of common FAs that comprised more than 5% of total proportional contribution (see Figure 2.2). This reduced PCA explained 43.0% (PC1) and 24.1% (PC2) of variation among samples. The pattern observed with the FA subset was similar to that seen when the full suite of FA were considered; however, the phylogenetic groupings were even more distinct. Overall, division-level differences were supported more strongly than other taxonomic levels between both the full FA (PERMANOVA, DF = 2, SS = 4.007, F = 53.363, p(perm) = 0.001) and reduced FA (PERMANOVA, DF = 2, SS = 4.065, F = 62.708, p(perm) = 0.001) analyses, and species-level effects were more clearly pronounced than by order or family (Table 2.5).

Table 2.5: PERMANOVA table for results across all biomarker and taxonomic analyses.

Biomarkers		df	SS	F	*P* (perm)
FA only					
division					
	Division	2	4.007	53.363	0.001
	Residual	103	3.868		
	Total	105	7.875		
order					
	Order	15	5.687	15.596	0.001
	Residual	90	2.188		
	Total	105	7.875		
family					
	Family	21	6.568	20.105	0.001
	Residual	84	1.307		
	Total	105	7.875		
species					
	Species	30	7.138	24.221	0.001
	Residual	75	0.737		
	Total	105	7.875		
FA reduced					
division					
	Division	2	4.065	62.708	0.001
	Residual	103	3.338		
	Total	105	7.403		
order					
	Order	15	5.514	17.512	0.001
	Residual	90	1.889		
	Total	105	7.403		
family					
	Family	21	6.336	23.760	0.001
	Residual	84	1.067		
	Total	105	7.403		
species					
	Species	30	6.788	27.610	0.001
	Residual	75	0.615		
	Total	105	7.403		
SI only					
division					
	Division	2	0.262	10.794	0.001
	Residual	89	1.080		
	Total	91	1.342		
order					
	Order	11	0.624	6.312	0.001
	Residual	80	0.718		
	Total	91	1.342		
family					
	Family	17	1.016	13.584	0.001
	Residual	74	0.326		
	Total	91	1.342		
species					
	Species	24	1.136	15.410	0.001
	Residual	67	0.206		
	Total	91	1.342		
Reduced FA + SI					
division					
	Division	2	0.298	13.269	0.001
	Residual	73	0.818		
	Total	75	1.116		
order					
	Order	11	0.596	6.667	0.001
	Residual	64	0.520		
	Total	75	1.116		
family					
	Family	17	0.889	13.344	0.001
	Residual	58	0.227		
	Total	75	1.116		
species					
	Species	24	1.002	18.609	0.001
	Residual	51	0.114		
	Total	75	1.116		
All FA + SI					
division					
	Division	2	0.301	13.289	0.001
	Residual	73	0.826		
	Total	75	1.126		
order					
	Order	11	0.602	6.677	0.001
	Residual	64	0.525		
	Total	75	1.126		
family					
	Family	17	0.896	13.267	0.001
	Residual	58	0.230		
	Total	75	1.126		
species					
	Species	24	1.005	18.437	0.001
	Residual	51	0.116		
	Total	75	1.121		

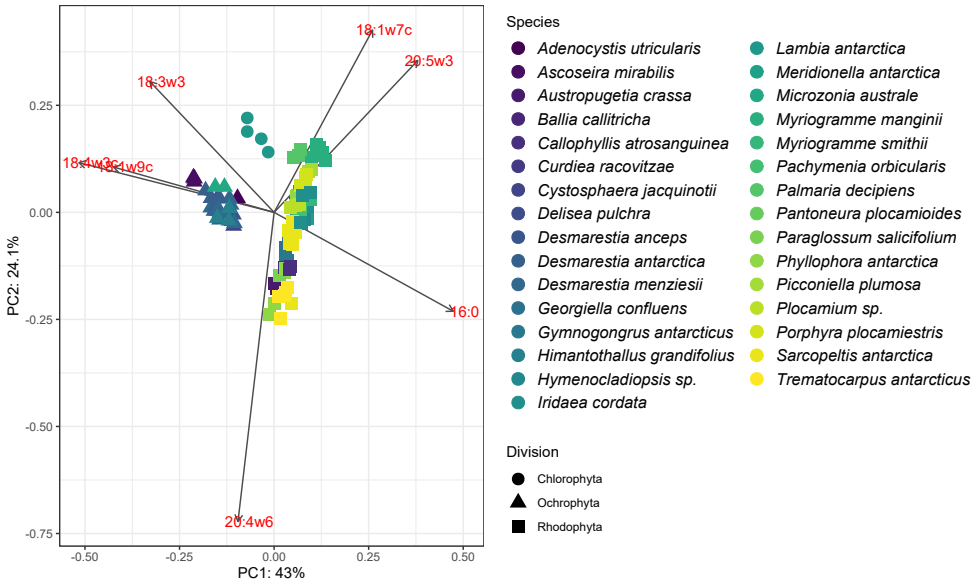


Figure 2.6: Principle Component Analysis (PCA) of all macroalgal samples based on a reduced set of seven fatty acids as determined by a Similarity Percentage (SIMPER) analysis. See Figure 2.5 for details on the PCA arrangement.

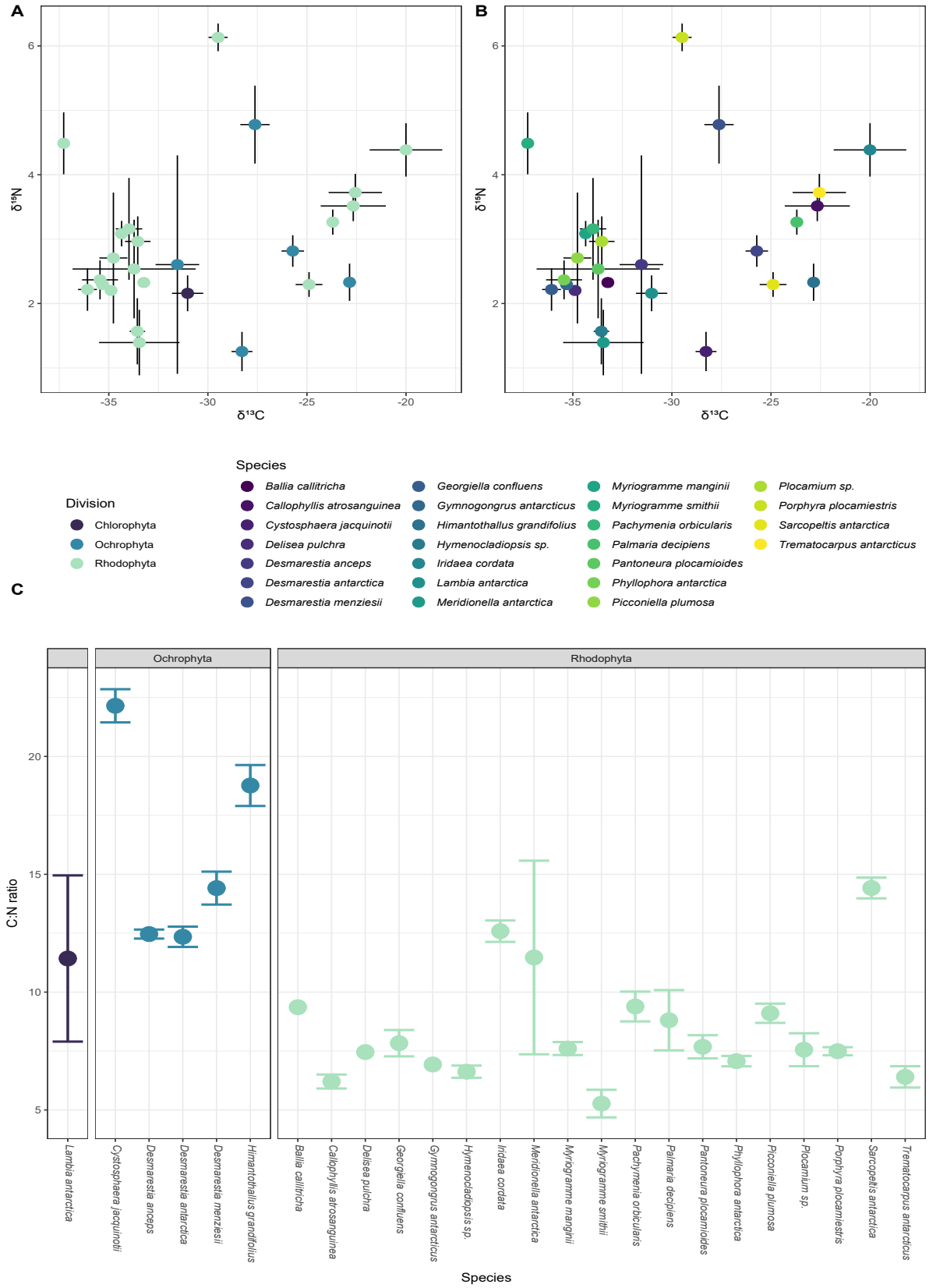
Table 2.6: SIMPER analysis of contribution of fatty acids to driving differences across all samples, through 80% of cumulative variation.

FA	average	sd	cumulative sum
20:5ω3	0.081	0.060	0.226
20:4ω6	0.072	0.059	0.427
16:0	0.048	0.035	0.562
18:3ω3	0.028	0.040	0.639
18:4ω3	0.026	0.029	0.712
18:1ω9	0.023	0.027	0.778
18:1ω7	0.015	0.019	0.820

Stable isotope values were variable across the Rhodophyta and Ochrophyta, with $\delta^{13}C$ ranging from -37.3‰ to -20.0‰ (mean -30.1‰, Rhodophyta) and -31.5‰ to -22.9‰ (mean -

27.2‰, Ochrophyta) (Table 2.3). The $\delta^{13}C$ range among the Rhodophyta was particularly large and included two broad groupings, one with values highly depleted in ^{13}C and another grouping with $\delta^{13}C$ values more similar to those of many Ochrophyta (Figure 2.7a,b). The green alga *L. antarctica* and one Ochrophyta (*Desmarestia anceps*) aligned closely with the ^{13}C-depleted Rhodophyta group. $\delta^{15}N$ ranged from 1.4‰ to 6.1‰ (mean 3.0‰, Rhodophyta), and 1.3‰ to 4.8‰ (mean 3.1‰, Ochrophyta; Figure 2.7a,b, Table 2.3). The single Chlorophyta had a mean $\delta^{13}C$ of -31.0‰ and a mean $\delta^{15}N$ of 2.2‰. Four macroalgal species were distinct with mean $\delta^{15}N$ values >4‰; these were the Ochrophyta *Desmarestia menziesii*, and the Rhodophyta *Iridaea cordata*, *Myriogramme smithii*, and *Porphyra plocamiestris*. The C:N ratios across all macroalgal divisions were variable but overall lower in the Rhodophyta than the other divisions with means ranging from 5.3 to 14.4 (mean 8.8, Rhodophyta), 12.3 to 22.2 (mean 15.7, Ochrophyta), and 11.4 (Chlorophyta) (Figure 2.7c). Differences in C:N ratio were significantly different between Rhodophyta and Ochrophyta, but not between Chlorophyta and the other divisions (ANOVA, DF = 2, MS = 392.5, F 30.49, $p < 0.0001$; Tukey-test, p-adj. < 0.0001).

Figure 2.7: Biplot of mean values for δ15N and δ13C with standard error bars coded by Division (A) and Species (B). Mean C:N ratio (C) is also shown with mean and standard error and each species (column) is coded by Division as in panel A.


A
B
δ¹⁵N
δ¹³C
Species
Division
Chlorophyta
Ochrophyta
Rhodophyta
Ballia callitricha
Callophyllis atrosanguinea
Cystosphaera jacquinotii
Delisea pulchra
Desmarestia anceps
Desmarestia antarctica
Desmarestia menziesii
Georgiella confluens
Gymnogongrus antarcticus
Himantothallus grandifolius
Hymenocladiopsis sp.
Iridaea cordata
Lambia antarctica
Meridionella antarctica
Myriogramme manginii
Myriogramme smithii
Pachymenia orbicularis
Palmaria decipiens
Pantoneura plocamioides
Phyllophora antarctica
Picconiella plumosa
Plocamium sp.
Porphyra plocamiestris
Sarcopeltis antarctica
Trematocarpus antarcticus
C
C:N ratio
Ochrophyta
Rhodophyta
Species

Cluster analysis of stable isotope values ($\delta^{13}C$, $\delta^{15}N$) and the C:N ratio resulted in four groupings of algae, although these did not resolve distinctly along the phylogenetic divisions (Figure 2.8, see Table 2.4 for taxonomic information). Although taxonomic groupings were not readily apparent in nMDS and clusters, PERMANOVA revealed significant Division, Order, and Family groupings (Figure 2.9, Table 2.6). A PCA analysis of macroalgal samples based only on carbon and nitrogen SI values and C:N ratios explained 43.9% (PC1) and 33.7% (PC2) of variation among samples (Figure 2.10). This PCA also showed clear division-level groupings although the separation of Rhodophyta and Ochrophyta along PC1 was not as large as it was in the ordination of macroalgal species based on FA analysis (see Figure 2.5). Analyses across all samples provided support for all taxonomic groupings; however, species-level effects were strongest (PERMANOVA, DF = 24, SS = 1.136, F = 15.410, p(perm) = 0.001; Table 2.5).

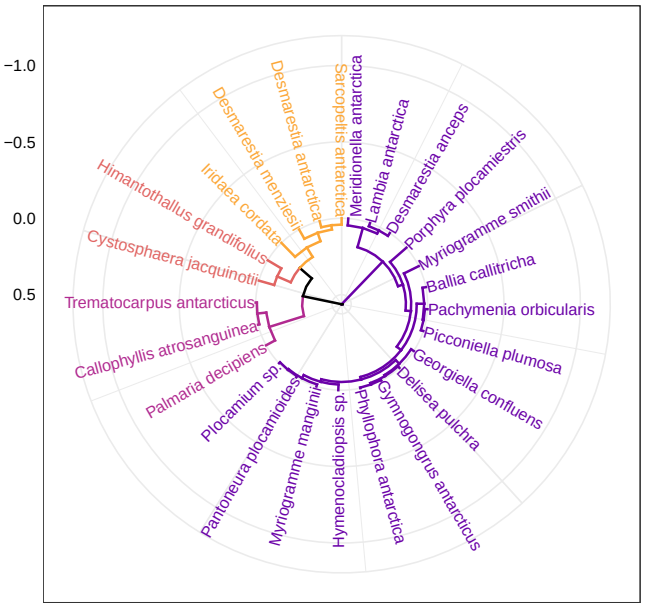


Figure 2.8: Cluster diagram of macroalgal species based on their average carbon and nitrogen stable isotope values as well as their C:N ratio. Colors represent major groupings of algal species, with four major groupings identified, which do not group according to phylum.

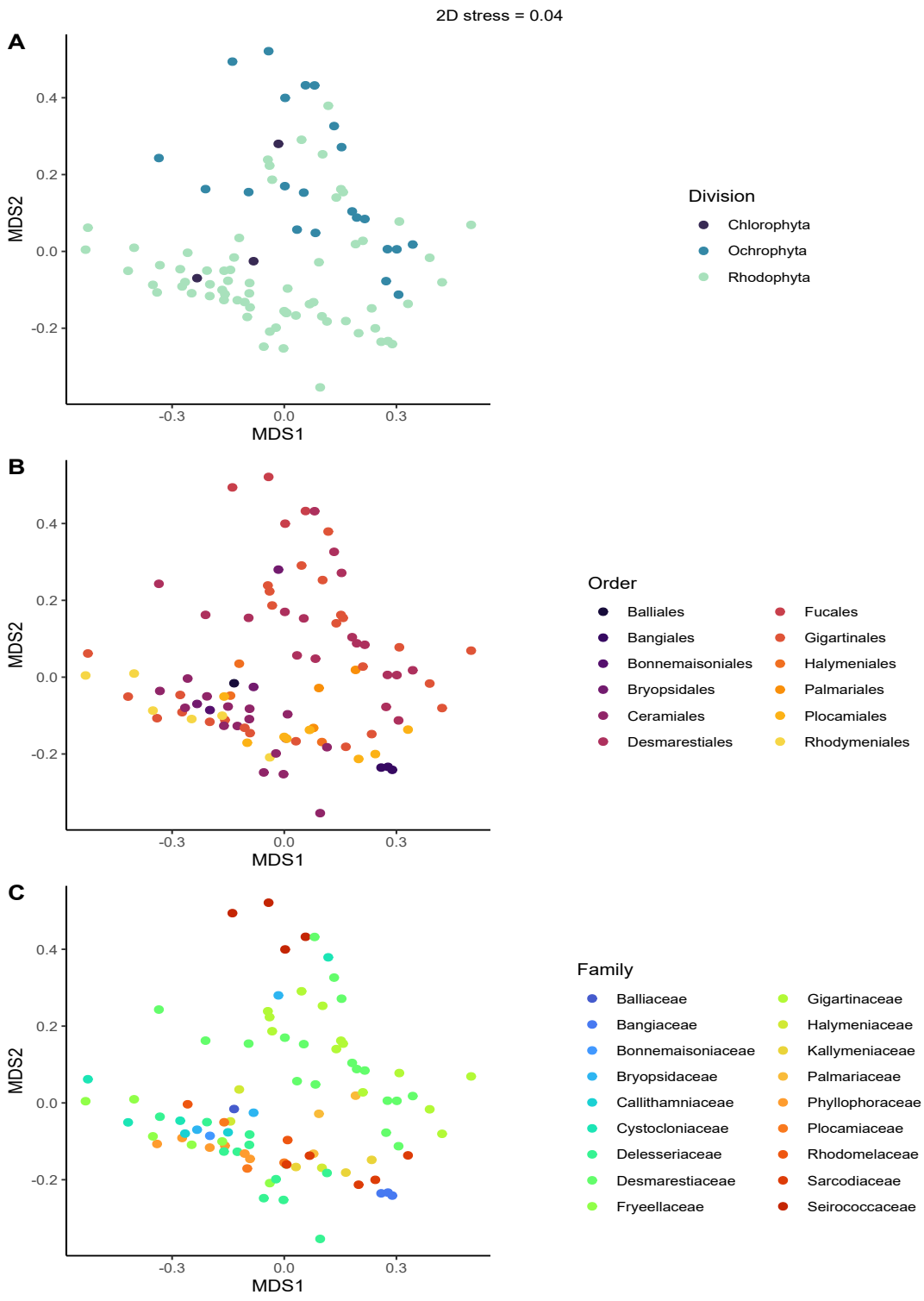


Figure 2.9: nMDS of all macroalgal SI profiles coded by Division (A), Order (B), and Family (C).

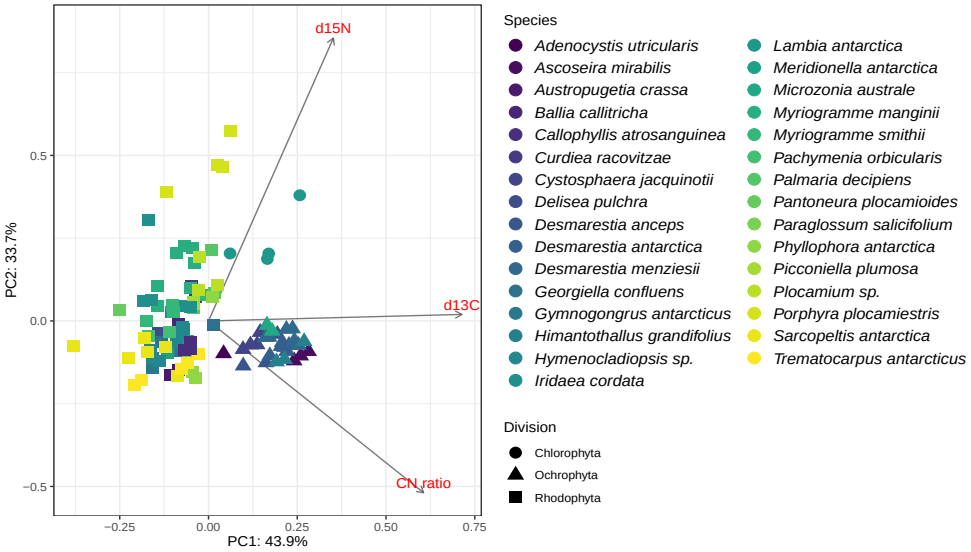


Figure 2.10: Principle Component Analysis (PCA) of all macroalgal samples based on the full suite of 40 fatty acids as well as the carbon and nitrogen stable isotope values and the C:N ratio. See Figure 2.5 for details on the PCA arrangement.

A combined PCA based on either total or reduced FA with carbon and nitrogen SI and C:N ratio data from samples that were analyzed for both types of biomarkers (Figure 2.11, 2.12) produced very similar patterns as seen in the PCA based solely on total or reduced FAs (Figure 2.5), with clear delineation of the macroalgal divisions. The combination of both biomarkers slightly increased the explanatory power for PC1 (16.5%) and PC2 (9.5%), and created a larger separation between Ochrophyta and Rhodophyta (Figure 2.11) compared to the FA-only PCA (Figure 2.5). These groupings were maintained, though not as tightly, when comparing the reduced FAs combined with SI (Figure 2.12) with the reduced-FA only PCA (Figure 2.6). It showed a stronger effect at the species-level (PERMANOVA, DF = 24, SS = 1.002, F = 18.609, p(perm) = 0.001) than the division-level, which was in contrast to the reduced FA-only groupings that had stronger division than species groupings (Table 2.5).

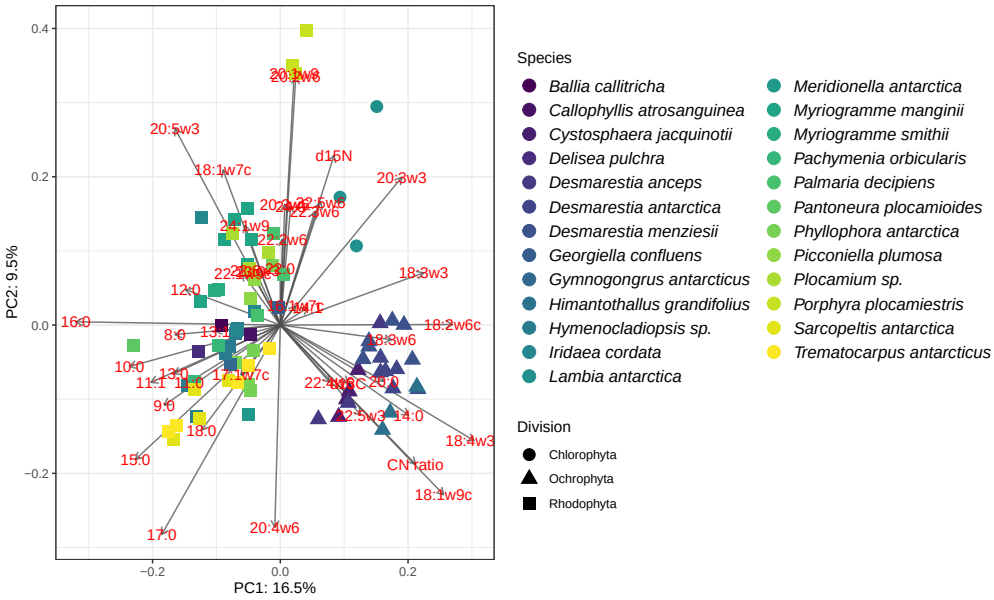


Figure 2.11: Principle Component Analysis (PCA) of all macroalgal samples based on the full suite of 40 fatty acids as well as the carbon and nitrogen stable isotope values and the C:N ratio. See Figure 2.5 for details on the PCA arrangement.

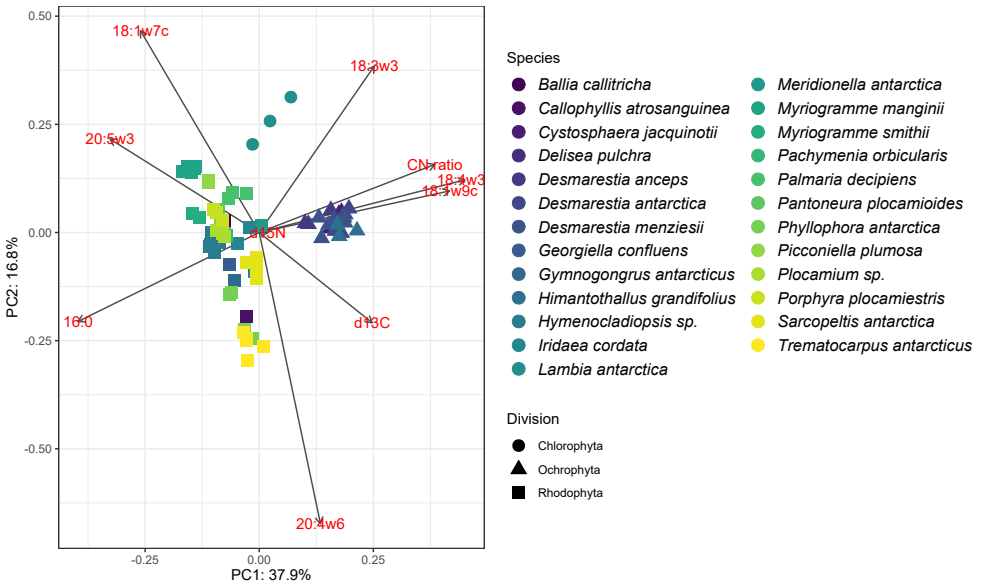


Figure 2.12: Principle Component Analysis (PCA) of all macroalgal samples based on the reduced suite of 40 fatty acids as well as the carbon and nitrogen stable isotope values and the C:N ratio. See Figure 2.5 for details on the PCA arrangement.

The morphologically similar Ochrophyta species *Desmarestia menziesii* and *D. anceps,* and the Rhodophyta *Phyllophora antarctica* and *Callophyllis atrosanguinea* were tested for differences based on FA profiles and carbon and nitrogen SI composition. *D. menziesii* and *D. anceps* were significantly different from one another with regards to both their FA profiles (PERMANOVA, DF = 1, SS = 0.03, F = 3.04, p = 0.0425), and SI values (PERMANOVA, DF = 1, SS = 0.01, F = 5.73, p = 0.015). However, *P. antarctica* and *C. atrosanguinea* were not significantly different from one another with regards to their FA profiles (PERMANOVA, DF = 1, SS = 0.01, F = 0.40, p =0.7658), but were different in stable isotope values (PERMANOVA, DF = 1, SS = 0.07, F = 38.61, p =0.0187).

4. DISCUSSION

In this study, we quantified the FA profiles and SI values of 31 Antarctic algal species to assess their biochemical and biogeochemical composition and the possible value of using these metrics as biomarkers to differentiate macroalgal species in food web applications. Our work extended the number of published Antarctic macroalgal FA profiles by 14 species not covered in previous studies (Graeve et al. 2002, Santos et al. 2017, Aumack et al. 2017, Schmid et al. 2018, Schram et al. 2019, Berneira et al. 2020). As has been established for macroalgae elsewhere (Galloway et al. 2012), the FA profiles of WAP macroalgal species differed phylogenetically by the major algal divisions of Chlorophyta, Ochrophyta, and Rhodophyta, and the separation was robust also on the order and even the family levels. The grouping by division was preserved when only a small subset of FAs was applied, including a number of PUFAs, most of them EFAs. SI information was less specific to the taxonomic affiliation of the species, although

divisional-level separation was still noticeable. We suggest that the combination of the two biomarker approaches could be especially valuable for differentiating macroalgal species groups in food web studies.

Macroalgal species from the WAP varied in their proportional FA compositions, but all contained high proportions of PUFAs, often comprising more than 50% of the total FA. The Rhodophyta in our study were largely composed of the SAFA 16:0 (mean 27.9%) and the PUFA 20:5ω3 (mean 35.5%), which is generally comparable to means previously published for Antarctic red algae (30.9% and 28.8%, respectively; see Table 2.7; averaged from Graeve et al. 2002, Santos et al. 2017, Aumack et al. 2017, Schmid et al. 2018, Berneira et al. 2020). However, 20:4ω6 was more prominent in our samples (mean 20.0%) compared to previously published Antarctic Rhodophyta (9.0%, references as above, Table 2.7), although 20:4ω6 has been identified as a major FA in Rhodophyta elsewhere (e.g., Dalsgaard et al. 2003, Kumari et al. 2013, Sohrabipour 2019). 16:1ω7 was reported as one of the top five contributing FA for Antarctic Rhodophyta in the literature (mean 6.5%, Table 2.7) but was negligible when considering the same set of species in our study (Table 2.7). However, when only considering new Rhodophyta species not previously investigated for FAs, 16:1ω7 was detectable (mean 2.7%). Some differences to previous Antarctic macroalgal records could be based on the fact that FA are reported in percentages instead of absolute concentrations, making these mean values dependent on the total number of FAs detected. In our study, we targeted 40 FAs, while other studies analyzed between 11 and 35 FAs (Graeve et al. 2002, Santos et al. 2017, Aumack et al. 2017, Schmid et al. 2018, Berneira et al. 2020), although the identity of the major FA contributors should still be similar, with just differences in the exact percentages.

Table 2.7: Most abundant FA found in samples ranked by taxonomic division and source of values (published = published literature values, literature species = species in our dataset with published counterparts, new species = species in our dataset with no published profiles, both = overall mean of all species within our dataset). Only FA included in our analyses were extracted from the literature.

	Ochrophyta				Rhodophyta			
		this study				this study		
Rank	published*	literature species	new species	both	published†	literature species	new species	both
1	20:4ω6 (16.0%)	20:4ω6 (19.5%)	20:4ω6 (20.8%)	20:4ω6 (19.8%)	16:0 (30.9%)	20:5ω3 (40.4%)	20:5ω3 (28.7%)	20:5ω3 (35.5%)
2	20:5ω3 (15.7%)	20:5ω3 (18.6%)	20:5ω3 (17.9%)	20:5ω3 (18.5%)	20:5ω3 (28.8%)	16:0 (27.6%)	16:0 (28.4%)	16:0 (27.9%)
3	16:0 (15.2%)	18:4ω3 (11.8%)	16:0 (15.7%)	16:0 (12.6%)	20:4ω6 (9.0%)	20:4ω6 (14.1%)	20:4ω6 (28.3%)	20:4ω6 (20.0%)
4	18:4ω3 (12.0%)	16:0 (11.8%)	18:4ω3 (11.7%)	18:4ω3 (11.8%)	18:1ω9 (6.6%)	18:1ω7 (3.5%)	18:1ω9 (2.7%)	18:1ω9 (2.7%)
5	18:1ω9 (10.5%)	18:1ω9 (11.7%)	18:3ω3 (11.7%)	18:1ω9 (10.7%)	16:1ω7 (6.5%)	18:1ω9 (2.7%)	16:1ω7 (2.7%)	18:1ω7 (2.6%)

* values from Aumack et al. 2017, Berneira et al. 2020, and Graeve et al. 2002

† values from Aumack et al. 2017, Berneira et al. 2020, Graeve et al. 2002, Santos et al. 2017, and Schmid et al. 2018

The identity as well as the relative proportion of the main FA contributors in Ochrophyta in our study were extremely similar to those reported in the literature (Table 2.7). The Ochrophyta in our study as well as those reported in the literature (Graeve et al. 2002, Aumack et al. 2017, Berneira et al. 2020) contained high proportions of the same set of FA: PUFAs 20:4ω6 (19.8% in our study vs. 16.0% in the literature) and 20:5ω3 (18.5% vs. 15.7%), the SAFA 16:0 (12.6% vs. 15.2%) as well as the PUFAs 18:4ω3 (11.8% vs. 12.0%) and 18:1ω9 (10.7% vs. 10.5%). In part, this consistency may be based on the large overlap in species between the literature and our study, including six out of the eight total Ochrophyta we investigated (see Table 2.4). However, we added not only two new species (*Cystosphaera jacquinotii* and *Microzonia australe*) in our investigation but these species belong to different orders (Fucales and Syringodermatales, respectively) that have not been included in any previous analysis. Despite minor differences in the rank order of the main contributing FA depending on which species set is included (Table 2.7), the very high consistency we found in FA composition even after adding representatives of two new orders confirms the conservative FA makeup of Ochrophyta. This does not only apply to the Antarctic Ochrophyta but is typical of the main FA

composition of Ochrophyta in general from a variety of regions and climate regimes (e.g., Khotimchenko 1998, Khotimchenko et al. 2002, Dalsgaard et al. 2003, Sohrabipour 2019 and references therein).

Chlorophyta are the least diverse macroalgal division in the Antarctic, with many species only occurring at lower latitudes along the WAP (Oliveira et al. 2020). Our study only contained one green algal species, *Lambia antarctica*, which was only previously investigated for its FA composition by Graeve et al. (2002). FA composition for this species matched well for some of the main FA contributors in our study, such as the SAFA 16:0 (19.8% in Graeve et al. 2002 and 20.3% in this study). It matched reasonably well for 18:3ω3 (23.7% in Graeve et al. 2002 and 31.6% in this study), but it differed considerably for 18:2ω6, which contributed nearly a quarter (22.3%) of FAs in Graeve et al (2002), but only 6.3% in our analysis. Conversely, 20:5ω3 was the third-largest contributing FA in *L. antarctica* in our study while this FA was only represented with 5.0% in Graeve et al. (2002). *L. antarctica* is a siphonous, unicellular species, so it is possible that cell content is lost to various degrees when damaged during collection, and the thallus also disintegrates rapidly. This may contribute to some of the differences in FA profiles we saw to the previously published record.

Marine macroalgae are a polyphyletic group, where the major divisions differ in many of their cellular and molecular properties (Chapman et al. 2012, Belghit et al. 2017). This explains the consistency of differences in major FA profiles among the three main divisions, which has been reported numerous times for macroalgae worldwide (e.g., Dunstan et al. 2005, Hanson et al. 2010, Galloway et al. 2012, Kumari et al. 2013). This strong divisional grouping has also been observed previously for Antarctic macroalgae (Graeve et al. 2002) and our addition of 14 newly

profiled species supported this finding. Some previous studies have documented similar separations on the order and even the family level for non-Antarctic species (e.g., Galloway et al. 2012, Kumari et al. 2013). Here, we documented, for the first time, that Antarctic macroalgal FA profiles are similarly conservative on the order and family levels.

A subset of seven FAs described patterns of phylogenetic differentiation equally well if not more distinctly than the full suite of 40 FAs. The FAs that were driving differences among macroalgal divisions were primarily EFAs, including 20:5ω3, 20:4ω6, 18:4ω3, 18:1ω9, 18:3ω3, and 20:3ω3. Only one SAFA (16:0) contributed strongly to these patterns. A similar observation was made for Northwest Pacific macroalgae, where robust phylogenetic separation was also observed when only applying EFA (Galloway et al. 2012). EFAs are typically used as key markers in food web analysis as they cannot be synthesized in most higher consumers (Budge et al. 2006, Brett et al. 2016). These EFAs also are routinely recorded in the published literature of macroalgal FAs, allowing the published profiles to be 'mined' for applications in trophic analyses. A caveat of this approach may be that, despite the overwhelming consistency reported in the literature for characteristic FA profiles of macroalgal divisions, there are reports that macroalgal FAs can vary seasonally in content and relative proportions, which may make taxon-specific profiles more difficult to resolve (Hernández-Carmona et al. 2009, Dethier et al. 2013, Barbosa et al. 2020). However, macroalgae living in narrow temperature windows may be limited in their ability to modulate their FA profiles (Barkina et al. 2019), suggesting that Antarctic macroalgal FA profiles, especially EFA, are likely reasonably consistent over time. As FA analyses can be labor and time intensive, a targeted FA approach, including relying on

published macroalgal FA values, could allow a broader application of Antarctic macroalgal FAs in food web studies.

When solely considering SI and C:N ratios, macroalgal species resolved into three major groupings that did not fully align with phylogenetic groups or match those obtained based on FA profiles. Instead, groupings seemed to be driven more by physiological properties of the species. Specifically, $\delta^{13}C$ values are strongly influenced by the carbon uptake mechanisms of the macroalgal species, resulting in a separation of macroalgal species into two groups based on their $\delta^{13}C$ values. Despite generally employing C3 photosynthesis, some marine florideophyte Rhodophyta lack pyrenoids and carbon-concentrating mechanisms (CCMs), rendering them highly depleted in ^{13}C, typically resulting in $\delta^{13}C$ values of -30‰ or lower (Raven et al. 2002, 2005, 2012). Some Antarctic red algae, however, can have low $\delta^{13}C$ values despite being able to use bicarbonate and having CCMs (Beardall & Roberts 1999). The largest macroalgal grouping in our analysis were species with such low $\delta^{13}C$ values, suggesting that underlying physiological differences in photosynthetic processes drive this separation. While the occurrence of macroalgae with low $\delta^{13}C$ values is typically rare, it was a fairly widespread phenomenon in our dataset. This is confirmed by similar SI records in previous studies of several of the same Antarctic macroalgal species as examined in this study (e.g., Fischer & Wiencke 1992, Dunton 2001). Interestingly, one Ochrophyta (*D. anceps*) and the sole Chlorophyta in our study (*L. antarctica*) also grouped with this low $\delta^{13}C$ value group. However, we are not aware of CCMs in marine brown or green algae, suggesting there may be other physiological processes at play. Accordingly, the $\delta^{13}C$ values also were the strongest driver separating algal species with our PCA based on SI data and C:N ratios.

Some of the macroalgal species formed a lose grouping based on high $\delta^{15}N$ values. Of particular note is *P. plocamiestris*, which had high mean $\delta^{15}N$ values >6‰. The $\delta^{15}N$ values of macroalgae can be driven by environmental conditions, such as inorganic nitrogen availability in the surrounding waters (Viana & Bode 2013, Lemesle et al. 2016) or selective release of nitrogen during photorespiration (Kim et al. 2013). The former is unlikely to be a driving factor in Antarctic waters as anthropogenic influences of eutrophication are not present on larger spatial scales, and point sources such as guano from penguin or other seabird colonies that could be implicated in the isotope values of marine organisms (Rossi et al. 2019) were not present in our sampling locations. High tissue nitrogen SI values have been associated with protein breakdown during photorespiration in shallow-water macroalgae, especially during emersion in intertidal algae (Kim et al. 2013). While we did not collect *P. plocamiestris* intertidally, it is a very shallow-water species that could experience periodic emergence, which could drive its very high $\delta^{15}N$ value.

We also included the C:N ratio of macroalgae in the ordination based on SI tracers as an added dimension, a not uncommon approach when applying dietary mixing models including primary producer sources (e.g., Wen et al. 2016, Brett et al. 2016), although it should be noted that the C:N ratio itself is not a dietary tracer. But briefly, C:N ratios were generally low, reflecting high tissue nitrogen concentrations driven by the nitrogen-replete waters of coastal Antarctica (Grotti et al. 2001). C:N ratios in our study were lower in Rhodophyta than Ochrophyta, confirming earlier findings by Weykam et al. (1996), although differences in thallus structure have also been implicated in driving C:N ratios in Antarctic macroalgae and likely contributed to the variability we observed in the C:N ratios in the Rhodophyta. The higher C:N

ratios in the large Ochrophyta are indicative of high levels of structural cell components (cell walls, phlorotannins, e.g., Iken et al. 2007). Thus, C:N ratios added to the divisional separation of macroalgae when combined in multivariate ordinations with the stable carbon and nitrogen isotope markers.

The underlying processes driving macroalgal FAs versus SI values among macroalgal species are quite different, with FAs primarily driven by metabolic pathways rooted in phylogenetic identity, while stable isotope values are usually more influenced by environmental factors such as light, temperature and the ability to sequester inorganic carbon (Wiencke & Fischer 1990, Fischer & Wiencke 1992, Dalsgaard et al. 2003, Guest et al. 2010, Mackey et al. 2015). In this context, it is of note that we did not find any spatial trends either in FA profiles or SI values of select macroalgal taxa across an environmental gradient spanning >3 degrees latitude and a range of sea ice cover along the WAP (Galloway and Iken, unpublished data). This indicates that the data provided here for both biomarkers are representative of the taxa in general. We, therefore, expected the combination of the two biomarkers to add to the ability to separate macroalgal groupings, ultimately for the purpose of tracing food web connections. This has been increasingly suggested or applied in several other systems across the world (e.g., Hanson et al. 2010, Kelly & Scheibling 2012, Dethier et al. 2013), where the combination of multiple biomarkers aided in finer trophic resolution of benthic primary producers. We found that the combination of FA profiles with SI markers and C:N ratios only slightly improved the separation of macroalgal species or groupings in multivariate space. This may be explained by the much larger number of FA compared to isotope markers. It is possible that the more nuanced SI

separation of macroalgae based on physiological processes is overwhelmed by the strong phylogenetic origins of FA profiles that also underlie some of the SI patterns.

This does not mean that a combination of FA and stable isotope biomarkers would not be useful in Antarctic coastal food web studies, and we propose the benefit of a combined approach for several reasons. For one, SI are commonly used to analyze trophic connections (Peterson & Fry 1987, Layman et al. 2007), and while there is still uncertainty about fractionation and trophic enrichment (Post 2002), our understanding of SI in food webs is probably greater than that of FA. There is still a need for controlled experimental work to better understand FA uptake and trophic transfer (Galloway & Budge 2020). An example of the usefulness of a combined approach has been shown for Antarctic amphipods feeding on macro- and microalgae, where general food sources identified by SI data were confirmed and refined by FA analysis (Aumack et al. 2017). Also, the different underlying processes driving FA and SI in macroalgae can be particularly useful when aiming to differentiate the contribution of phylogenetically close macroalgal species to a consumer. Based on FA, those species would likely be difficult to trace but if they differ profoundly in their SI composition, the combination of the two biomarkers could prove very useful. For example, the two red algal species, *Phyllophora antarctica* and *Callophyllis atrosanguinea,* were almost identical in their FA profiles, and they also are morphologically difficult to distinguish (Wiencke & Clayton 2002). Isotopically, however, these two species were vastly different, with *P. antarctica* having very low $\delta^{13}C$ values of around -37‰ compared to -23‰ in *C. atrosanguinea* in our study, similar to values reported previously (e.g., Norkko et al. 2004, Marconi et al. 2011 for genus-level value for *Callophyllis*). Therefore, using multiple lines of evidence in tracing macroalgal species in the coastal food web could

prove especially useful. Therefore, comprehensive data set across a large number of macroalgae for both FAs and SI provided in the present work provides an important baseline for the future application of these tracers in Antarctic food web studies.

BRIDGE

In Chapter II I focused on the chemical ecology of a suite of nearshore macroalgae in Antarctica. In Chapter III I consider a suite of nearshore macroalgae along the West Coast of North America and how these communities change across a seascape in conservation and adjacent comparison areas.

CHAPTER III

MULTI-YEAR MONITORING OF MACROALGAL ABUNDANCE AND DIVERSITY WITHIN THE OREGON MARINE RESERVE SYSTEM AND COMPARISON AREAS

From Whippo R, Fields SA, Aylesworth L (2022). SCUBA kelp report. Oregon Department of Fish and Wildlife Marine Resources Program. Newport, OR. https://ecologyreports.oregonmarinereserves.com/Data_Files/7.%20Collaborations/SCUBA%20Algal%20Swath%20Report/ODFW_markdown_algae.html

1. INTRODUCTION

The importance of large seaweeds, or macroalgae, in coastal ecosystems cannot be understated. Distributed worldwide, they form the base of many marine food webs, provide habitat for a diverse collection of species across many life stages, and comprise some of the most productive marine ecosystems in our oceans (Seitz et al. 2014, Krause-Jensen & Duarte 2016, Duarte 2017, Lefcheck et al. 2019). Macroalgae form mosaics of habitat above the tide line, as well as in deeper subtidal waters. In Oregon there are more than 50 common low intertidal and subtidal macroalgal species that are used as shelter or food by ecologically and commercially important species including rockfish, salmon, sea urchins, and abalone (Seitz et al. 2014, Krieg et al. 2019, Lefcheck et al. 2019).

While much research has described the diversity and ecology of intertidal macroalgae in Oregon, there has been relatively little investigation into subtidal macroalgal communities,

which potentially comprise a large portion of the habitable coastline (Menge et al. 1993, 2005, Bracken & Nielsen 2004). This is due, in large part, to the logistical difficulties of accessing near-shore submerged habitat exposed to the open ocean. Assumptions about subtidal macroalgal diversity in Oregon have therefore been primarily based on intertidal algal observations across the coast, which are much more robust and have been summarized in numerous places (Krieg et al. 2019). Prior to the formation of the Oregon Marine Reserves monitoring program in 2010, the only data available on subtidal macroalgal communities came from disparate sources. These data included approximately 50 SCUBA surveys by the Partnership for Interdisciplinary Studies of Coastal Oceans (PISCO) from 2001-2004 (Carr et al. 2020), a contracted benthic resources summary by The Port Orford Ocean Resources Team (POORT) and ODFW in 2009, and satellite imagery targeting canopy-forming kelps in Oregon collected semimonthly since 1984 that has only recently been synthesized (Hamilton et al. 2020). The remote-sensing data synthesis in particular is useful as it provides a continuous record of kelp cover on the Oregon Coast for the last 35 years, however, it targets only the large canopy forming kelp *Nereocystis luetkeana* leaving many sub-canopy species unaccounted for. While these historical surveys capture snapshots of algal communities on a broad scale, they lack continuity in the case of the PISCO data, algal abundance in the POORT report (presence/absence only), and assemblage-level accounting in the case of the satellite data. What is needed is a continuous, fine-scale multi-species inventory of subtidal algal resources along the Oregon Coast.

Although global canopy kelps are in decline, there is evidence that Oregon's canopy kelp communities have increased in area since 2014, or at least remained stable (Krumhansl et al. 2016, Hamilton et al. 2020). Yet the state and trajectory of Oregon's subtidal algal communities which are associated with these canopy kelps is unknown. Warming sea surface temperatures

and increasing sea urchin populations have been linked with large-scale macroalgal declines on the West Coast (Rogers-Bennett & Catton 2019). This speaks to an urgency to characterize our local macroalgal populations, as Oregon is in the transition zone between recently decimated kelp populations to the south, and more stable populations to the north (Pfister et al. 2017, Beas-Luna et al. 2020). In addition, macroalgal communities have been shown to have differential responses to upwelling depending on the strength and duration of the event (Hessing-Lewis & Hacker 2013). As patterns of upwelling along the Oregon coast continue to shift with climate change, it is vital to implement long-term, targeted monitoring of Oregon's subtidal algal resources to inform the state of these valuable macroalgal communities and how they might respond (Rykaczewski et al. 2015).

The goal of the ODFW Marine Reserves Ecological Monitoring program is to provide such long-term monitoring to track changing nearshore communities over time. The reserves were put in place with the expectation of preserving biodiversity and resilience of the coastal ecosystem. While systems such as these have been effective for fish and invertebrate communities in many areas around the world, less is known about their effects on macroalgal assemblages (Halpern 2003, Molloy et al. 2009). Studies suggest that it may be difficult to detect community-wide algal trends even over a decade or more of protection, although targeting focal species may provide more answers than simply considering total biomass or other gross indicators of production (Barrett et al. 2009, Medrano et al. 2020).

As a first step to implementing long-term monitoring of the marine reserves' and associated comparison areas' marine flora and fauna, ODFW contracted CA-based PISCO divers to start benthic SCUBA surveys in 2010 and 2011 at the Redfish Rocks and Otter Rock Marine Reserves. These surveys included a targeted kelp swath survey – focused on commonly observed

kelp species (brown algae) - and a benthic habitat survey (uniform point count (UPC)) focused on broad structural groups of mostly red algae). After this initial survey effort, it was determined that contracting PISCO to conduct future surveys was not a feasible long-term strategy, so ODFW worked with PISCO and local partners to build a volunteer dive program in Oregon based on PISCO methods. Starting in 2013, the Oregon Marine Reserves (ORMR) volunteer dive team began collecting monitoring data targeting macroalgae, fish, and other associated invertebrates. The Marine Reserve Program and local partners have worked hard to sustain the pool of well-trained volunteer divers that currently collect data at four of the five marine reserve (Cape Perpetua has no hard bottom habitat in diveable depths).

The data summarized in this report are from the kelp swath surveys, for data on benthic habitat and cover from UPC surveys, please see the SCUBA habitat and cover appendices for the Redfish Rocks, Otter Rock, Cascade Head and Cape Falcon Marine Reserves. The kelp swath surveys target several common taxa identified to genera or species level including: *Alaria marginata*, *Costaria costata*, *Desmarestia* sp., Saccharina latissima, Laminaria setchellii, *Nereocystis luetkeana*, *Pleurophycus gardneri*, and *Pterygophora californica*. Of these, the ODFW Marine Reserves program selected *Nereocystis luetkeana* and *Pterygophora californica* as focal species based on their ecological, economic or management importance. For more information please refer to the (Update) Methods Appendix detailing focal species selection. These data are a starting point to describe diversity, abundance, and distribution of subtidal Oregon kelps, however, they are limited in their descriptions due to the depths, spatial extent, and temporally irregular intervals at which they were conducted.

1.1 Knowledge gaps

Given the dearth of information available on the diversity and distribution of subtidal macroalgal communities along the Oregon Coast, there are several key knowledge gaps that can be prioritized to provide information as a starting place to create a clearer picture of the state of these communities. These gaps include:

1. The abundance and distribution of key subtidal macroalgae. This includes canopy kelps as well as perennial sub-canopy species.
2. The stability of macroalgal abundance and distribution through time. Where do we find macroalgae, and are assemblages of species consistent across years?
3. Associations between fish, invertebrates, and macroalgal habitats. Do we see associated changes in animal communities associated with macroalgae? Do these associations change across space and through time?
4. The overall diversity of subtidal macroalgae. What is the overall diversity of all subtidal macroalgae in Oregon?
5. The oceanographic, climatic, and ecological factors controlling macroalgal diversity, composition, and abundance. There are many potential drivers of macroalgal community state that are likely operating across multiple scales (Lamy et al. 2018; Conser and Shanks 2019).

The first three gaps are being partially addressed by by the OR Marine Reserves monitoring program, though methods can be streamlined, augmented, and targeted from lessons learned since monitoring of the reserves began. The last two are opportunities for future work that could be addressed though various means, including ‘bio-blitz’-style survey efforts, DNA analysis, and modeling.

1.2 Research questions

- What kelp species have been observed at each marine reserve and comparison area?
- How have these kelp communities changed over time?

2. TAKEAWAYS

Here we present the major takeaways from our SCUBA kelp swath analyses across Oregon's Marine Reserves.

2.1 All targeted kelp swath species have been observed across the marine reserves, but their presence varied among sites and years.

Over the course of the Marine Reserve Monitoring Program all major groups of kelps targeted–including 'focal species' (*N. luetkeana*, *P. californica*) and other species of interest (*Desmarestia* sp., *C. costata*, *L. setchellii*, *S. latissima*, and *A. marginata*)–were detected, although the detection of those species varied among marine reserves, comparison areas, and years. The highest total counts of kelps within a marine reserve totaled across all years were found at Otter Rock, followed by Redfish Rocks and Cascade Head. No kelps were detected at Cape Falcon. *P. californica* and *A. marginata* are found almost exclusively in the shallow subtidal <10 meters depth so it's not surprising that they were not frequently detected at any of the sites.

2.2 Monitoring data suggest declines over time, but likely attributable to methodological shifts, not true biological changes.

Summary data from both the Redfish Rocks Marine Reserve and Otter Rock Marine

Reserve suggest precipitous kelp declines between 2011 and later survey years. However, the low detection rate of kelp in later years and generally shallower transects (half the depth at Otter Rock) in earlier years (2010-2011) do not allow us to rule out methodological differences causing the declines observed. In general, a decline was detected at Cascade Head as well, but the low counts of kelps, even in earlier years, do not allow us to draw any conclusions. No kelps were detected at Cape Falcon in any survey so trends cannot be determined. At the Otter Rock Marine Reserve, the early PISCO surveys counted 3,136 kelp observations over two years, whereas the later two survey years counted only 107 individuals. If we compare the early two years to the later two years, we noted a difference in average transect depth, where the 2010-2011 transects were on average five meters shallower than the 2017-2019 transects. At Redfish Rocks Marine Reserve, we also observe very low detection rates of kelp in the later two years of surveys (2015, 2019), compared to the earlier years (2010, 2011). While the differences in depth between these years was not significant, safety concerns of diving in the open ocean limited access to kelp beds in these later years (L. Aylesworth, pers. comm). It is possible that the results may reflect a genuine decline in *N. luetkeana* in both regions, though other regional data on *N. luetkeana* populations through 2018 suggests they are increasing or stable, indicating there may be methodological differences at play (Hamilton et al. 2020). Furthermore, there are no observations in the literature of any decline on the order of what is observed in the data, if these were indeed true biological declines. Macroalgal communities up until 2010 had been fairly stable in estuaries across Oregon, though macroalgae are sensitive to temperature and the marine heatwave of 2015 could have negatively impacted these populations (Hessing-Lewis & Hacker 2013, Straub et al. 2019), this is not supported by other regional *N. luetkeana* data (Hamilton et al. 2020).

2.3 Adapting SCUBA surveys to open ocean conditions in Oregon limits our ability to survey prime subtidal kelp habitat.

On the surface, it appears there was a drastic decline in kelp detection between initial survey efforts and subsequent survey years for all sites except Cape Falcon where no kelp was detected. However, with the transition from the PISCO dive team to an Oregon-based volunteer dive team, surveys were adjusted for safety concerns around live-boating in nearshore conditions. This resulted in requiring a down-line divers must use to descend/ascend, avoiding the shallowest target depth (5 m), and avoiding *N. luetkeana* beds. Algal communities are concentrated in the shallows to access light for photosynthesis; surveys in later years moved away from targeting the shallower 5 m depths at which we'd expect to find dense nearshore macroalgal communities along the Oregon coast. Of the remaining two target depths (12.5 and 20 m) only one of those has the potential for algae communities as the photic zone is frequently shallower than 20 m during summer months (Small & Menzies 1981, Steneck & Dethier 1994). Safety concerns of diving at such shallow depths in exposed open ocean environments, and the requirement to maintain contact with a descent line limited access to prime subtidal algal habitats at some sites occasionally, and at other sites entirely.

These safety restrictions led to targeting the edges of *N. luetkeana* beds or other hard bottom substrate without *N. luetkeana* beds at the Otter Rock and Redfish Rocks Marine Reserves. The implementation of monitoring efforts at the Cascade Head Marine Reserve (2014) and Cape Falcon Marine Reserve (2016) provided additional challenges, as these sites are not known to contain the rich *N. luetkeana* beds of the other two marine reserve sites, and targeting the edge of such beds was not possible. These factors combined with the irregular sampling

intervals and randomly stratified transect locations make it difficult to draw any conclusions about the state of kelp populations over time.

For these reasons, the program is not currently able to detect change over time in kelp communities apart from generalized presence/absence metrics. These data should be considered snapshots of limited transects at a particular place in time, though they provide a starting place for future survey work.

2.4 While abiotic characteristics are generally similar between reserves and comparison areas, more data is necessary to assess the suitability from a kelp perspective.

From the kelp monitoring data we cannot say whether or not the comparison areas are appropriate reference sites for assessing change over time at the marine reserves. The ODFW Marine Reserves program did select comparison areas based on similar abiotic conditions (depth range, size, habitat types, oceanographic conditions), but further data is required to determine if comparison areas are appropriate from a kelp perspective.

2.5 While methodological shifts in kelp monitoring proved to be challenging for analyses, data provides a 'snapshot' of subtidal kelps in Oregon.

The challenges of our kelp data have been outlined above, but we were able to collect some baseline data that provides a snapshot of more rarely sampled kelps, especially subcanopy kelps. With the recommendations below our program aims to improve the consistency of our kelp data collection.

3. RECOMMENDATIONS

3.1 A move towards permanent transects for kelp surveys is needed to confidently detect future trends in brown algae with SCUBA surveys.

Algal communities can be ephemeral, and random stratified selection of survey sites, particularly those that encompass substrate that we would not expect to have associated macroalgal communities, may not be representative of the population dynamics at play. Therefore, the installation of several permanent transects along appropriate substrate (i.e. - bedrock, consolidated rock) in extant kelp habitat will allow temporal variation to be tracked. These can be supplemented occasionally by additional surveys for annual 'snapshots' of the community.

3.2 Eliminate 20 m kelp surveys, reconsider adding 5 m sites where appropriate.

Kelp communities are concentrated in the shallows to access light for photosynthesis. The depths of the surveys (5 m, 12.5 m, 20m) only capture two depth contours at which we'd expect to find dense nearshore macroalgal communities along the Oregon coast (5 m, 12.5 m), as the photic zone is frequently shallower than 20 m during summer months (Small & Menzies 1981, Steneck & Dethier 1994). To address this shortcoming, more shallow surveys should be conducted to access the likely range of canopy, and subcanopy kelp Additionally, future analysis could focus on 12.5 m data only to explore trends, with this depth-bin having the most overlap between PISCO and ODFW surveys.

3.3 Conduct surveys during peak of kelp production season (July – August).

The lack of consistent sampling across years poses a challenge in detecting patterns of

kelp community dynamics. Surveys along permanent transects should be conducted at a minimum of once per year, preferably at the peak of macroalgal production season (June-August) as is standard in other macroalgal monitoring programs (Pfister et al. 2017, Byrnes & Reed 2018) to ensure more comparability from year to year and increases the likelihood of sampling the full kelp community.

3.4 Consider adding in UAV surveys at Redfish Rocks and Otter Rock Marine Reserves to improve tracking of the focal species, N. luetkeana.

Additional surveys of *N. luetkeana*, one of the 'focal' species identified by ODFW for monitoring, can also be tracked using unmanned aerial vehicle (UAV, aka drone) technology. Drones have become and increasingly affordable and reliable way to quantify canopy kelps such as *N. luetkeana* in nearshore areas (Tait et al. 2019, Thompson 2021). These remote sensing techniques has also been used to monitor many types of marine vegetation and can be accomplished by surface crew concurrently with SCUBA transects and other shore- or boat-based surveys.

4. METHODS

4.1 Field surveys

All dive surveys were conducted from a boat within the Oregon Marine Reserve System and in associated comparison areas. The overall survey design for the initial surveys consists of multiple survey sites on shallow (< 20m depth) rocky reef habitat within each reserve area and corresponding comparison sites outside of the reserve. Comparison areas outside of each reserve were chosen based on similar depths, habitat, size, oceanographic conditions and historical

fishing pressure similar to each reserve. In 2013, PISCO methods and datasheets were modified to adjust for common Oregon species, frequently challenging sea states, and safety needs given the volunteer diver team (avoiding shallower depths, avoiding kelp bed locations, and requiring downlines).

Kelp swath surveys were conducted concurrently with invertebrate and benthic habitat and cover surveys where depths of 12.5 and 20 m were targeted . Once the site was located with GPS, a video lander was deployed from the boat with a buoyed downline. Divers would descend on the downline and use the lander as the anchor-point for their transect tapes (a required OSU safety requirement). A visibility check was conducted on each dive with a minimum acceptable visibility of 1 m. A 30 m transect was laid along contiguous rocky substrate following the depth contour of interest and following the natural curves of the substrate. Transect depths were kept within 1.5 m of the target depth and any changes in substrate type were noted during data collection. Transects did not overlap with other transects and the surveys were aborted if more than 5 continuous meters of sand were encountered. Divers noted all algal species of interest within 1 m on either side of the transect including:

- *Nereocystis luetkeana* (>1 m stipe length)
- *Pterygophora californica* (>30 cm stipe length)
- *Pleurophycus gardneri* (>30 cm stipe length)
- Laminaria setchellii / Saccharina latissima1 (>30 cm stipe length)
- *Costaria costata* (any size)
- *Desmarestia* sp. (any size)
- *Alaria marginata* (any size)

Algae were subsampled if encountered in excess of 30 individuals per 10 m-swath segment. Subsampling ended at the completion of each swath column (i.e., every 10m), and regular counting resumed.

4.2 Data analysis

All data used in this analysis were complete 30 meter transects (i.e. equal area surveyed), thus abundance is explored at as counts. Survey effort and basic abundance relationships among sites, years, and species were visualized in boxplots and tables. Higher-level statistical analysis proved difficult due to the sparse nature of the data (zero-rich dataset) and irregular sampling intervals (skipped years, unbalanced sampling effort). While simple statistical comparisons were made between dive parameters with ANOVA and post-hoc Tukey tests, any higher-level analyses with factors of interest (site, year, reserve vs. comparison area) could only be achieved by pooling data sets or comparing data that were not statistically robust, which was not a realistic or informative line of inquiry. As such, descriptive visualizations were created to suggest trends and provide a starting point for future survey design refinement. Because we adapted original PISCO protocols for long-term use in Oregon nearshore water, we decided to test data collected with the original PISCO protocol against those collected with the modified Oregon Marine Reserves (ORMR) method. To determine if overall mean transect depth was different between the protocols among sites a multi-way ANOVA was run. Significant differences in site-specific depths were detected using a post-hoc Tukey test. All survey and species counts were visualized in R using the tidyverse, gt, and viridis packages (Wickham et al. 2019, Garnier et al. 2021, Iannone et al. 2022, R Core Team 2022).

5. RESULTS

5.1 Data overview

Between 2010 and 2019 there were a total of 254 kelp swath survey transects completed in the Oregon Marine Reserves and associated comparison areas (Table 3.1). Kelp was counted on 102 of the 254 total transects (~40% overall detection rate) at every site except Cape Falcon, where no kelp was detected.

Reserves and their associated comparison areas were surveyed annually on a rotating basis. Three of the reserves have four years of survey data (Redfish Rocks (2010, 2011, 2015, 2019); Otter Rock (2010, 2011, 2017, 2019); Cascade Head (2013, 2014, 2017, 2018)), and despite several efforts over multiple years, only one year of survey data is available for Cape Falcon (2017). Kelp transects within a single reserve and comparison area ranged from 1-31 in a single year, with an average of 20 transects conducted per site on years when it was surveyed.

5.2 Protocol comparison

Between 2010 and 2019 there were a total of 254 kelp swath survey transects completed in the Oregon Marine Reserves and associated comparison areas (Table 3.1). Kelp was counted on 102 of the 254 total transects (~40% overall detection rate) at every site except Cape Falcon, where no kelp was detected.

Reserves and their associated comparison areas were surveyed annually on a rotating basis. Three of the reserves have four years of survey data (Redfish Rocks (2010, 2011, 2015, 2019); Otter Rock (2010, 2011, 2017, 2019); Cascade Head (2013, 2014, 2017, 2018)), and despite several efforts over multiple years, only one year of survey data is available for Cape Falcon (2017). Kelp transects within a single reserve and comparison area ranged from 1-31 in a

single year, with an average of 20 transects conducted per site on years when it was surveyed.

Table 3.1: Number of SCUBA kelp swath transects per year across all regions including marine reserves and comparison areas completed as part of Marine Reserves Ecological Monitoring. All surveys conducted in 2010-2011 followed original PISCO dive protocols. Those from 2013 onward followed a modified ORMR protocol.

	Region			
Year	Redfish Rocks	Otter Rock	Cascade Head	Cape Falcon
2010	20	4	NA	NA
2011	26	20	NA	NA
2013	0	0	3	NA
2014	0	0	3	NA
2015	11	0	0	0
2017	0	23	24	28
2018	0	0	58	0
2019	18	16	0	0

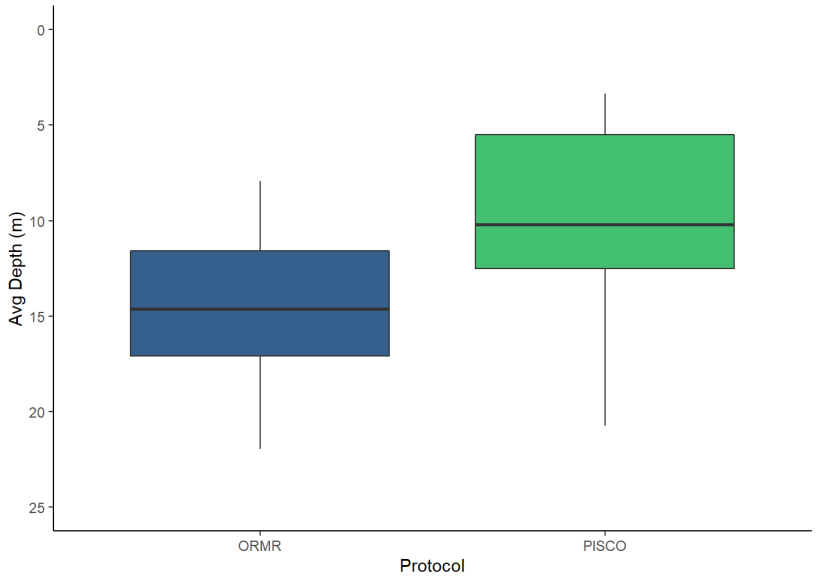


Fig. 3.1: Average depth of kelp swath transects between ODFW and PISCO survey teams.

5.3 Data by site

5.3.1 REDFISH ROCKS

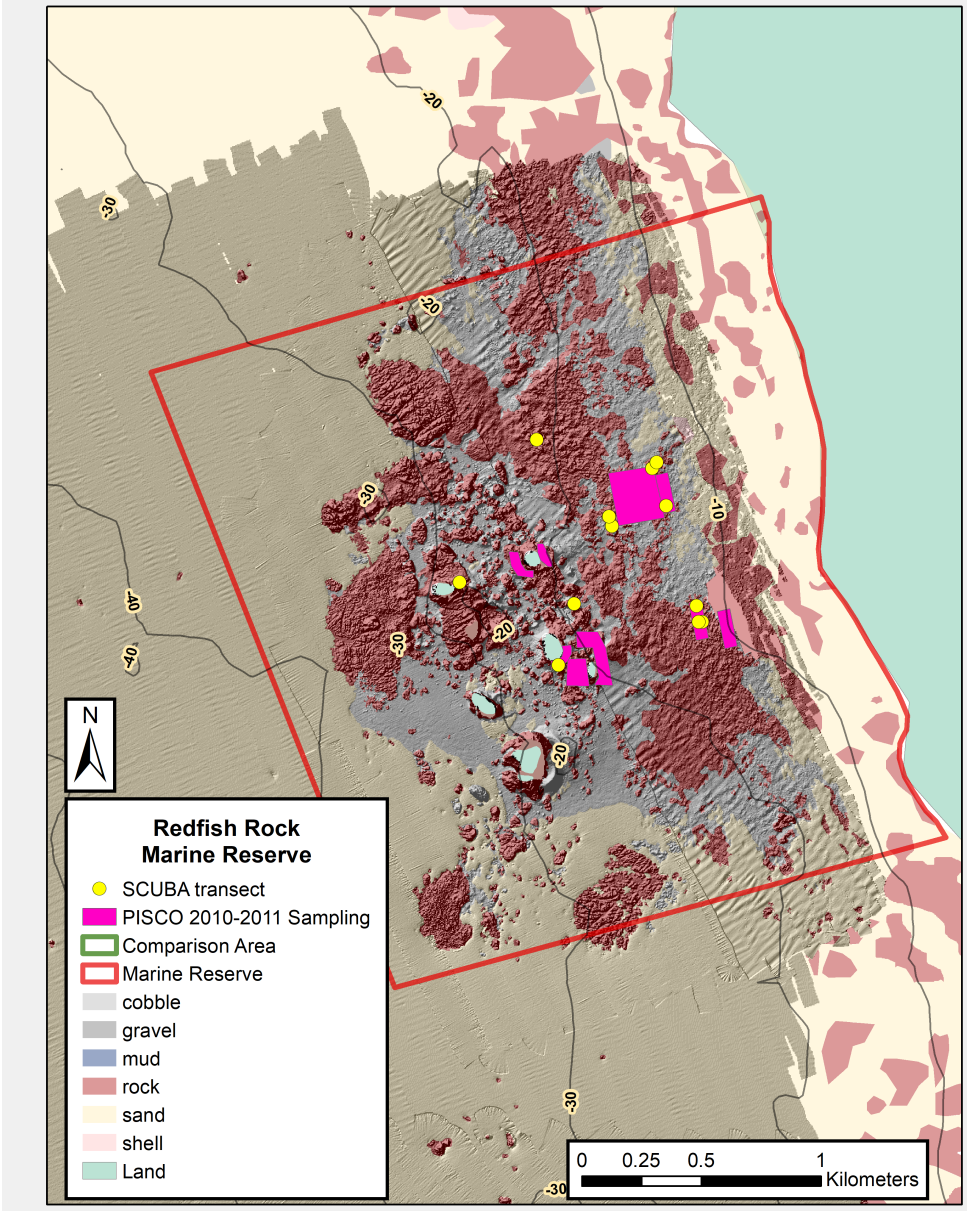


Fig. 3.2: Map of SCUBA transect locations at Redfish Rocks Marine Reserve.

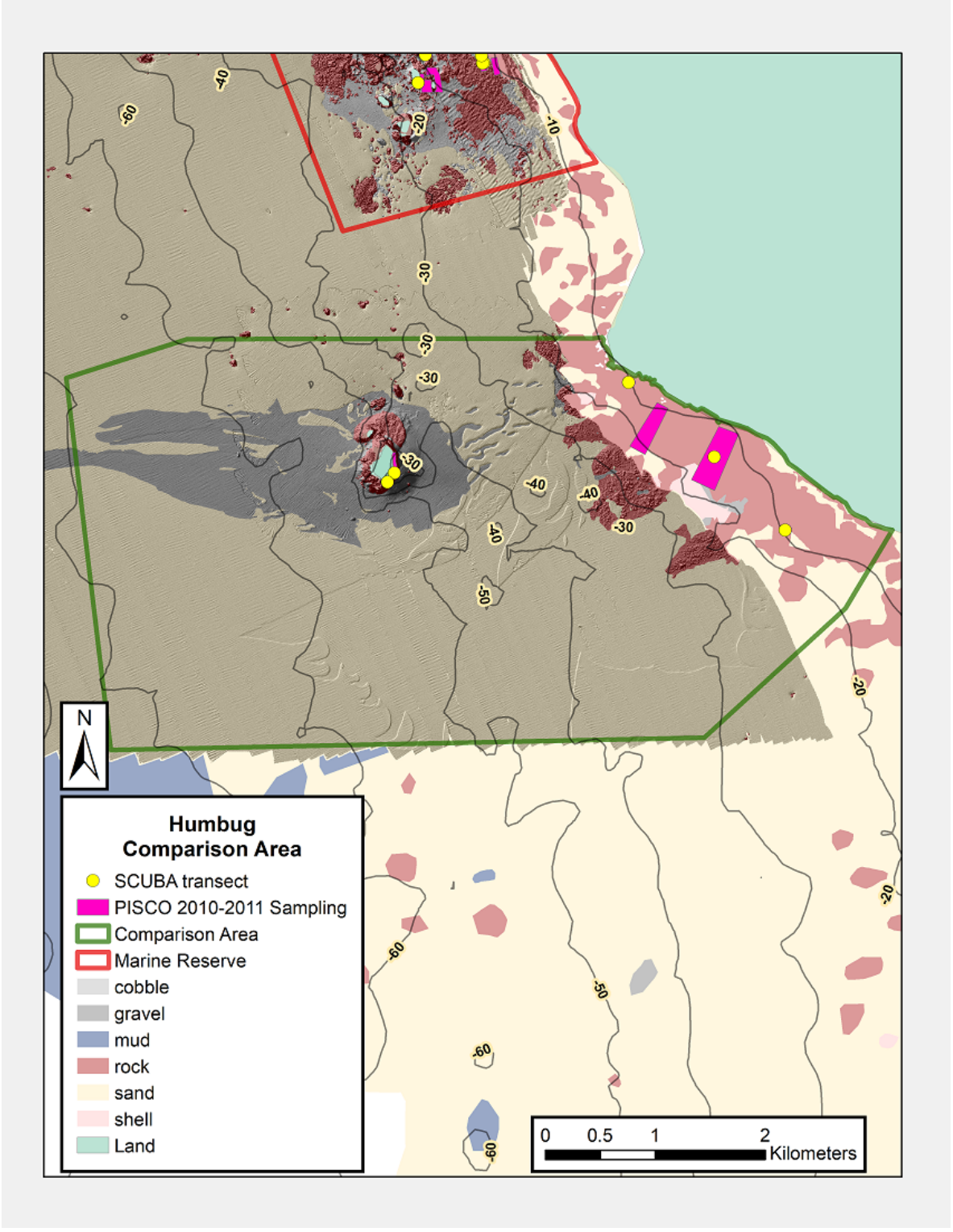


Fig. 3.3: Map of SCUBA transect locations at Humbug Comparison Area.

A total of 75 kelp transects were conducted at Redfish Rocks, and Humbug Comparison Area over four years (2010, 2011, 2015, 2019; Table 3.2, Figure 3.4).

Table 3.2: Total number of SCUBA kelp swath transects conducted in Redfish Rocks Marine Reserve and Humbug Comparison Area between 2010 and 2019.

	Site	
Year	Redfish Rocks MR	Humbug CA
2010	15	5
2011	16	10
2015	9	2
2019	12	6

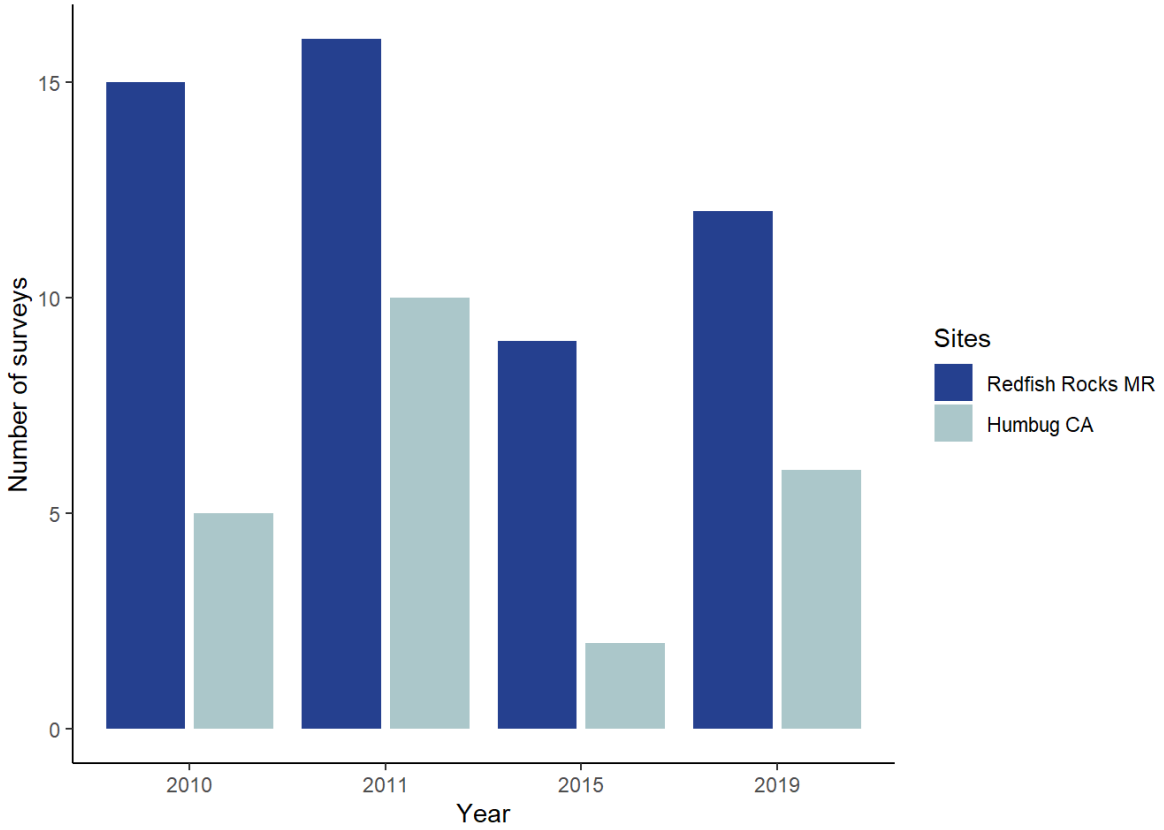


Fig. 3.4: Total SCUBA kelp swath surveys conducted by year in the Redfish Rocks Marine Reserve (dark blue), and Humbug Comparison Area (lighter blue).

Mean depths of PISCO and ORMR protocols differed between the marine reserve and

comparison area, though none of these differences were statistically significant (Table 3.3).

Table 3.3: Mean depth in meters of SCUBA kelp swath transects conducted with PISCO protocols (2010, 2011) and modified OR (ORMR) protocols (2015, 2019). None of the depth comparisons between protocols or sites were statistically significant (post-hoc Tukey, $p > 0.05$).

	Site	
Protocol	Redfish Rocks MR	Humbug CA
ORMR	14.01	13.85
PISCO	12.71	10.79

Across all years and protocols the focal species *N. luetkeana* was the most abundant alga of all species surveyed within the Redfish Rocks Marine Reserve and in the Humbug Comparison Area (Figure 3.5). Focal species *P. californica* was detected also in three of the four survey years, along with associated species *L. setchellii* and *P. gardneri*. No kelp was detected inside or outside the reserve in 2019.

- Mean depth of transects did not differ significantly between the PISCO and ORMR surveys.
- The focal species *N. luetkeana* and *P. californica*, along with other common species *P. gardneri* and *L. setchellii* were detected in the marine reserve between 2010-2015, but not detected at all in 2019. Observations in the comparison area followed a similar pattern.
- Observed declines in *N. luetkeana* may be due to methodological differences between the PISCO and ORMR surveys. Specifically the avoidance of *N. luetkeana* beds by ORMR due to safety considerations.

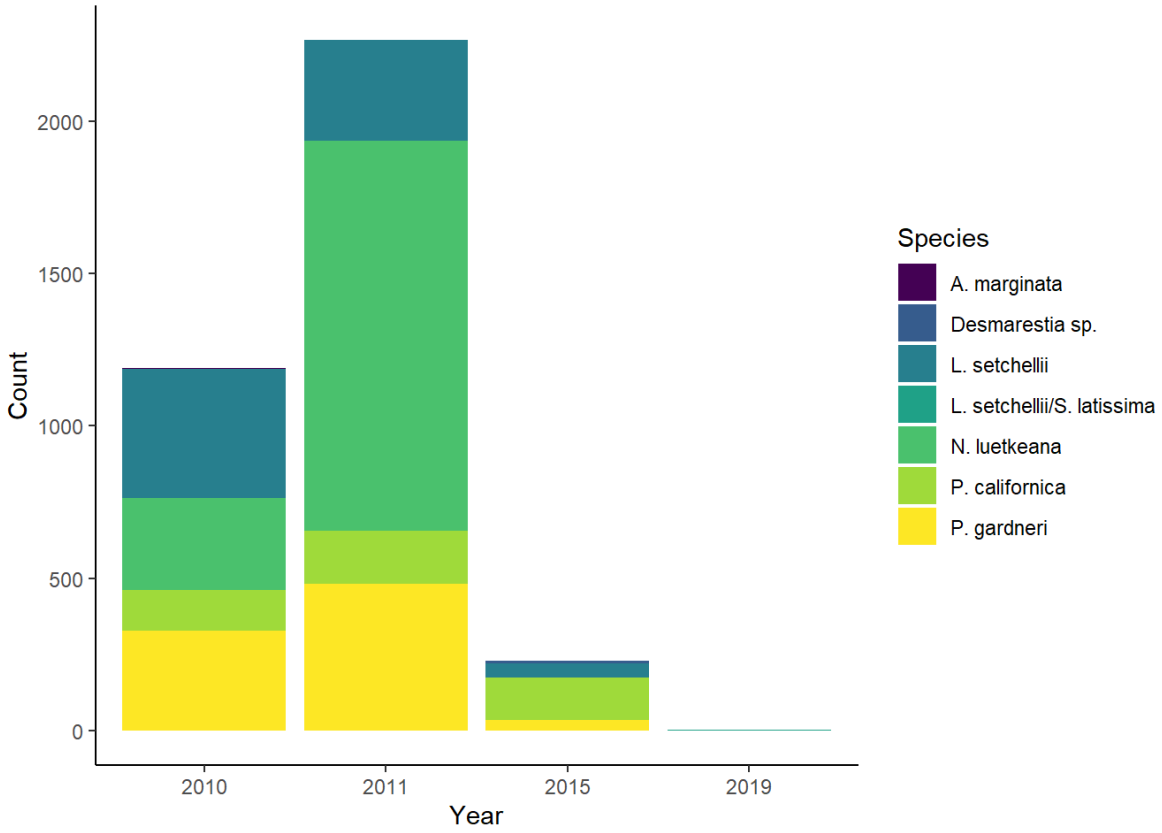


Fig. 3.5: Kelp species counts observed in the Redfish Marine Rocks Reserve and Humbug Comparison Area (combined) by year.

The focal species *N. luetkeana* was found in relatively high abundance within the Redfish Rocks Marine Reserve in 2010 and 2011 along with associated species *P. gardneri* and *L. setchellii*, although *L. setchellii* was found less frequently in 2011 with a total decline in detection of 76% (Table 3.4, Figure 3.6). Focal species *P. californica* peaked in 2015 with 139 individuals counted, but was not detected at all in the following survey year of 2019. In fact, no kelp was detected at Redfish Rocks Marine Reserve at all in 2019.

A total count of 1013 individual *N. luetkeana* were found in the Humbug Comparison Area in 2011, the second highest detection of *N. luetkeana* across all years and sites second only to Otter Rock Marine Reserve in the same year, and the highest count within a comparison area

Table 3.4: Abundance of focal species (*N. luetkeana*, *P. californica*) and other common kelp from SCUBA swath surveys within the Redfish Rocks Marine Reserve by year. Total counts are summed across 30 m transects.

Species	2010	2011	2015	2019
N. luetkeana	**211**	**266**	**0**	**0**
P. californica	**48**	**37**	**139**	**0**
P. gardneri	268	270	23	0
Desmarestia sp.	0	0	0	0
C. costata	0	0	0	0
L. setchellii	234	56	26	0
A. marginata	5	0	0	0
L. setchellii/S. latissima	0	0	0	0

(Table 3.5, Figure 3.6). Algal detection was reduced greatly in 2015 and 2019, similar to the marine reserve, however none of the focal species *P. californica* was detected in Humbug Comparison Areas as it was in the marine reserve. Overall, kelp detection between the marine reserve and comparison areas tended to be species-specific in terms of abundance within and across years. The only consistent pattern was the drastic reduction in detection between 2010/11 and 2015/19. The common species *Desmarestia* sp. was also detected in small quantities in 2015/19.

Table 3.5: Abundance of focal species (*N. luetkeana*, *P. californica*) and other common kelp from SCUBA swath surveys within the Humbug Comparison Area by year. Total counts are summed across 30 m transects.

	Year			
	2010	2011	2015	2019
Humbug CA				
N. luetkeana	**91**	**1013**	**0**	**1**
P. californica	**83**	**136**	**0**	**0**
P. gardneri	62	212	12	0
Desmarestia sp.	0	0	8	1
C. costata	0	0	0	0
L. setchellii	189	275	21	0
A. marginata	0	0	0	0
L. setchellii/S. latissima	0	0	0	3

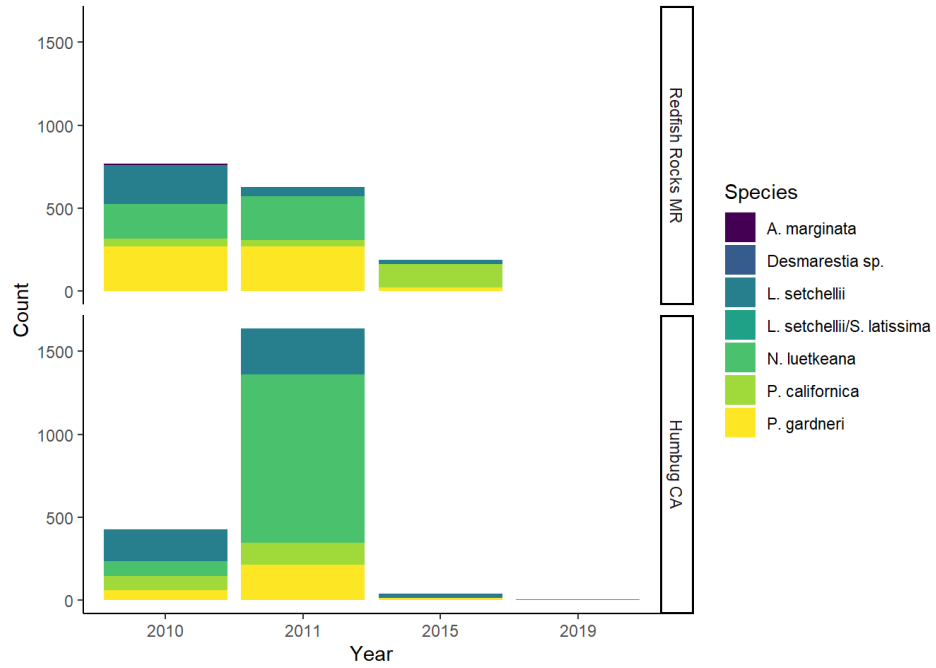
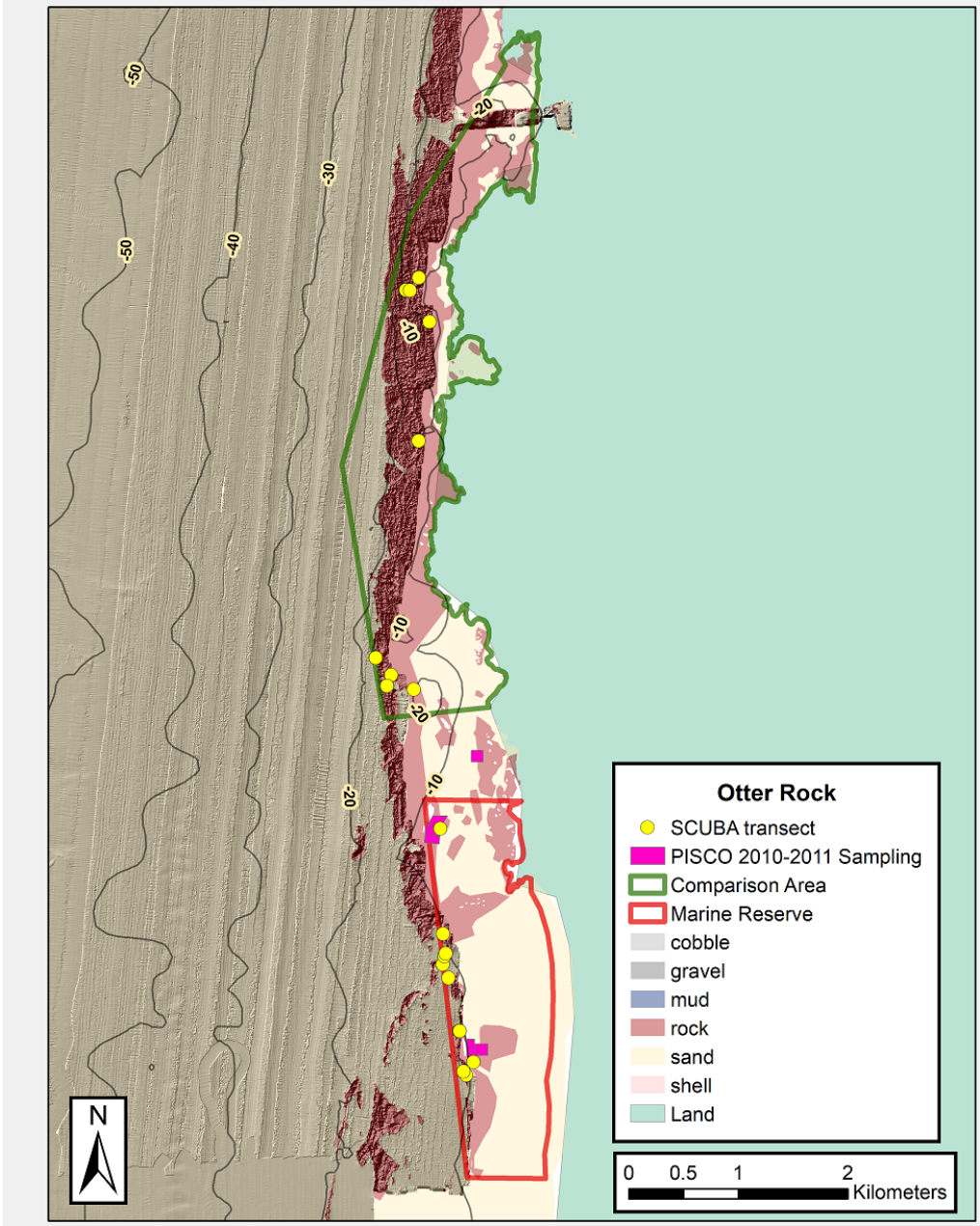


Fig. 3.6: Kelp species counts observed in Redfish Rocks Marine Reserve and Humbug Comparison Area from SCUBA kelp swath surveys by year. Total counts are summed across 30 m transects.

5.3.2 OTTER ROCK

Fig. 3.7: Map of SCUBA transect locations at Otter Rock Marine Reserve.


-50
-30
-40
-50
-20
-10
-10
-20
-10
-20
Otter Rock
SCUBA transect
PISCO 2010-2011 Sampling
Comparison Area
Marine Reserve
cobble
gravel
mud
rock
sand
shell
Land
N
0
0.5
1
2
Kilometers

A total of 63 transects were conducted at Otter Rock over four years of survey effort (2010, 2011, 2017, 2019; Table 3.6, Figure 3.8).

Table 3.6: Total number of SCUBA kelp swath transects conducted in Otter Rock Marine Reserve and Cape Foulweather Comparison Area between 2010 and 2019.

	Site	
Year	Otter Rock MR	Cape Foulweather CA
2010	4	0
2011	14	6
2017	15	8
2019	8	8

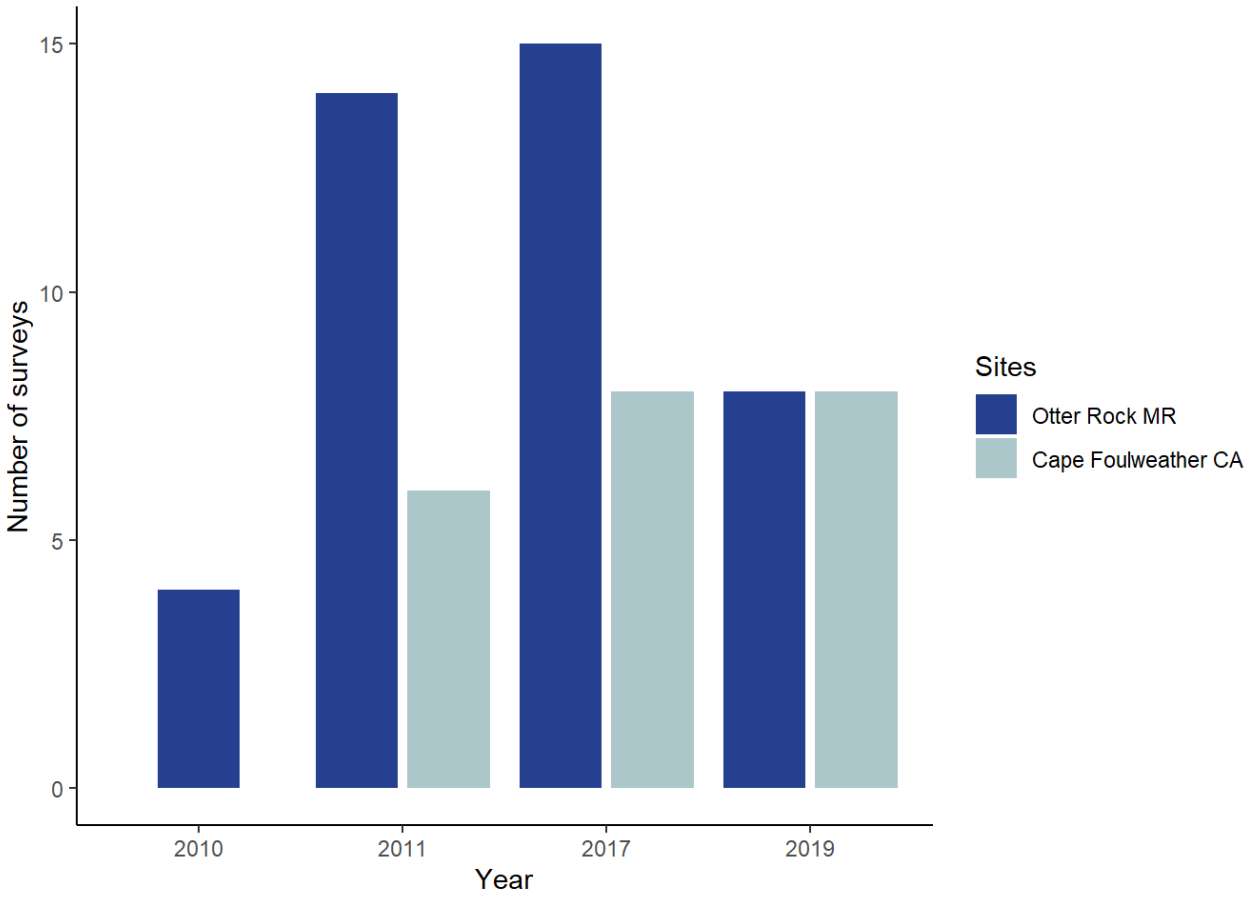


Fig. 3.8: Total SCUBA kelp swath surveys conducted by year in the Otter Rock Marine Reserve (dark blue), and Cape Foulweather Comparison Area (lighter blue).

Mean depths of PISCO and ODFW transects differed significantly among the Marine Reserve and Comparison Area transects. PISCO transects were reliably shallower than the ODFW transects with a mean difference of 5.57 m within the Marine Reserve (n = 41) and 7.05 m in the Cape Foulweather Comparison Area (n = 22; Table 3.7).

Table 3.7: Mean depth of kelp surveys conducted at Otter Rock by PISCO (2010, 2011) and modified OR (ORMR) protocols (2017, 2019). Depths between teams were different in both the Marine Reserve and Comparison Area (post-hoc Tukey, ** $p < 0.001$, *** $p < 0.0001$).

	Site	
Protocol	Otter Rock MR***	Cape Foulweather CA**
ORMR	11.73	12.13
PISCO	6.16	5.08

Across all years the common species *L. setchellii* and *S. latissima* were the most consistently detected at Otter Rock, while focal species *P. californica* and *N. luetkeana* were the most abundant overall in 2010 and 2011 (Fig 8). This was also the only site where the alga *Costaria costata* was found inside or outside the Marine Reserve system.

- Transect depths were significantly shallower using the PISCO protocol (2010, 2011) by 5.57 m and 7.05 m in the marine reserve and comparison area respectively.
- The Cape Foulweather Comparison Area was the only site among all the regions surveyed where the alga *Costaria costata* was detected (2011, 2019).
- Kelp detection was greater by an order of magnitude in the marine reserve than in the associated comparison area in 2011. This was driven primarily by *N. luetkeana* and *P. californica*. While detection remained higher within the marine reserve in 2017 and 2019, overall counts were greatly reduced.

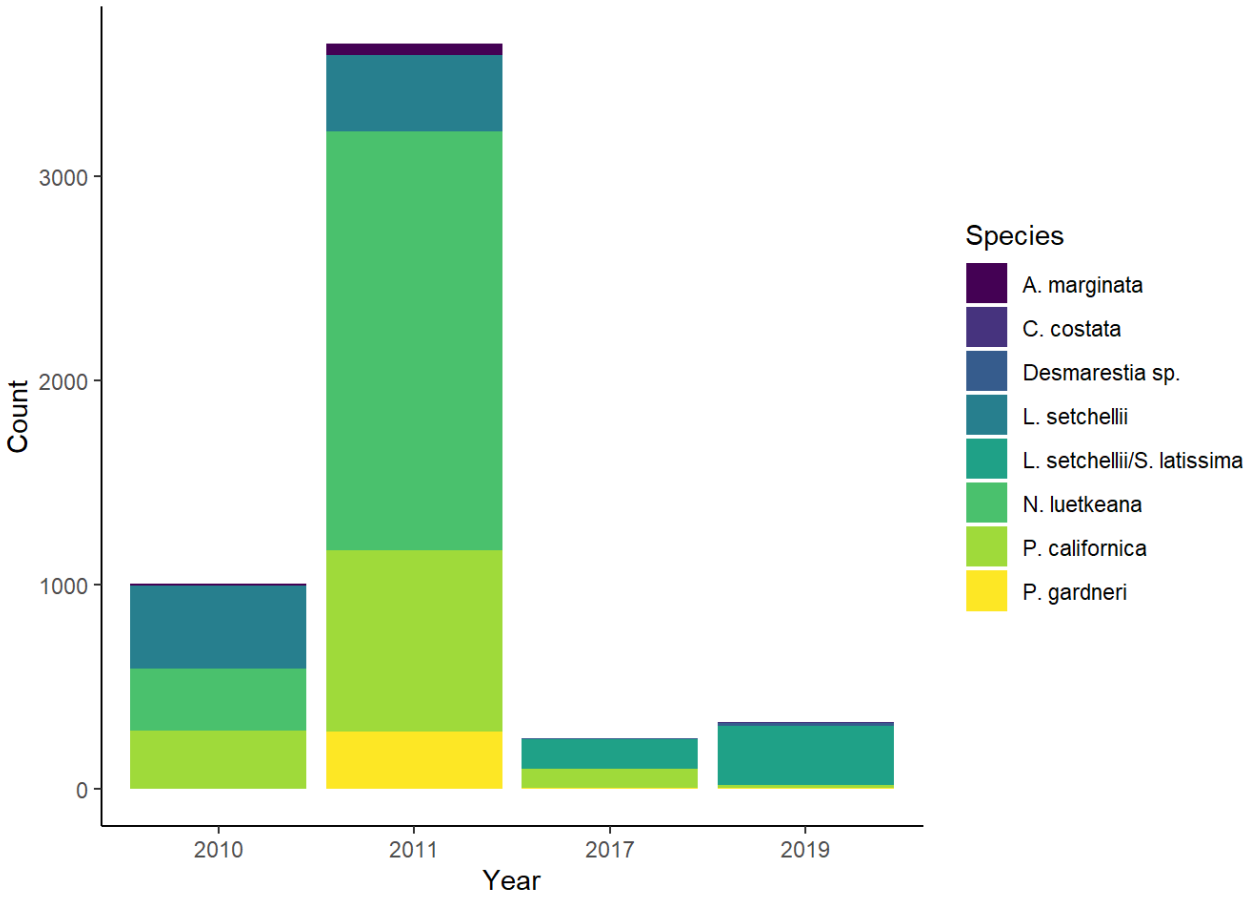


Fig. 3.9: Kelp species counts observed in the Otter Rock Marine Reserve and Cape Foulweather Comparison Area (combined) by year.

Inside the Otter Rock Marine Reserve the focal species *P. californica* was detected in every survey year. Likewise, the common alga *P. gardneri* was also detected each year, albeit in much lower quantities (Table 3.8, Figure 9). Within the marine reserve *N. luetkeana* was detected at its highest level with a count of 1702 individuals across 14 transects. The alga *Desmarestia* sp. was also detected in 2017.

Table 3.8: Abundance of focal species (*N. luetkeana*, *P. californica*) and other common kelp from SCUBA swath surveys within the Otter Rock Marine Reserve by year. Total counts are summed across 30 m transects.

Species	2010	2011	2017	2019
N. luetkeana	**305**	**1702**	**0**	**1**
P. californica	**283**	**846**	**92**	**14**
P. gardneri	3	217	7	1
Desmarestia sp.	0	0	3	0
C. costata	0	0	0	0
L. setchellii	404	354	0	0
A. marginata	9	53	1	0
L. setchellii/S. latissima	0	0	145	280

In the Cape Foulweather Comparison Area both *N. luetkeana* and *P. californica* were detected in 2011, though *P. californica* was detected at lower levels than in the marine reserve the same year (Table 3.9, Figure 3.10). Only a single observation of *P. californica* was made in 2017 and 2019, and *N. luetkeana* was not detected at all after 2011. Other species including *P. gardneri*, *Desmarestia* sp., *C. costata*, *L. setchellii*, and *S. latissima* were detected at very low level sporadically across the 2011 and 2019 survey years. No kelp whatsoever was detected at Cape Foulweather Comparison Area in 2017.

Table 3.9: Abundance of focal species (*N. luetkeana*, *P. californica*) and other common kelp from SCUBA swath surveys within the Cape Foulweather Comparison Area by year. Total counts are summed across 30 m transects.

	Year		
	2011	2017	2019
Cape Foulweather CA			
N. luetkeana	**348**	**0**	**0**
P. californica	**40**	**0**	**1**
P. gardneri	65	0	5
Desmarestia sp.	0	0	15
C. costata	1	0	4
L. setchellii	22	0	0
A. marginata	0	0	0
L. setchellii/S. latissima	0	0	8

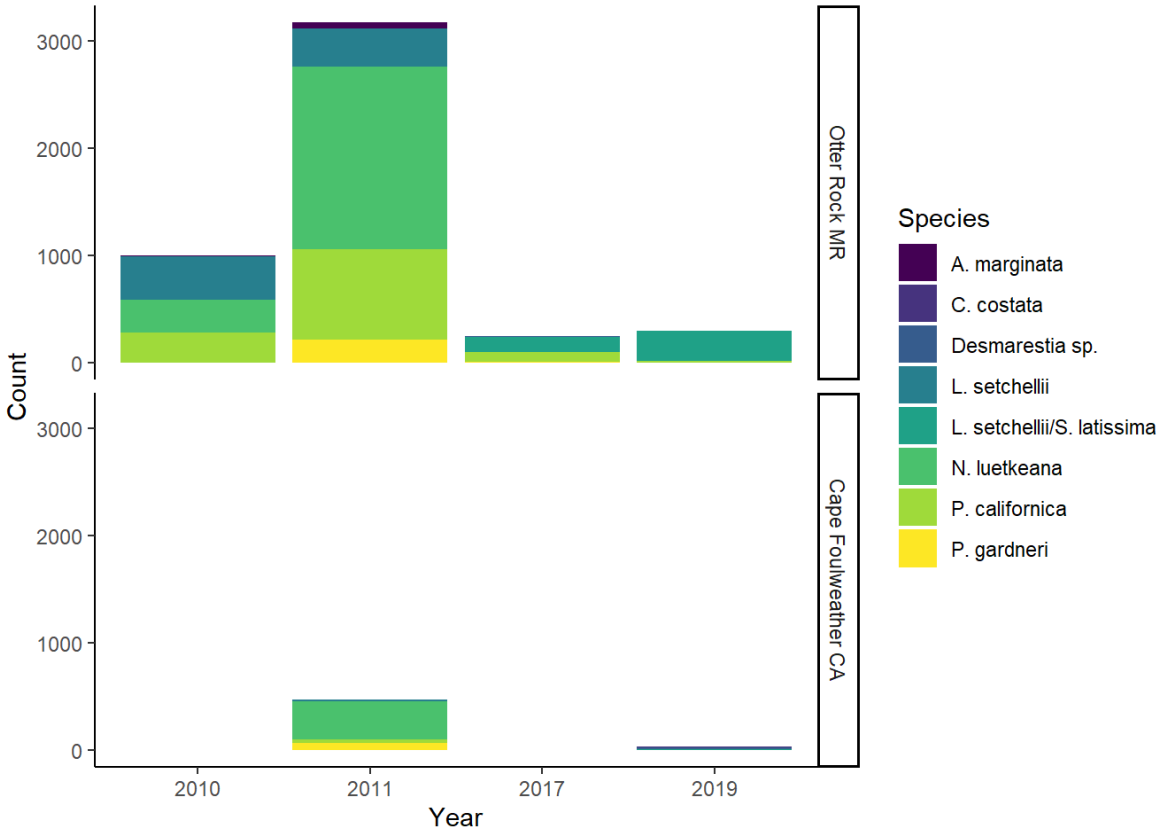


Fig. 3.10: Kelp species counts observed in Otter Rock Marine Reserve and Cape Foulweather Comparison Area from SCUBA algal swath surveys by year. Total counts are summed across 30 m transects.

5.3.3 CASCADE HEAD

A total of 88 transects were conducted at Cascade Head over four years of sampling effort (2013, 2014, 2017, 2018; Table 3.10, Figure 3.11-3.14).

Cascade Head Marine Reserve and associated comparison areas were not surveyed with the original PISCO protocols as other sites in 2010 and 2011. To detect if there are mean differences in transect depths across years, ODFW transects were binned into either a 2013-2014 or 2017-2018 category. These binned year groups were tested for differences in the same way as

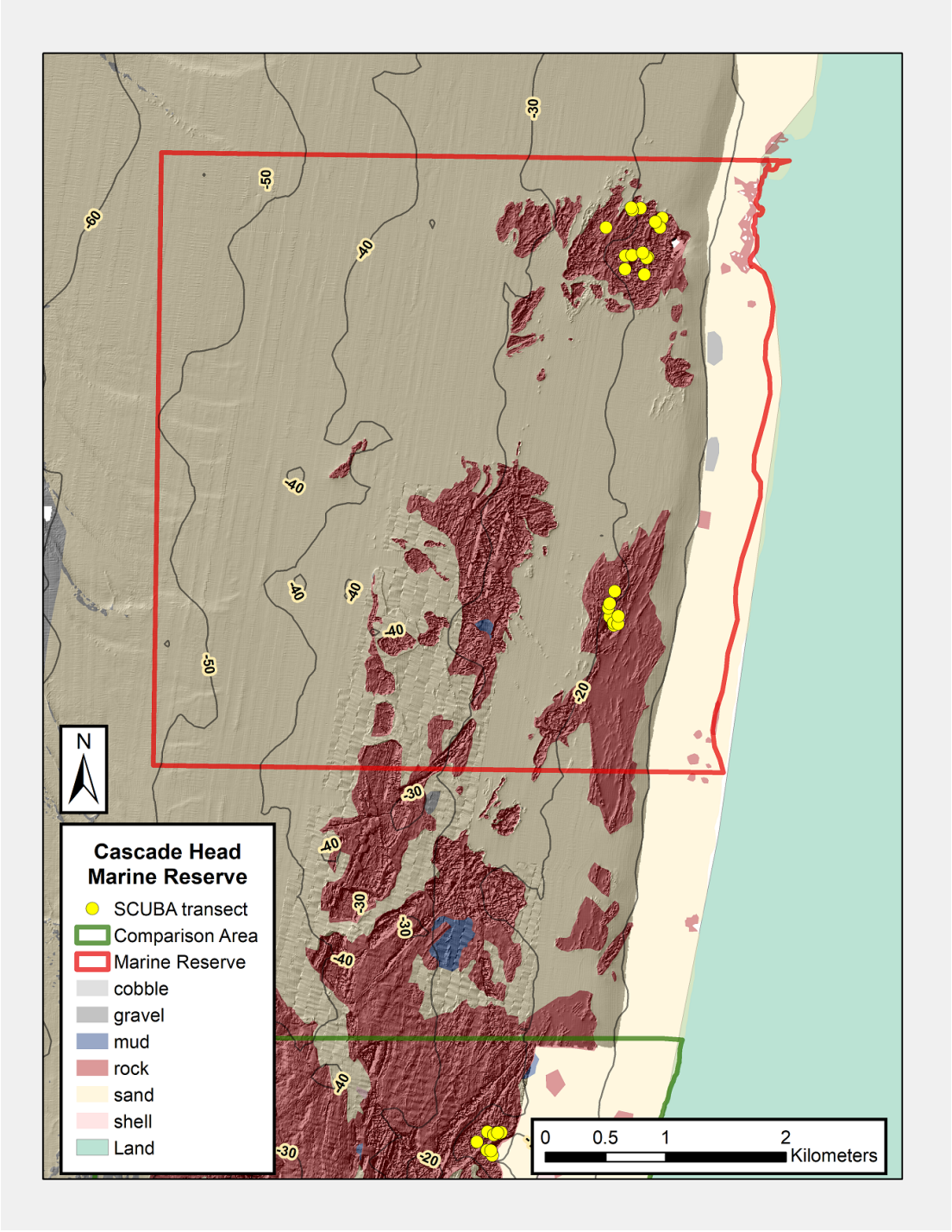


Fig. 3.11: Map of SCUBA transect locations at Cascade Head Marine Reserve.

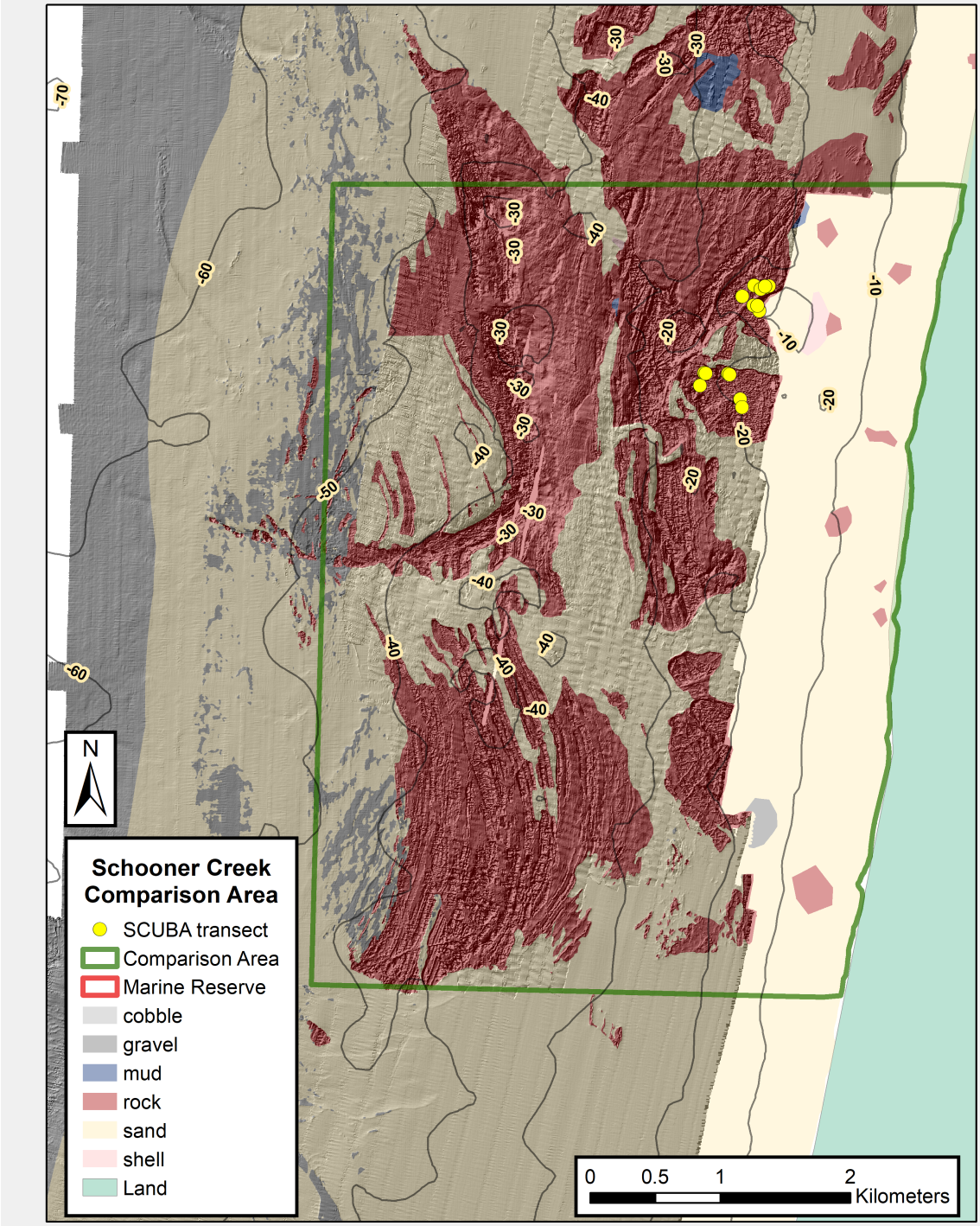


Fig. 3.12: Map of SCUBA transect locations at Schooner Creek Comparison Area.

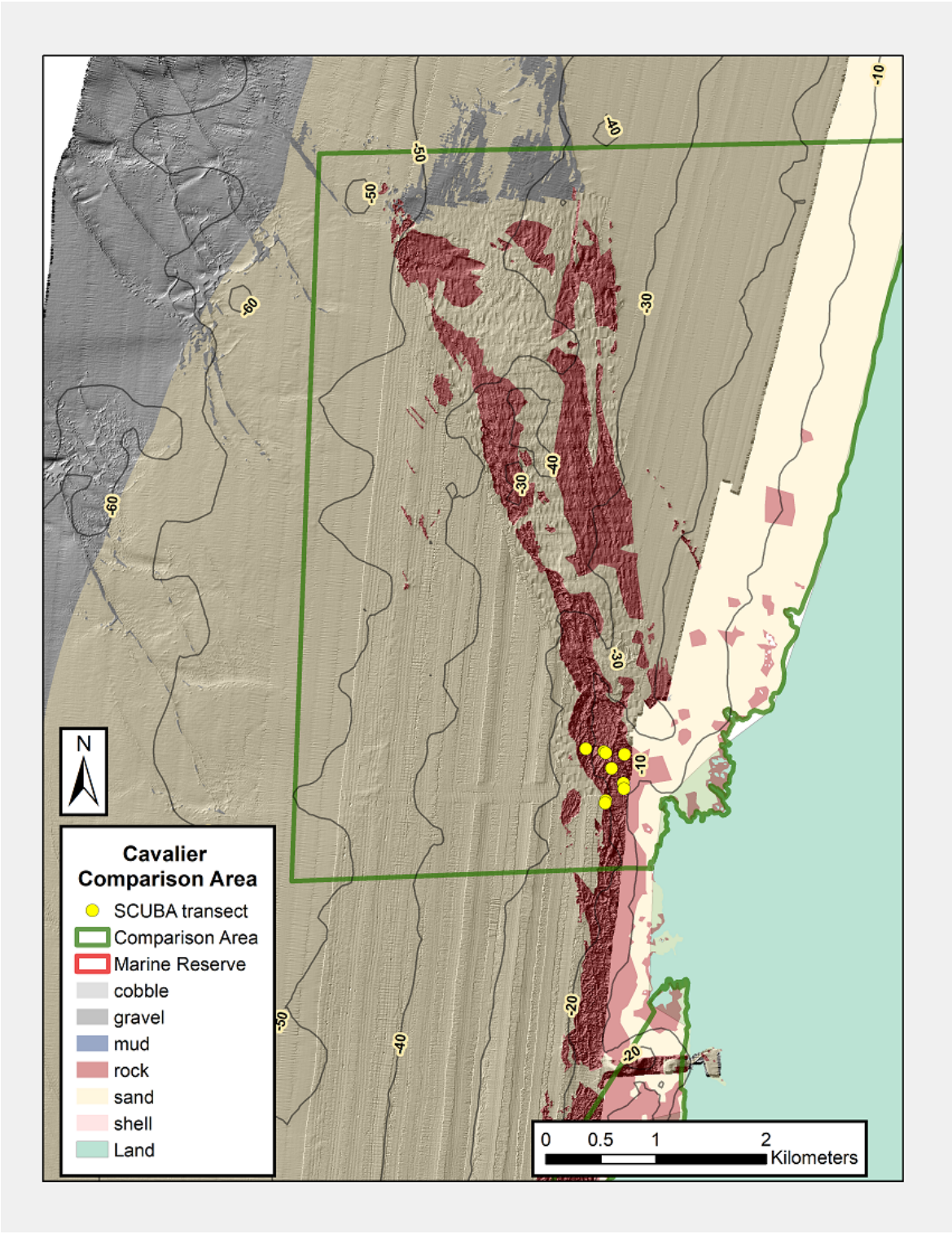


Fig. 3.13: Map of SCUBA transect locations at Cavalier Comparison Area.

Table 3.10: Total number of kelp transects conducted in Cascade Head Marine Reserve and Schooner Creek and Cavalier Comparison Areas between 2013 and 2018.

	Site		
Year	Cascade Head MR	Cavalier CA	Schooner Creek CA
2013	0	3	0
2014	1	0	2
2017	14	4	6
2018	27	13	18

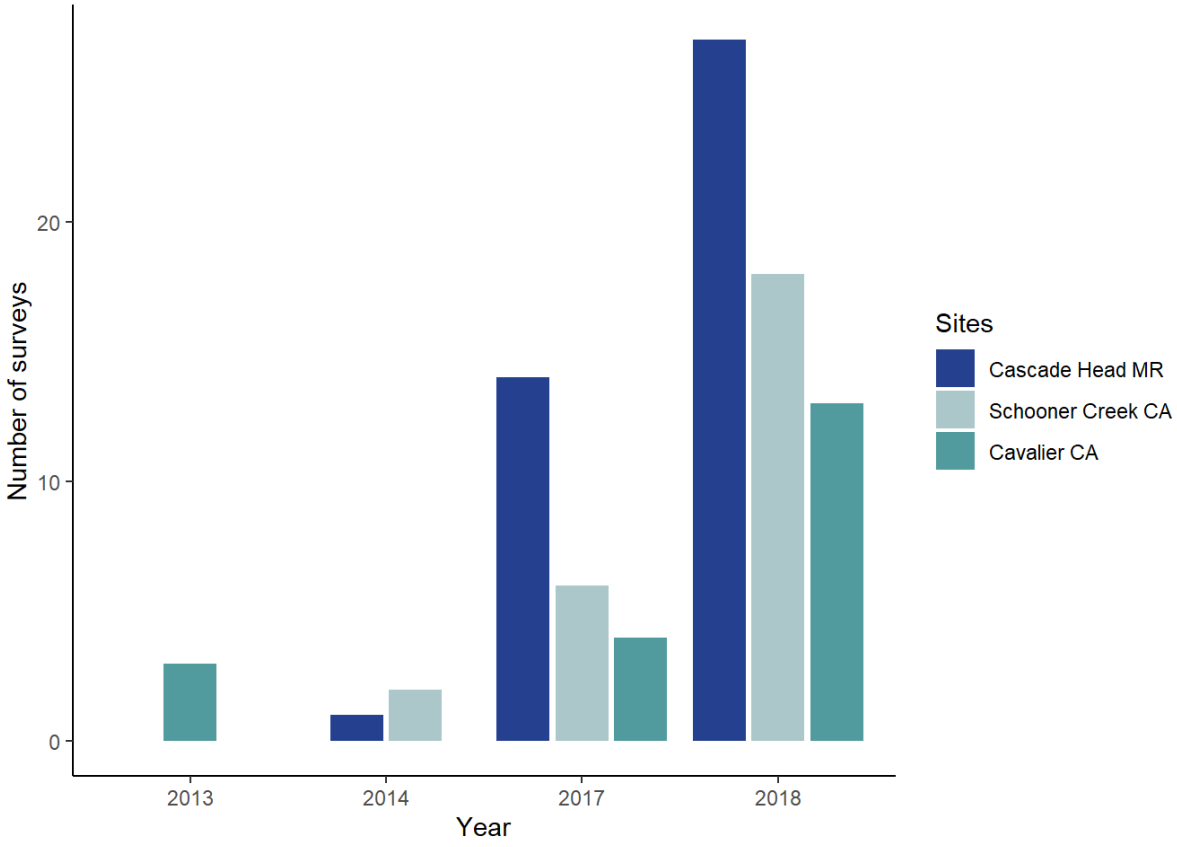


Fig. 3.14: Total SCUBA kelp swath surveys conducted by year in the Cascade Head Marine Reserve (dark blue), and comparison areas (lighter blue).

the PISCO and ORMR protocols in other marine reserves and comparison areas. Mean depths of ODFW transects differed significantly across years, but not across sites within years (Table

3.11). The 2013-2014 transects were 3.98 m shallower on average than the 2017-2018 transects.

Table 3.11: Mean depth of kelp surveys conducted at Cascade Head by the Marine Reserve Research Dive Team (ORMR) binned into 2013-2014 and 2017-2018 groups. Mean depth was different between years, but not among sites (ANOVA, df = 1, F = 14.010, ** p < 0.001).

	Site		
Year	Cascade Head MR	Cavalier CA	Schooner Creek CA
2013-2014**	12.8	10.06	13.26
2017-2018**	15.7	16.08	16.28

Across all years inside and outside the Cascade Head Marine Reserve the most common kelp detected were *Desmarestia* sp., *L. setchellii*, *N. luetkeana*, and *P. gardneri* (Figure 3.15). Both focal species (N luetkeana and *P. californica*) were only detected in 2017, and a gradual decrease in overall detection of all kelp can be seen between 2013 and 2018.

- Surveys were consistently shallower in 2013-2014 than in 2017-2018, though no clear pattern in kelp detection other than an overall decline in 2018 was apparent.
- The focal species *N. luetkeana* and *P. californica* were only detected in the region in 2017 within the marine reserve.
- The alga *Desmarestia* sp. was detected at the highest rate across all years and regions within the Cavalier Comparison Area at 71 individuals counted in 2013.

  Within the Cascade Head Marine Reserve the focal species *N. luetkeana* and *P. californica* were only detected in 2017, while the other algae *P. gardneri* and *L. setchellii*/*S. latissima* were only detected in small quantities in 2014/17 and 2017/18 respectively (Table 3.12; Figure 3.16). *N. luetkeana* had the highest detection count at 45, which was found on just two transects. No other kelp species were detected in any year.

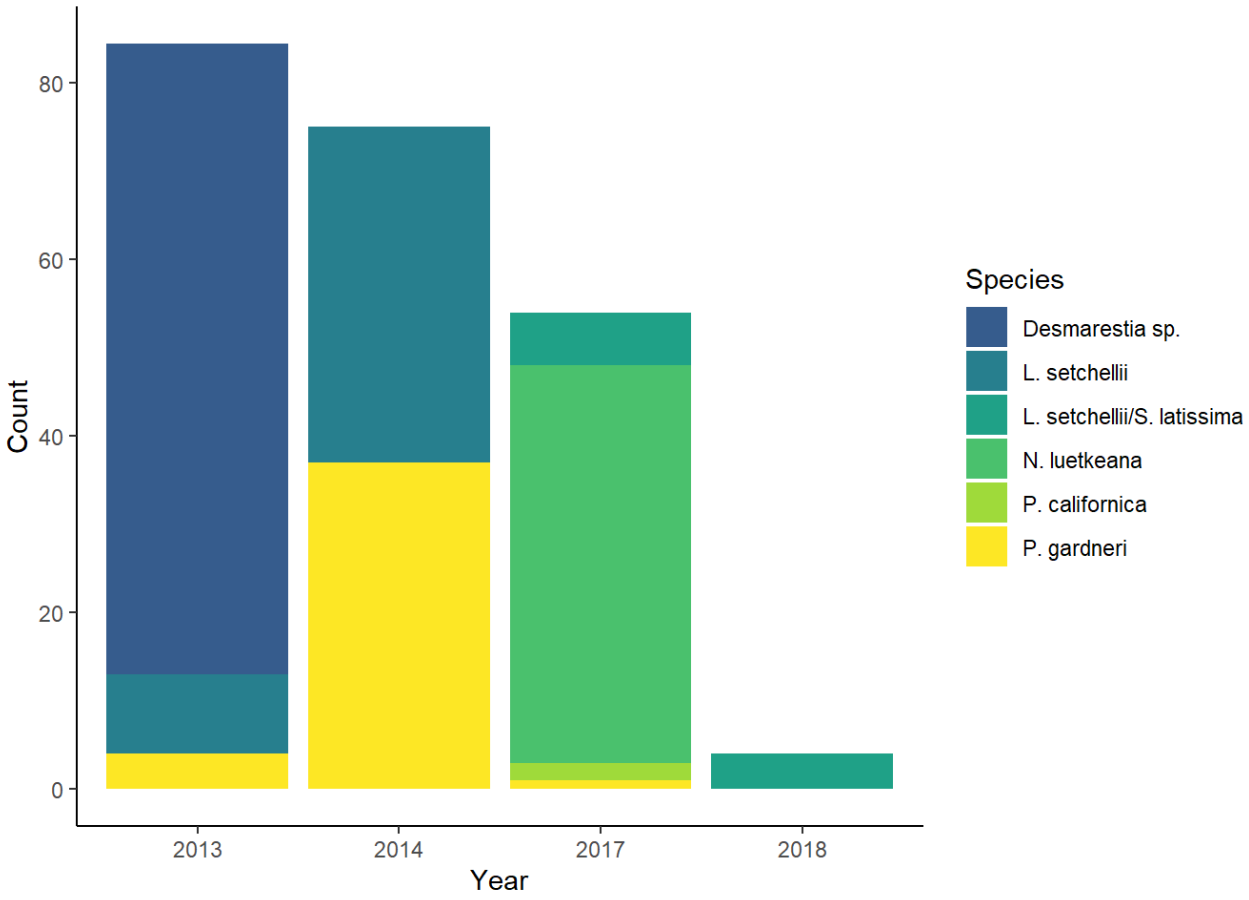


Fig. 3.15: Kelp species counts observed in the Cascade Head Reserve and comparison areas (combined) by year.

The only species detected at the Cavalier and Schooner Creek Comparison Areas were *P. gardneri*, *Desmarestia* sp., L. setchellii/*S. latissima* (Table 13, Figure 13). Detection rate were generally higher in 2013-14 with 150 total counts of kelp across all comparison areas, but only 2 detections in 2017-18.

Table 3.12: Abundance of focal species (*N. luetkeana*, *P. californica*) and other common kelp from SCUBA swath surveys within the Cascade Head Marine Reserve by year. Total counts are summed across 30 m transects.

Species	2010	2011	2017	2019
N. luetkeana	**305**	**1702**	**0**	**1**
P. californica	**283**	**846**	**92**	**14**
P. gardneri	3	217	7	1
Desmarestia sp.	0	0	3	0
C. costata	0	0	0	0
L. setchellii	404	354	0	0
A. marginata	9	53	1	0
L. setchellii/S. latissima	0	0	145	280

Table 3.13: Abundance of focal species (*N. luetkeana*, *P. californica*) and other common kelp from SCUBA swath surveys within the Cavalier and Schooner Creek Comparison Areas by year. Total counts are summed across 30 m transects.

	Year			
	2013	2014	2017	2018
Cavalier CA				
N. luetkeana	**0**	**NA**	**0**	**0**
P. californica	**0**	**NA**	**0**	**0**
P. gardneri	4	NA	0	0
Desmarestia sp.	71	NA	0	0
C. costata	0	NA	0	0
L. setchellii	9	NA	0	0
A. marginata	0	NA	0	0
L. setchellii/S. latissima	0	NA	0	0
Schooner Creek CA				
N. luetkeana	**NA**	**0**	**0**	**0**
P. californica	**NA**	**0**	**0**	**0**
P. gardneri	NA	28	0	0
Desmarestia sp.	NA	0	0	0
C. costata	NA	0	0	0
L. setchellii	NA	38	0	0
A. marginata	NA	0	0	0
L. setchellii/S. latissima	NA	0	0	2

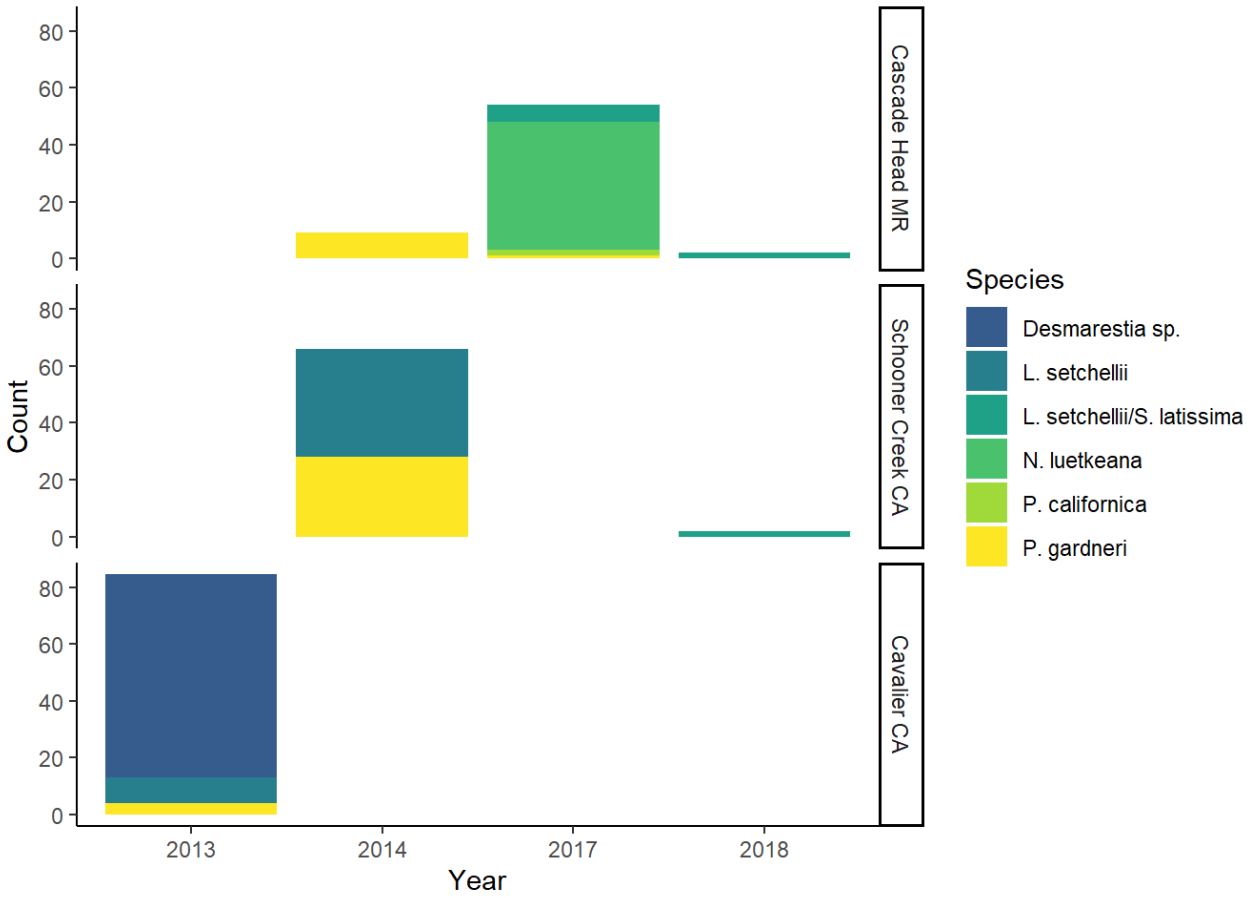


Figure 3.16: Kelp species counts observed in Cascade Head Marine Reserve and associated comparison areas from SCUBA kelp swath surveys by year. Total counts are summed across 30 m transects.

5.3.4 CAPE FALCON

A total of 28 transects were conducted at Cape Falcon over one year of sampling effort (2017; Table 14, Figure 15), despite multiple attempts over several years to reach the Marine Reserve and comparison areas. Mean depths of transects were not significantly different among the marine reserve and comparison area transects with an overall mean depth of 14.2 m (Table 15).

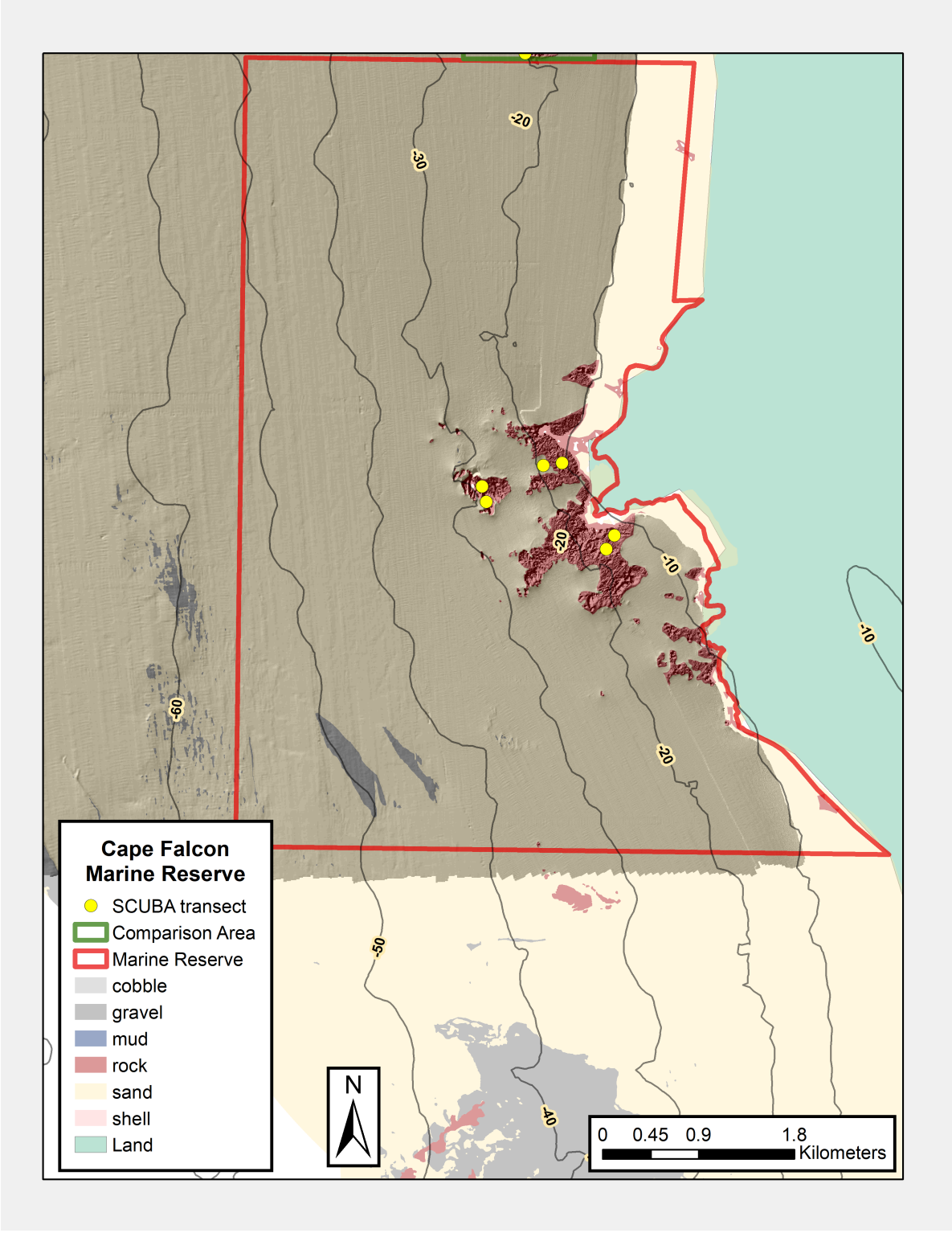


Fig. 3.17: Map of SCUBA transect locations at Cape Falcon Marine Reserve.

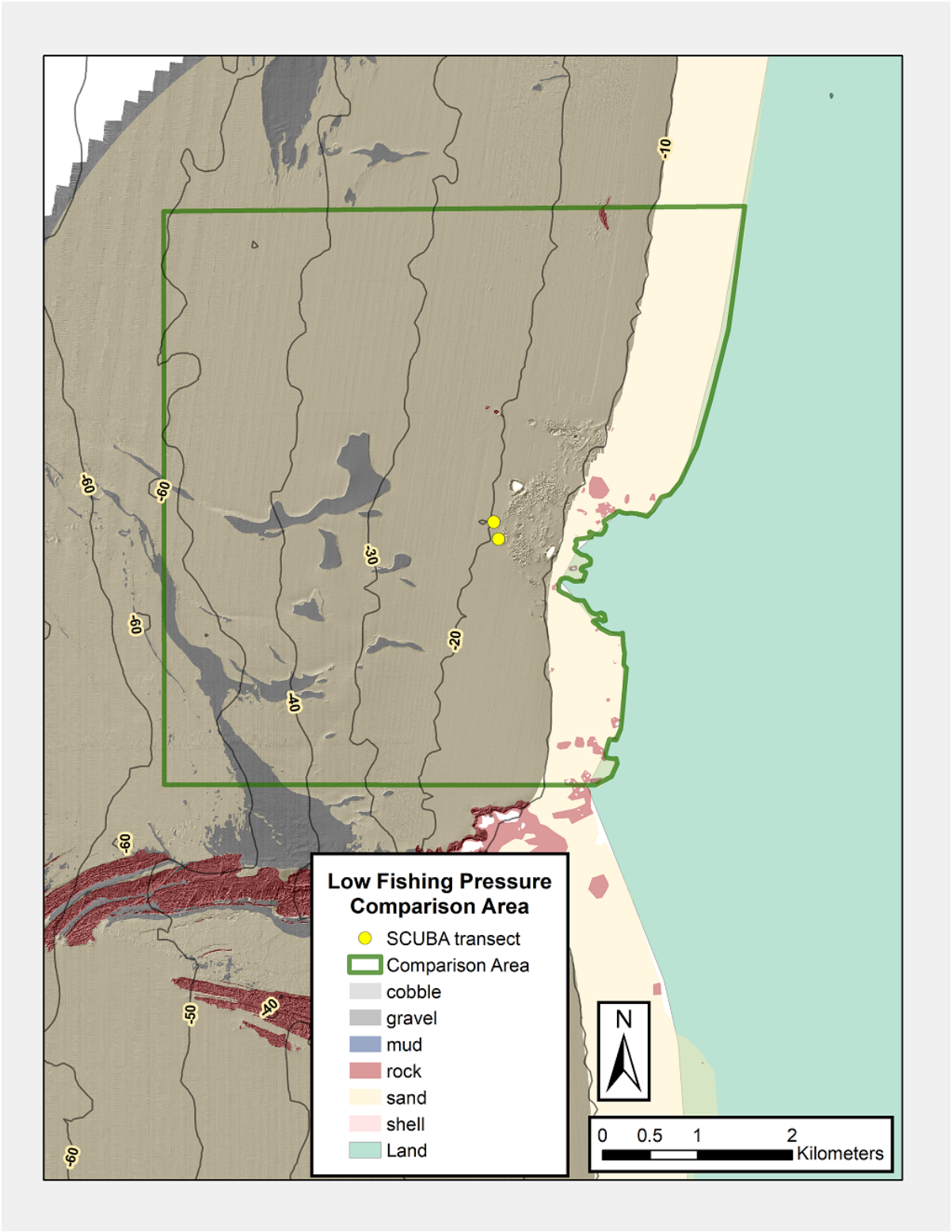


Fig. 3.18: Map of SCUBA transect locations at Low Fishing Pressure Comparison Area.

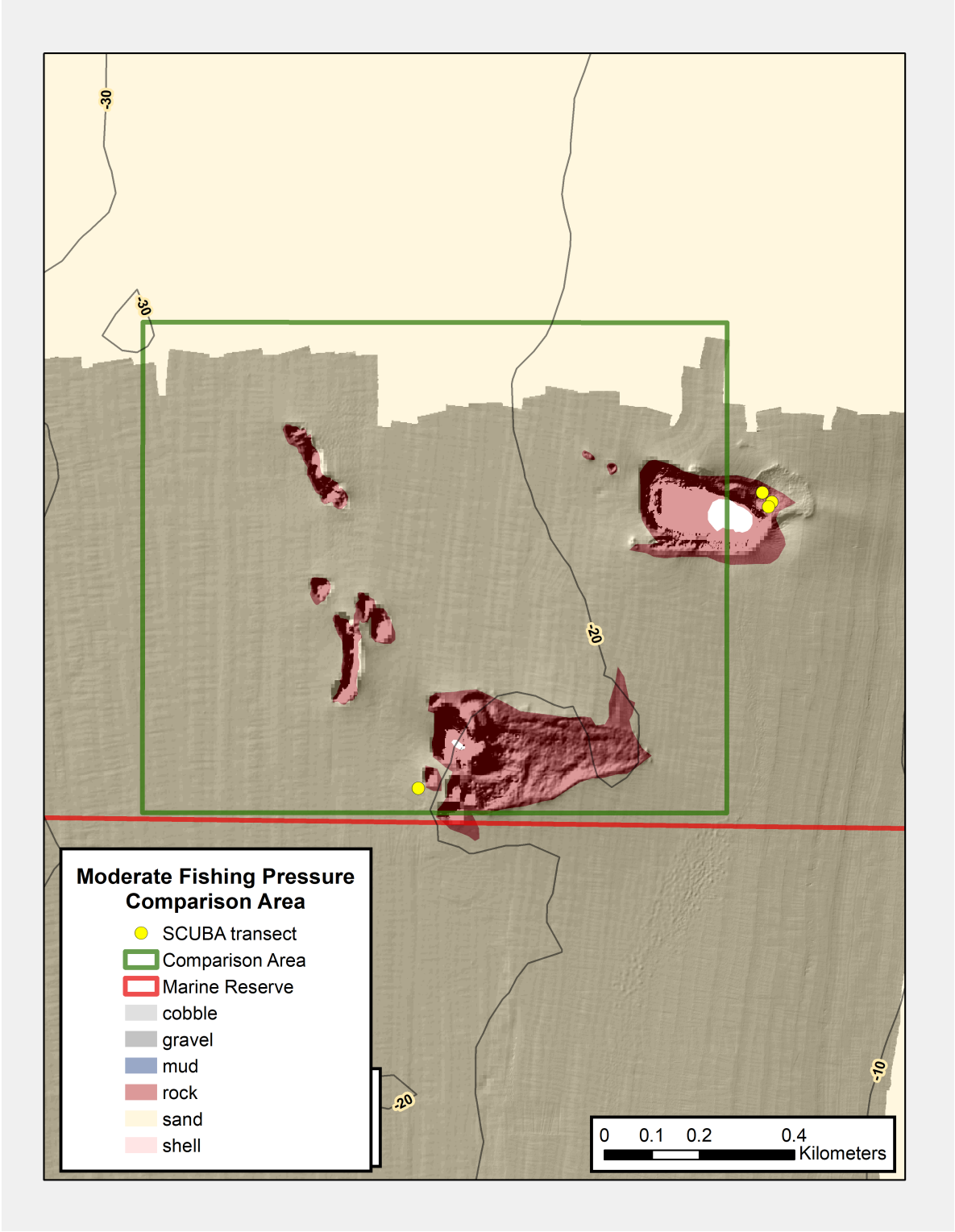


Fig. 3.19: Map of SCUBA transect locations at Moderate Fishing Pressure Comparison Area.

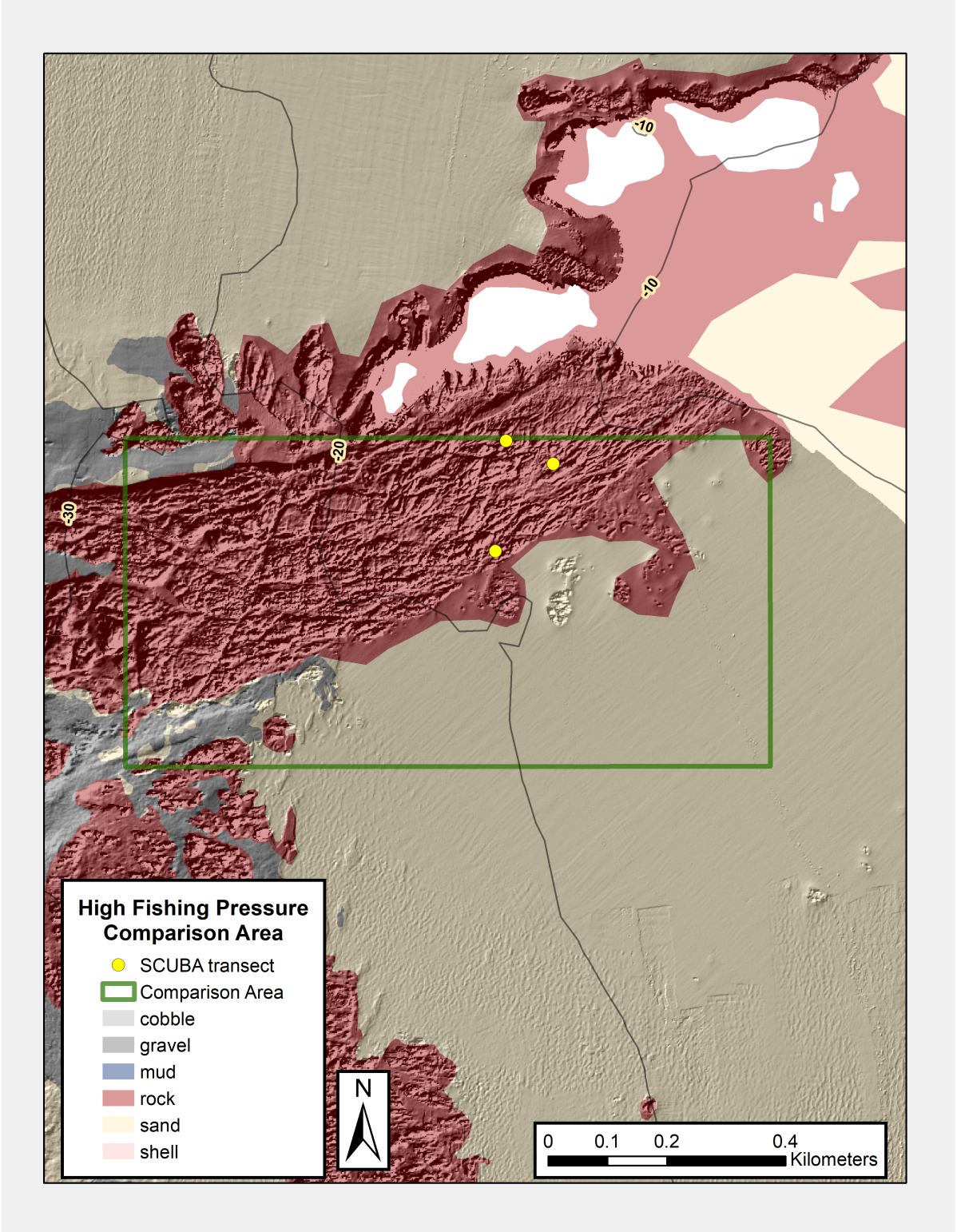


Fig. 3.20: Map of SCUBA transect locations at High Fishing Pressure Comparison Area.

Table 3.14: Total number of SCUBA kelp swath transects conducted in Cape Falcon Marine Reserve and Low Fishing Pressure and Moderate Fishing Pressure Comparison Areas since surveys began.

	Site		
Year	Cape Falcon MR	Low Fishing Pressure CA	Moderate Fishing Pressure CA
2017	12	4	12

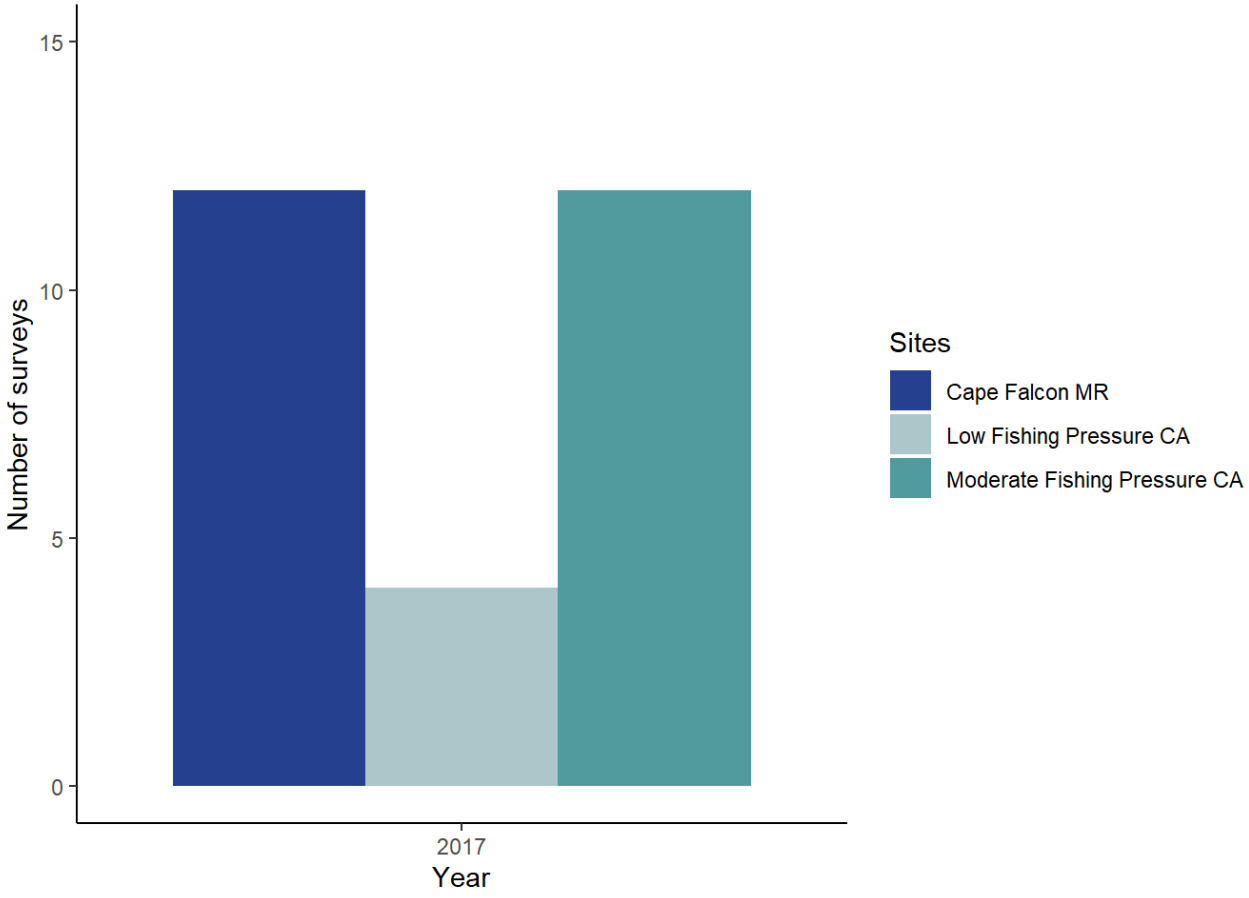


Fig. 3.21: Total SCUBA kelp swath surveys conducted by year in the Cape Falcon Marine Reserve (dark blue), and associated comparison areas (lighter blue).

Table 3.15: Mean depth of kelp surveys conducted at Cape Falcon by the Marine Reserve Research Dive Team (ORMR) separated into the Marine Reserve and associated comparison areas. Mean depth was not different among the marine reserve and comparison areas (post-hoc Tukey Test, $p > 0.05$).

	Site		
Year	Cape Falcon MR	Low Fishing Pressure CA	Moderate Fishing Pressure CA
2017	14.22	16.54	15.04

- Due to the logistical difficulties of accessing Cape Falcon by SCUBA, kelp swath surveys were only conducted in 2017 within the marine reserve and at associated comparison areas.
- Although 28 SCUBA kelp swath surveys were conducted in the region, no kelp was detected on any transect.

BRIDGE

This Chapter addressed the status of macroalgae abundance and diversity within and near a marine reserve system. In the next chapter, I focus on an important generalist predator in kelp forests that plays an important role in kelp forest health, the sunflower sea star *Pycnopodia helianthoides.*

CHAPTER IV

FEEDING PREFERENCES OF THE SUNFLOWER SEA STAR *PYCNOPODIA HELIANTHOIDES*

This study contains unpublished coauthored material that was conceived by me with conceptual input from Aaron Galloway. Field collections were made by myself and coauthor Kindall Murie. I was responsible for all experimental lab work with support from Kindall Murie. All statistical analyses were done by myself, and I wrote the manuscript with editorial input from all authors.

1. INTRODUCTION

Predator-prey interactions are a cornerstone of ecosystem function and can control a suite of processes including primary productivity (Estes & Duggins 1995), nutrient cycling (Schmitz et al. 2010), and patterns of biodiversity (Ellingsen et al. 2015). These interactions are modulated by the environment in which they occur (Draper & Weissburg 2019), as well as through the specific behaviors and physiological states of the species involved in the interaction (Schmitz 2017). The reciprocal responses of prey-to-predator and predator-to-prey can change outcomes of these interactions through various mechanisms including predator avoidance strategies (e.g., crypsis, escape; Heithaus et al. 2009, Spyksma et al. 2017) or prey preferences, including prey switching (Murdoch 1969). Prey switching is a common strategy predicted by optimum foraging theory when a predator pursues atypical prey because their preferred prey item is scarce or absent in the environment, or when more desirable prey become available (Prokopenko et al. 2023). Depending on the capacity of a particular predator to switch prey, prey switching may be easier than other strategies such as long-range foraging, or alteration of hunting methods to obtain rare or evasive prey (Vallina et al. 2014). In habitats in which scarcity of potential prey is

not a factor, other properties of available prey such as handling time, relative nutritional value, or ease of capture dictate the choices predators make to pursue the prey item (Pyke et al. 1977, Ostfeld 1982).

The choices that predators make can dictate environmental outcomes such as the formation of alternate stable states (Beisner et al. 2003) or changes in community diversity (Douglass et al. 2008), which are often influenced by the relative abundance of prey in the environment (Hughes & Croy 1993). Other factors including predator-prey size ratios (Kalinkat et al. 2011), spatial overlap (Kempf et al. 2008), and ontogeny (i.e., juvenile versus adult; Elliott 2006) can also play important roles in prey selection, and thus, the resulting ecological effects. In addition, geographically widely distributed predator species may display different diet preferences based upon local (i.e., 10's of km) environmental factors that may not be generalizable to their entire range (Byrne et al. 2019).

In kelp forests, which are highly productive habitats that are biodiverse and contain large quantities of biomass compared to many adjacent systems, predator-prey interactions play important roles in maintaining community structure (Dayton 1985, Sala & Graham 2002). Generalist predators in kelp forests have a wide range of prey items among which they can choose or potentially switch (Steneck et al. 2002), from small mobile fishes and invertebrates to encrusting organisms. Prey species identity can be very important when considering how predation can influence a community, as particular prey species play different roles in the ecosystem across spatial scales, from centimeters to kilometers. For example, prey species such as chitons grazing on encrusting species clear occupied space on small (cm) scales and allow the settlement of new larvae (Elahi & Sebens 2013). On larger (km) spatial scales, some grazers such as sea urchins can denude entire kelp forests (Hamilton & Caselle 2015). The consumption

of prey with different ecological functions can, therefore, result in community-wide effects that act across spatial scales, even resulting in trophic cascades, dependent upon what function their prey fulfills within the ecosystem (Ripple et al. 2016).

The sunflower seastar *Pycnopodia helianthoides* (hereafter *Pycnopodia*), is a widely distributed generalist consumer in North Pacific kelp forests and has been implicated as a primary predator of multiple taxa including bivalves and echinoderms (Mauzey et al. 1968, Duggins 1983). They have often been cited as a major predator of grazing sea urchins, including purple (*Strongylocentrotus purpuratus*, Bonaviri et al. 2017), green (*Strongylocentrotus droebachiensis*, Schultz et al. 2016), and red (*Mesocentrotus franciscanus*, Nishizaki & Ackerman 2007) urchins, all of which are, in turn, primary grazers of kelp. This *Pycnopodia*-urchin-kelp relationship is becoming more and more recognized as a potentially major driver of kelp forest health (Galloway et al. 2023), particularly since *Pycnopodia* populations were dramatically reduced, and in some places completely extirpated, by seastar wasting disease from 2012-2015 (Harvell et al. 2019, Hamilton et al. 2021). *Pycnopodia*'s decline was concurrent with an increase in grazing urchin populations, likely from release of predation pressure, and a subsequent decline in kelp forest cover in many areas across the West Coast of North America (Burt et al. 2018, Rogers-Bennett & Catton 2019). The resulting mosaic habitat of remaining kelp forest patches and urchin barrens is marked by an overall reduction of biomass in formerly-productive kelp communities (Rogers-Bennett & Catton 2019). Starved urchins within the barrens that are interspersed among the remaining kelp maintain this alternate stable state by continuously grazing on newly recruiting kelp, inhibiting the growth and development of kelp and other macroalgae (Spindel et al. 2021). The biodiversity within urchin barrens also is lower than kelp forests, which further decreases ecosystem functioning in barrens (Gabara et al. 2021).

Therefore, grazing control of urchins through generalist predators such as *Pycnopodia* could be critical in maintaining important ecosystem functions.

Direct consumptive effects of *Pycnopodia* on the urchins in Pacific Northwest kelp forests are an important pathway for grazer control (Galloway et al. 2023). However, this grazer control depends directly on *Pycnopodia* prey selection. There are relatively few studies that describe the 'wild diet' of *Pycnopodia* or explicitly test their preference of particular prey types *in situ,* and the majority of field studies quantifying the effect of *Pycnopodia* on urchins is largely correlative in nature (e.g., Schultz et al. 2016, Bonaviri et al. 2017, Burt et al. 2018, Rogers-Bennett & Catton 2019). Qualitative observations of *Pycnopodia* feeding in the wild have indicated that they actively prey on urchins, bivalves, and gastropods, while also scavenging on fish, other seastars, seabirds, octopus, large crabs (e.g., *Cancer productus*, Galloway personal observation) and seals (*Phoca vitulina*, Whippo personal observation). This wide range of diets has resulted in *Pycnopodia* most frequently being identified as 'generalist predators' (Duggins 1983) and 'facultative scavengers' (Brewer & Konar 2005). Of course, these are potentially complimentary terms, and it is likely that *Pycnopodia* plays different trophic roles depending upon the depth at which they are found (i.e., intertidal versus subtidal), geography (i.e., local prey spectrum), and season. It is also likely that *Pycnopodia,* like other sea star species, can specialize in particular prey types depending upon various physiological and ecological drivers including ontogeny (Manzur et al. 2010), prey density (McClintock & Lawrence 1985), and intra- and interspecific competition (Gaymer et al. 2002, Storero et al. 2020). The latter is of particular interest as competitor identity is often linked to habitat type (e.g., predators that specialize on hard-bottom versus soft-bottom prey). *Pycnopodia* are known

to regularly cross habitat-type boundaries, particularly between hard- and soft-bottom substrates (Mauzey et al. 1968, Shivji et al. 1983, Sloan & Robinson 1983). However, the influence of ecological drivers such as competition and habitat type on *Pycnopodia* feeding preference and how their preferences may change as their habitats are altered (e.g., kelp forest to urchin barren) and the suite of competitors shift (e.g., repatriation of sea otters) is unknown.

To address some of these uncertainties, particularly related to the ecology of kelp forests, the feeding rates and relative preference of *Pycnopodia* on sea urchin species and other common benthic prey species from rocky nearshore habitats needs to be determined. This is especially true as the roles that *Pycnopodia*'s potential prey play in kelp forests vary, and *Pycnopodia* prey preferences may have dramatic, cascading consequences for the kelp forests they inhabit. Therefore, our goal for this study was to 1) review our state-of-knowledge about the *Pycnopodia* 'wild-diet' based on previously published research; 2) use a meta-analysis approach to detect quantitative generalities about *Pycnopodia* diets across part of their geographic range; and 3) directly determine *Pycnopodia* prey choice preferences and feeding rate in a cafeteria-style experiment using common potential prey items.

2. METHODS

2.1 Meta-analysis

Published records on *Pycnopodia* feeding, prey spectrum and prey preference were searched on Google Scholar and Web of Science using the keyword '*Pycnopodia helianthoides'*. Published peer-reviewed and non-peer reviewed (thesis) papers were selected based on the following criteria: the study described stomach contents of *Pycnopodia* observed or caught in the

field; stomach contents were quantified in some way (count, percent); at least phylum-level taxonomy was used to identify the consumed organisms; and the data were presented in a systematic way (i.e., a table, list, figure, etc.). Data from each study were extracted by hand and collated, either through extraction of raw values presented in the text, or estimation of values through visual analysis of figures using open-source imaging software (The GIMP Development Team, 2019). These data on *Pycnopodia* prey were then separated by geographic area and location of observation (subtidal, intertidal), then summarized and visualized using R and the tidyverse, viridis, maps, scatterpie, and ggpubr packages (Wickham et al. 2019, Garnier et al. 2021, Becker et al. 2022, R Core Team 2022, Yu 2022, Kassambara 2023). Prey categories were combined into broad phylogenetic groupings that would encompass most of the study results and allow comparative interpretation. Diet across studies was further analyzed for major prey contributors to diet and trends in observations across depths (see *statistical analyses* below).

2.2 Cafeteria experiment

Pycnopodia for the prey choice experiment were collected from the subtidal benthos at sites in the San Juan Archipelago through a permitting arrangement between the Friday Harbor Labs (FHL) and Washington State (Washington State House Bill 68, R.C.W.28.77.230, 1969 Revision R.C.W.28B.20.320) between June 26, 2022 and August 17, 2022. The collected *Pycnopodia* were held in flow-through sea water tanks (60 cm x 90 cm x 30 cm, WHD) prior to experimentation. As collections were opportunistic in nature due to the scarcity of *Pycnopodia*, holding times ranged between 2-43 days before experiments were run. During the holding time, *Pycnopodia* were fed a daily maintenance diet of mussels and were handled according to protocols described in Hodin et al. (2021). All *Pycnopodia* were returned to their point of origin

after the experiment was complete to minimize the impact that extraction could have on their local populations.

For the experiment, *Pycnopodia* were placed in outdoor flow-through sea tables (circular: 50 cm depth x 104 cm diameter) covered in black plastic sheeting to reduce light, and covered with an opaque lid. Temperature and salinity were monitored throughout each experimental trial. As tank numbers were limited, no more than three *Pycnopodia* could be tested at any one time, each in its own separate tank. No *Pycnopodia* was tested more than once for prey preferences, and all tanks were drained, scrubbed, rinsed with fresh water, and left to dry between uses. A total of 11 *Pycnopodia* with a mean diameter of 48.8 ± 4.7 cm were used in feeding trials. We note that larger sample sizes of individual stars might have been preferable but were not possible due to the endangered status and rarity of these animals in the wild (Gravem et al. 2021).

Putative prey species were chosen to include three common shallow subtidal urchin species found around the San Juan Islands (*Strongylocentrotus purpuratus, S. droebachiensis, Mesocentrotus franciscanus*), an additional non-urchin echinoderm (the sea cucumber *Apostichopus californianus*), and two molluscs including mussels (*Mytilus* spp.) and a chiton (*Cryptochiton stelleri*). Prey items were collected from the FHL surroundings throughout the experiments to maintain sufficient supply of each taxon. For each experimental run, two individuals of each prey species were measured for body size and placed in the tank with one *Pycnopodia* to allow direct interaction. Prey consumption was checked and recorded at least three times per day over a ten-day experimental period, as well as opportunistically throughout the experiment. All prey items were replenished as soon as an ‘envelopment’ event (defined below) occurred to ensure at least two of each prey species were available in the tank at all times. Envelopment was defined as the holding or covering of the prey item by the *Pycnopodia* so that

the prey item was no longer visible within the tank. Egestion was defined as the expulsion of non-digestible materials (tests, shells), or thick mucus (sea cucumber) by the *Pycnopodia* after a feeding event. Envelopment or egestion that occurred overnight was set as occurring at midnight of the current observation day. Total number, identity, and size of prey items consumed were calculated over each experimental period for each *Pycnopodia* trial, with egestion time set as the completion of a consumption event. *Pycnopodia* prey items were removed after ten days, but *Pycnopodia* were held and observed into the eleventh day to ensure that all egestion events were recorded.

2.3 Statistical analyses

The prey taxa documented across all meta-analysis studies were reduced to eight categories for analysis (Table 4.1). We developed a relative measure of importance for each of those categories across all studies, calculated separately for subtidal and intertidal observations as a 'weighted contribution factor' (WCF):

$$\mathrm{WCF} = \frac{p_i \cdot \log_{10}\left[N_j\right]}{\left(p_i \cdot \log_{10}\left[N_j\right]\right)_{max}}$$

Where p_i = percent of each of the observed prey items in the diet for study *i* (separated into intertidal/subtidal, if applicable), and N_j = number of *Pycnopodia* used to calculate those observations in study *i* (also separated into intertidal/subtidal, if applicable). The values were then constrained between 0 and 1 by dividing by the maximum value in the entire dataset for

ease of interpretation and to reduce the out-sized effect of outliers. Differences among WCFs were tested across all data, and separately for the intertidal and subtidal data with a Kruskall-Wallis test, a non-parametric analysis that is robust to non-normal distributions and heterogeneous variance in JMP v17.0.0 (SAS Institute Inc., Cary, NC, USA). Post-hoc Wilcoxon comparisons were made to determine food items that were driving differences in the observed diets.

To determine feeding preferences of *Pycnopodia* in cafeteria experiments we used Rodgers' preference index (Rodgers 1990), which accounts for order, rate, and total amount of each food item consumed, and is derived as the standardized area under the curve (AUC) of cumulative proportion eaten through time. This metric is considered appropriate for cafeteria-style experiments and provides a value to indicate preferences from 0 (avoided) to 1 (preferred; Gasperini et al. 2018). Differences in preference between prey types and variation in AUC were tested with a Kruskall-Wallis test and Dunn's post-hoc test, respectively. An additional analysis of the effect of urchin size on feeding choices was conducted using a linear mixed effects model (lme).

To further compare prey choices among individual *Pycnopodia*, the Shannon Diversity Index and Pielou's Evenness were measured for each *Pycnopodia* prey consumption. These analyses provide a single value for the diet of each *Pycnopodia*, measuring the diversity of prey items in the diet (Shannon diversity), and the distribution of prey consumed (evenness) (Cramer et al. 2020). These measures provide additional information as they take into account items that were offered, but not consumed, and evenness provides the additional power of the null expectation that all prey were consumed equally (diversity = 1.61, evenness = 1).

The *Pycnopodia* used in the cafeteria experiment were collected from five distinct areas, ranging from high-flow kelp forests with hard-bottom substrate to low-flow pilings with soft-bottom substrate. To determine if the location from which the *Pycnopodia* were collected had any meaningful effect on patterns of prey consumption, all *Pycnopodia* consumption patterns were tested against each other with PERMANOVA using location as a factor (Oksanen et al. 2018).

Table 4.1: List of all prey items identified within each study and the corresponding category it was assigned to for analysis in this meta-analysis. Species name were not updated with current taxonomy due to uncertainty around acutal identity of species, however, categories were broad enough to account for any species-level changes.

Prey Item	Assigned Category	Source
Acmaea spp.	Gastropods	Mauzey et al. 1968, Paul & Feder 1975
Alia carinata	Gastropods	Herrlinger 1983
Alpheus spp.	Crustaceans	Herrlinger 1983
Alvinia spp.	Gastropods	Shivji et al. 1983
Amphipoda	Crustaceans	Paul & Feder 1975
Amphissa sp.	Gastropods	Shivji et al. 1983
Amphissa versicolor	Gastropods	Herrlinger 1983
Anisodoris nobilis	Gastropods	Herrlinger 1983
Autolytus sp.	Other	Herrlinger 1983
Balanus spp.	Barnacles	Duggins 1983, Shivji et al. 1983, Paul & Feder 1975
Balcis sp.	Gastropods	Herrlinger 1983
Balcis thersites	Gastropods	Herrlinger 1983
Barleeia acuta	Gastropods	Herrlinger 1983
Barnacles	Barnacles	Sloan & Robinson 1983
Bittium sp.	Gastropods	Herrlinger 1983
Bivalves	Bivalves	Duggins 1983, Sloan & Robinson 1983
Bryozoans	Other	Duggins 1983
Calliostoma canaliculatum	Gastropods	Herrlinger 1983
Calliostoma ligatum	Gastropods	Herrlinger 1983, Shivji et al. 1983
Calliostoma supragranosum	Gastropods	Herrlinger 1983
Cancer branneri	Crustaceans	Herrlinger 1983
Cancer oregonensis?	Crustaceans	Paul & Feder 1975
Cancer productus	Crustaceans	Herrlinger 1983
Cancer sp.	Crustaceans	Herrlinger 1983
Cardium sp.	Bivalves	Paul & Feder 1975
Cheilostome colonies	Other	Herrlinger 1983
Chinocardium nuttallii	Bivalves	Paul & Feder 1975
Chinoecetes sp.	Crustaceans	Paul & Feder 1975
Chitons	Other Molluscs	Duggins 1983
Collisella ochracea	Gastropods	Herrlinger 1983
Collisella sp.	Gastropods	Herrlinger 1983
Conus californicus	Gastropods	Herrlinger 1983
Crabs	Crustaceans	Duggins 1983, Mauzey et al. 1968
Cucumaria lubrica	Other Echinoderms	Mauzey et al. 1968
Cymadusa uncinata	Crustaceans	Herrlinger 1983
Dermasterias imbricata	Other Echinoderms	Paul & Feder 1975
Diplodonta orbella	Bivalves	Shivji et al. 1983
Echiuroidea	Other	Paul & Feder 1975
Eptatretus stoutii egg case	Other	Herrlinger 1983
Eupentacta sp.	Other Echinoderms	Mauzey et al. 1968
Evasterias troschelii	Other Echinoderms	Paul & Feder 1975
Fusitriton oregonensis	Gastropods	Mauzey et al. 1968
Gastropods	Gastropods	Duggins 1983
Heart Urchin	Other Echinoderms	Paul & Feder 1975
Hemigrapsus oregonenesis	Crustaceans	Paul & Feder 1975
Hermit crabs	Crustaceans	Herrlinger 1983
Hiatella arctica	Bivalves	Paul & Feder 1975
Hinnites giganteus	Bivalves	Herrlinger 1983
Holothurians	Other Echinoderms	Duggins 1983
Humilaria kennerleyi	Bivalves	Mauzey et al. 1968
Hyas sp.	Crustaceans	Paul & Feder 1975
Hydroids	Other	Duggins 1983
Lacuna unifasciata	Gastropods	Herrlinger 1983
Leucosolenia eleanor	Other	Herrlinger 1983
Liparis mucosus	Other	Herrlinger 1983

Table 4.1 (cont.)

Macoma sp.	Bivalves	Paul & Feder 1975
Macoma spp.	Bivalves	Shivji et al. 1983
Megatebennus bimaculatus	Gastropods	Herrlinger 1983
Mitrella sp.	Gastropods	Shivji et al. 1983
Mitrella tuberosa	Gastropods	Herrlinger 1983
Modiolus carpenteri	Bivalves	Herrlinger 1983
Mya arenaria	Bivalves	Paul & Feder 1975
Mytilus californianus	Bivalves	Mauzey et al. 1968
Mytilus edulis	Bivalves	Duggins 1983, Paul& Feder 1975, Shivji et al. 1983
Nassarius mendicus	Gastropods	Herrlinger 1983
Natica sp.	Gastropods	Paul & Feder 1975
Nucula sp.	Bivalves	Paul & Feder 1975
Nuculana pernula	Bivalves	Paul & Feder 1975
Ocenebra minor	Gastropods	Herrlinger 1983
Ophiuroid pieces	Other Echinoderms	Herrlinger 1983
Ophiuroids	Other Echinoderms	Duggins 1983
Other	Other	Duggins 1983
Other Bivalves	Bivalves	Shivji et al. 1983
'Unlisted Crustaceans'	Crustaceans	Shivji et al. 1983
'Unlisted Gastropods'	Gastropods	Shivji et al. 1983
Pachycheles pubescens	Crustaceans	Herrlinger 1983
Pagurus spp.	Crustaceans	Paul & Feder 1975
Paracerceis cordata	Crustaceans	Herrlinger 1983
Parastichopus californicus	Other Echinoderms	Mauzey et al. 1968
Pectinaria sp.	Other	Paul & Feder 1975
Phascolosoma agassizii	Other	Herrlinger 1983
Phragmatopoma californica	Other	Herrlinger 1983
Pisaster ochraceus	Other Echinoderms	Mauzey et al. 1968
Pododesmus macroshisma	Bivalves	Mauzey et al. 1968
Protothaca staminea	Bivalves	Mauzey et al. 1968, Paul & Feder 1975, Shivji et al. 1983
Pugettia richii	Crustaceans	Herrlinger 1983
Pycnopodia Arms	Other Echinoderms	Paul & Feder 1975
Pycnopodia helianthoides	Other Echinoderms	Mauzey et al. 1968
Salps	Other	Duggins 1983
Saxidomus gigantea	Bivalves	Paul & Feder 1975
Saxidomus giganteus	Bivalves	Mauzey et al. 1968
Scale worm	Other	Herrlinger 1983
Seila montereyensis	Gastropods	Herrlinger 1983
Small Gastropods	Gastropods	Paul & Feder 1975
Spisula planulata	Bivalves	Herrlinger 1983
Strongylocentrotus droebachiensis	Urchins	Mauzey et al. 1968
Strongylocentrotus franciscanus	Urchins	Mauzey et al. 1968
Strongylocentrotus purpuratus	Urchins	Herrlinger 1983, Mauzey et al. 1968, Shivji et al. 1983
Strongylocentrotus sp.	Urchins	Paul & Feder 1975
Tegula brunnea	Gastropods	Herrlinger 1983
Tegula pulligo	Gastropods	Herrlinger 1983, Shivji et al. 1983
Tricolia pulloides	Gastropods	Herrlinger 1983
Triphora pedroana	Gastropods	Herrlinger 1983
Unidentified	Other	Herrlinger 1983
Unidentified Amphipods	Crustaceans	Shivji et al. 1983
Unidentified Chiton Plates	Other Molluscs	Paul & Feder 1975
Unidentified Crab Megalops	Crustaceans	Herrlinger 1983
Unidentified Crabs	Crustaceans	Shivji et al. 1983
Unidentified Dorid Nudibranch	Gastropods	Herrlinger 1983
Unidentified Holothuroid	Other Echinoderms	Herrlinger 1983
Unidentified Octopus Beaks	Other Molluscs	Paul & Feder 1975
Unidentified Ophiuroid	Other Echinoderms	Paul & Feder 1975
Unidentified sp.	Other	Paul & Feder 1975
Urchins	Urchins	Duggins 1983

3. RESULTS

3.1 Meta-analysis

Only six published datasets were identified that met the criteria for the literature search (Table 4.2). These studies spanned 24° of latitude along the West Coast of North America from Prince William Sound, Alaska in the north, to Pacific Grove, California in the south. The studies were all done within a 15-year period from 1968-1983, and sample sizes (number of *Pycnopodia* investigated*)* ranged widely from 7-425 individuals (Figure 4.1A).

A total of 114 prey taxa were reported across all studies (Table 4.1). Across all regions, bivalves and urchins tended to be the primary observed prey items, in both the intertidal (bivalve: WCF = 0.46 ± 0.50; urchin: WCF = 0.35 ± 0.40) and subtidal (bivalve: WCF = 0.24 ± 0.25; urchin: WCF = 0.21 ± 0.27). Gastropods also were a common although rarely dominant prey item. There was, however, much variation among studies (Figure 4.1B). Differences among all prey types were statistically significant only when considering WCF across all locations and depths (Kruskall-Wallis, Chi-square(1, 7) = 19.08, $p = 0.008$), with bivalves and gastropods found to be statistically more abundant than other prey items (Post-hoc Wilcox, $p < 0.05$). There were no obvious strong latitudinal trends in patterns of consumption, although the majority of bivalve consumption tended to occur in the more northern region and very little urchin consumption was observed at the southern most site. The dataset was also marked by large spatial gaps between most sites.

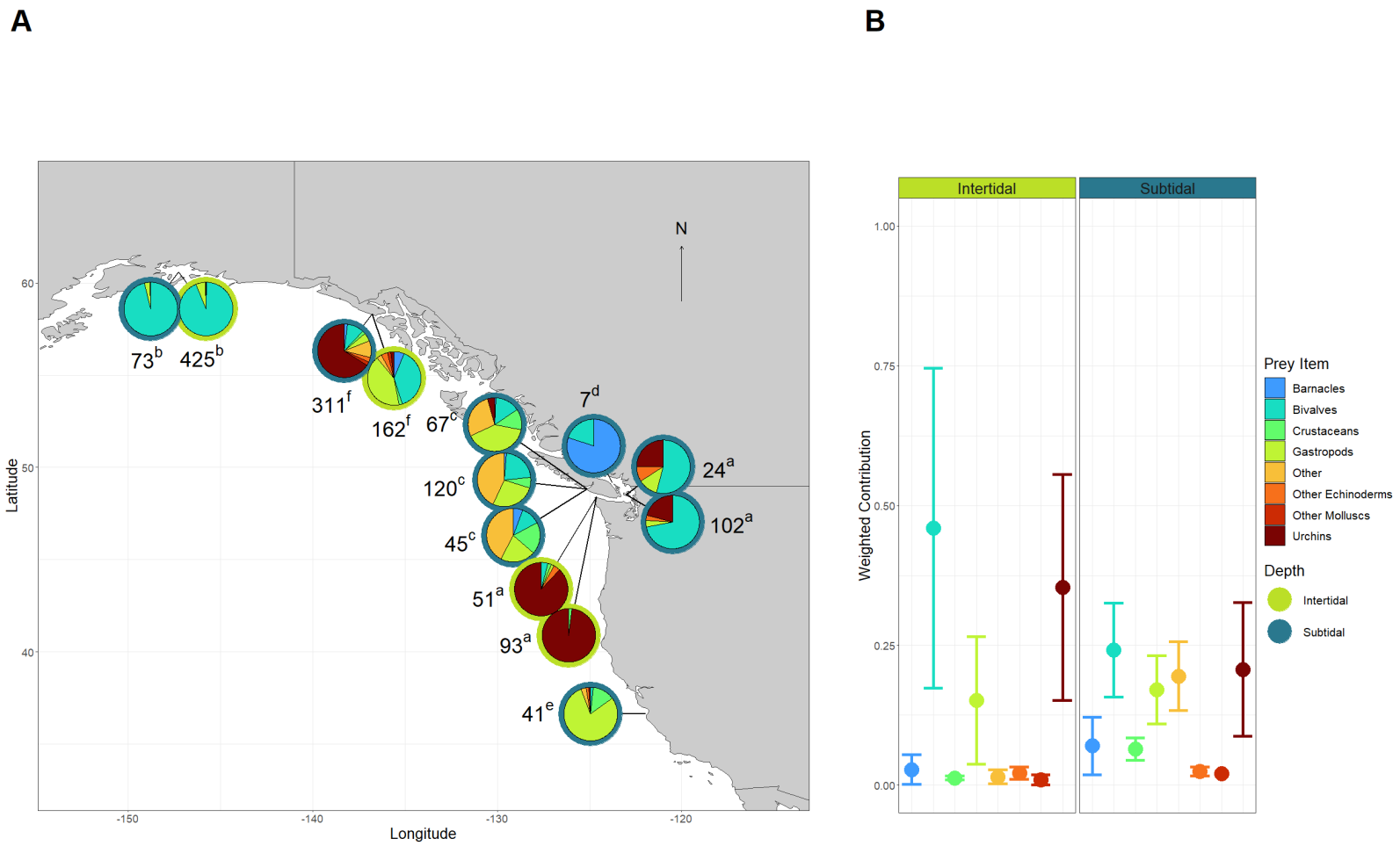


Figure 4.1: Summary of *Pycnopodia* wild-diet studies from the literature (A) and relative prevalence of diet contributions across studies (B). Includes six studies published between 1968 and 1983. Prey types are grouped into relevant categories and identified by location of data collection (intertidal – green circle/header, subtidal – teal circle/header). N of *Pycnopodia* used are listed as numbers next to each pie, superscript letter denotes study of origin (a = Mauzey et al. 1968, b = Duggins 1983, c = Shivji et al. 1983, d = Paul & Feder 1975, e = Herrlinger 1983, f = Sloan & Robinson 1983). Total diet is summarized as mean percentage across all *Pycnopodia* observed from each sampling unit within each study. Weighted contribution of prey items to diet across studies is shown as mean and standard error (B).

Table 4.2: Publications used in meta-analysis of *Pycnopodia* wild-diet. N is derived as number of *Pycnopodia* observed feeding within each study area. As some studies made observations across the seascape, there may be multiple N within each. Unit is the type of measurement extracted from the study (percent of gut contents, count of individual items in gut contents).

Reference	Region	Locale	Latitude	Longitude	N (Intertidal)	N (Subtidal)	Unit
Duggins 1983	Alaska	Torch Bay	58.3257	-136.7918	162	311	percent
Herrlinger 1983	Central California	Pacific Grove	36.6254	-121.9417	-	41	percent
Shivji et al 1983	British Columbia	Bamfield	48.8262	-125.1359	-	67 (exposed)	percent
					-	120 (intermediate)	percent
					-	45 (protected)	percent
					-	232	percent
Sloan & Robinson 1983	British Columbia	Gabriola Island	49.1649	-123.7820	-	7	percent
Paul & Feder 1975	Alaska	Prince William Sound	60.5937	-147.2879	425	73	count
Mauzey et al 1968	Washington	San Juan Islands	48.5380	-123.0003	-	24 (Vadas)	percent
					-	102 (Mauzey)	percent
		Outer Coast	48.3953	-124.6244	51 (Paine)	-	percent
					93 (Dayton)	-	percent

3.2 Cafeteria experiment

In the lab experiments, *Pycnopodia* demonstrated high individual-level variation in feeding quantity, ranging from only a single prey item consumed, to 27 consumed items over the course of a ten-day trial (Figure 4.2). They also showed individual variability in prey type selection, consuming ≤ 2 types of prey (n = 2 *Pycnopodia*), 3 types of prey (n = 5), or 4 types of prey (n = 4) (Figure 4.2). These selections were not consistent by prey species; species that were consumed most often when summed over all trials were green urchins (n = 49), purple urchins (n = 44), and mussels (n = 41). Red urchins were consumed 17 times and only a single cucumber was consumed. Chitons were never consumed in any of the feeding trials, though many developed scarring on their dorsal sides throughout the feeding trials, likely caused by attempted feeding by the *Pycnopodia*. On average, *Pycnopodia* consumed 0.41 ± 0.36 green urchins day^{-1}, 0.36 ± 0.41 purple urchins day^{-1}, 0.14 ± 0.15 red urchins day^{-1}, 0.34 ± 0.41 mussels day^{-1}, and 0.01 ± 0.03 cucumbers day^{-1} (means ± SD; Figure 4.3). When grouping urchin species together, *Pycnopodia* consumed on average 0.91 ± 0.63 urchins day^{-1}.

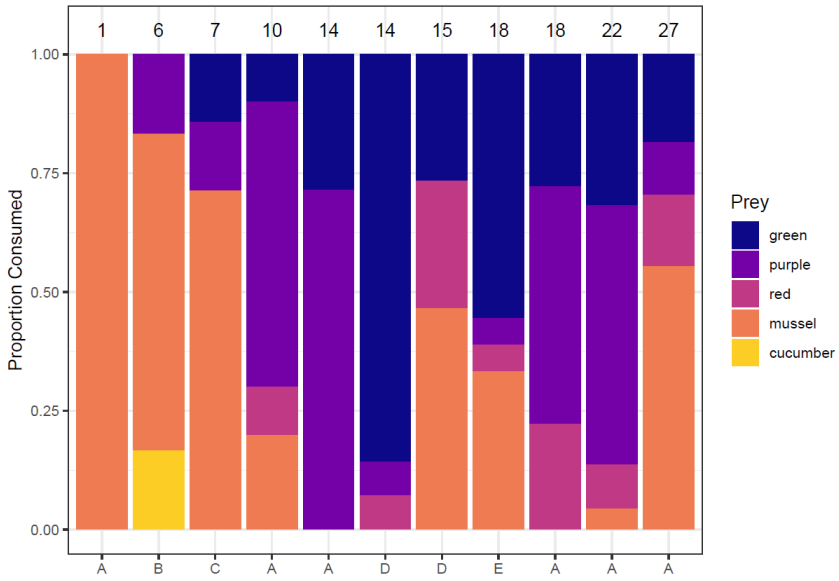


Figure 4.2: Proportional contribution of each prey type to individual *Pycnopodia*. Each column represents a single *Pycnopodia*, letters under each column indicate original collection location (A = Eagle Cove, B = O'Neal Island, C = Pt. Caution, D = Goose Island, E = FHL Docks). Numbers on top of each column indicate the number of total prey items consumed by each *Pycnopodia*.

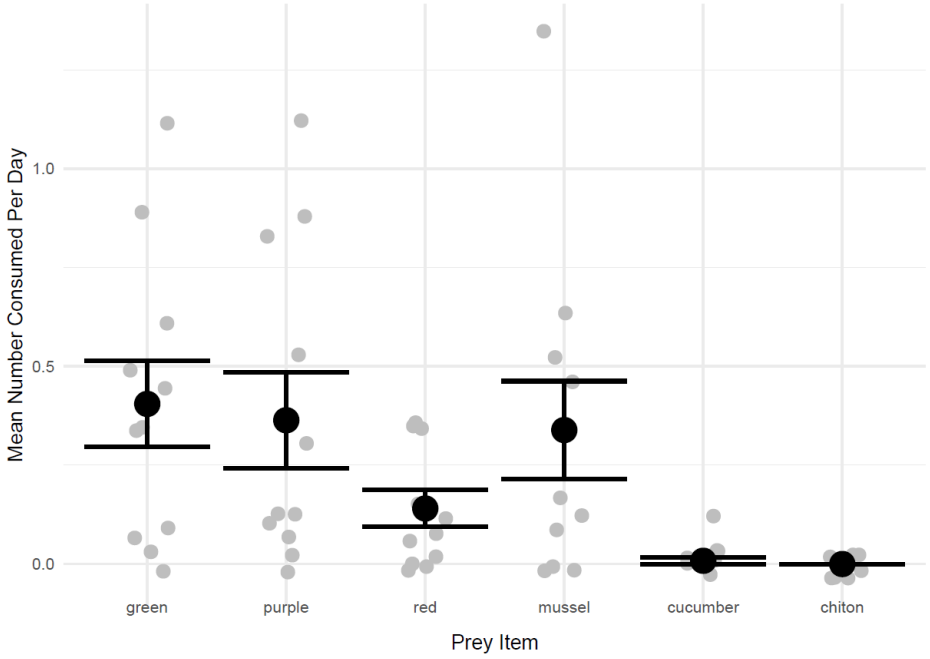


Figure 4.3: Mean number of prey items consumed per day by *Pycnopodia* across all cafeteria experiment trials. Bars indicate standard error and grey circles are individual consumption numbers for each of the 11 *Pycnopodia* tested.

Pycnopodia tended to consume smaller purple and red urchins than the mean size of those species that were offered (linear mixed effects, DF = 1, F Ratio = 4.14, p = 0.0432). They also consumed slightly larger green urchins than the mean size offered; however, this effect was not statistically significant (Figure 4.4). There was no interaction between urchin species and size preferences. The mean size of consumed urchins was identical for green (58.9 ± 10.7 mm) and purple (58.9 ± 10.1 mm) urchins, the two urchin species most often consumed.

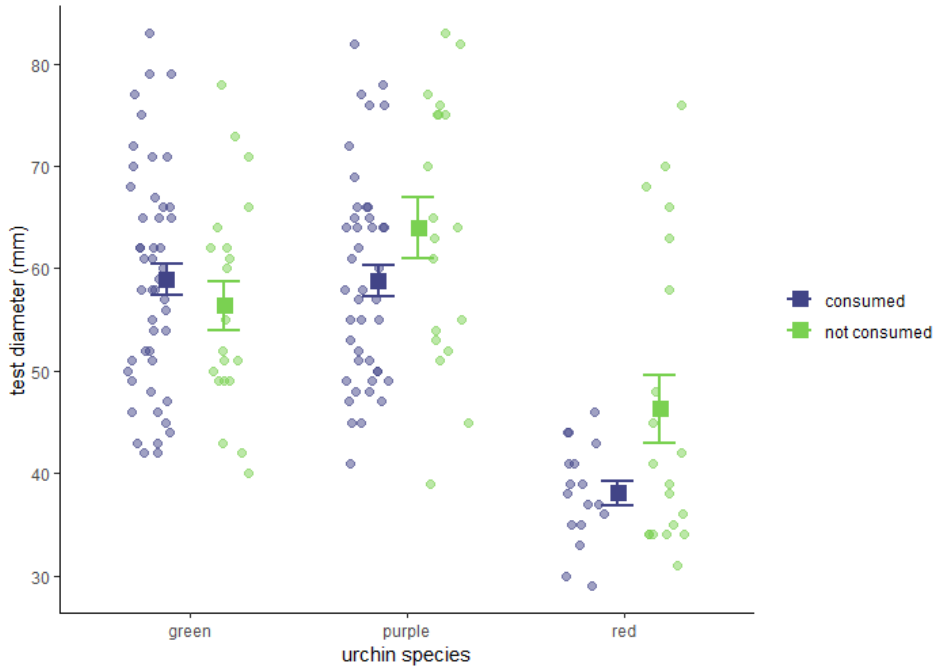


Figure 4.4: Test diameter of three urchin species that were either consumed (purple) or offered and not consumed (green) across all cafeteria feeding trials.

Time-to-first consumption of a prey species, order of species consumed, and total number of species consumed also varied among *Pycnopodia* individuals (Figure 2.5). Mean Rodgers' preference calculated as AUC indicated that mussels were the most preferred prey, followed by nearly equal preference for green urchins and purple urchins, then red urchins, and lastly,

cucumbers (Table 4.3). This preference was statistically significant with mussels, purple urchins, and green urchins being the most preferred prey (Kruskall-Wallis, chi-square(1, 4) = 16.65, p = 0.002; Dunn ranking p < 0.05). When considering individual *Pycnopodia* preferences, mussels were most often the number one ranked prey item by Rodgers' preference index (n = 6), followed by purple urchins (n = 4), then green urchins (n = 1; Table 4.4).

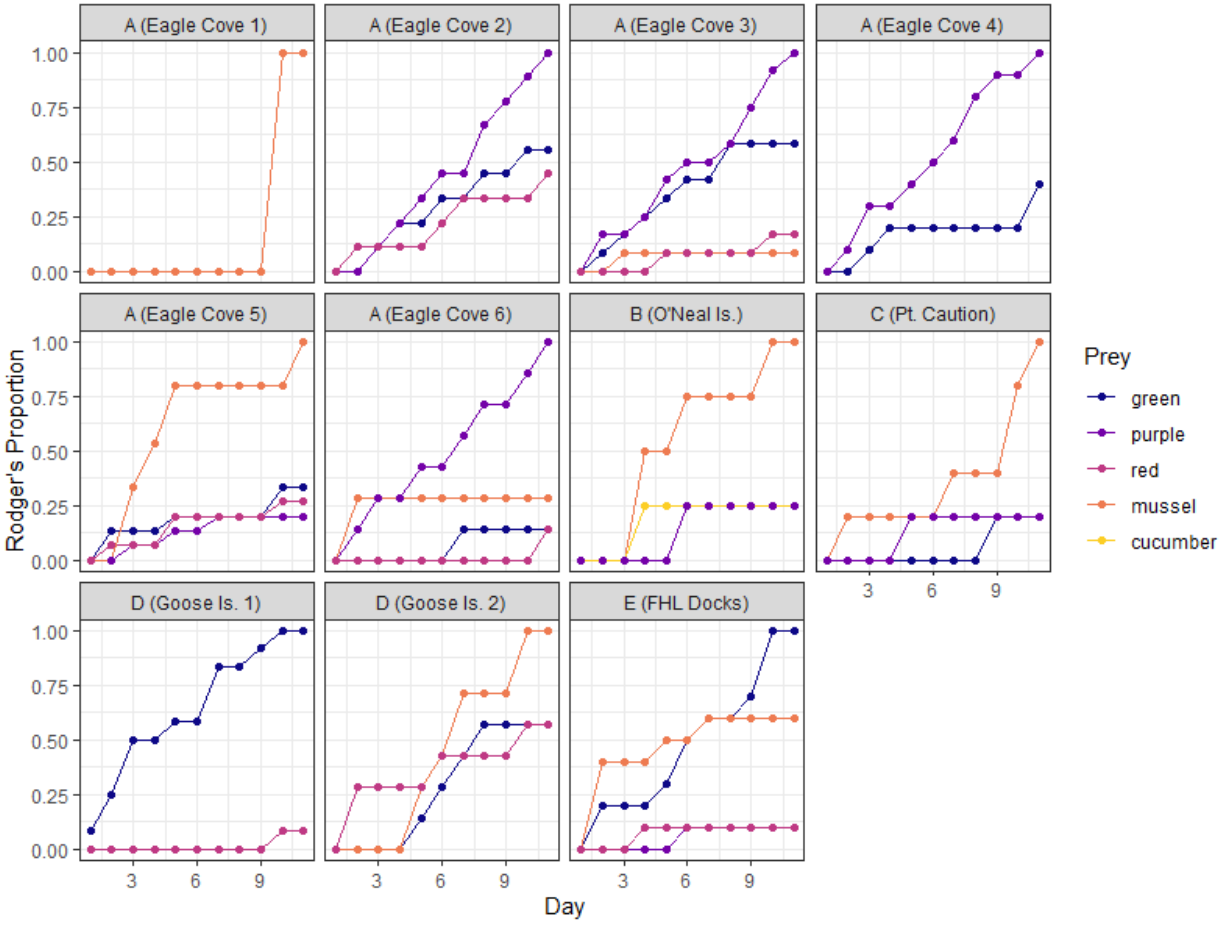


Figure 4.5: Cumulative consumption of all prey species by *Pycnopodia*. Boxes are individual *Pycnopodia*, and number of total prey items of each type consumed are listed by trial day (1-10).

Table 4.3: Mean rank of preference and standard deviation for each of the offered prey items (chiton is excluded as it was never consumed and had a value of zero). Prefence is calculated as area under the curve (AUC) as suggested by Rodgers 1990.

Rank	Prey	AUC (mean)	AUC (st. dev.)
1	mussel	0.609	0.474
2	green urchin	0.456	0.379
3	purple urchin	0.414	0.448
4	red urchin	0.177	0.274
5	cucumber	0.031	0.103

Table 4.4: Complete AUC values for each *Pycnopodia* and associated preference ranking.

Pycnopodia	Prey	AUC	Rank
FHL Docks	mussel	1.00	1
	green urchin	0.98	2
	red urchin	0.15	3
	purple urchin	0.11	4
	cucumber	0.00	5
Eagle Cove 1	mussel	1.00	1
	green urchin	0.00	2
	red urchin	0.00	2
	purple urchin	0.00	2
	cucumber	0.00	2
Eagle Cove 2	purple urchin	1.00	1
	green urchin	0.70	2
	red urchin	0.51	3
	mussel	0.00	4
	cucumber	0.00	4
Eagle Cove 3	purple urchin	1.00	1
	green urchin	0.78	2
	mussel	0.15	3
	red urchin	0.14	4
	cucumber	0.00	5
Eagle Cove 4	purple urchin	1.00	1
	green urchin	0.32	2
	red urchin	0.00	3
	mussel	0.00	3
	cucumber	0.00	3
Eagle Cove 5	mussel	1.00	1
	green urchin	0.31	2
	red urchin	0.26	3
	purple urchin	0.21	4
	cucumber	0.00	5
Eagle Cove 6	purple urchin	1.00	1
	mussel	0.55	2
	green urchin	0.13	3
	red urchin	0.01	4
	cucumber	0.00	5
Goose Is. 1	green urchin	1.00	1
	purple urchin	0.02	2
	red urchin	0.02	2
	mussel	0.00	3
	cucumber	0.00	3
Goose Is. 2	mussel	1.00	1
	red urchin	0.85	2
	green urchin	0.66	3
	purple urchin	0.00	4
	cucumber	0.00	4
O'Neal Is.	mussel	1.00	1
	cucumber	0.34	2
	purple urchin	0.25	3
	green urchin	0.00	4
	red urchin	0.00	4
Pt. Caution	mussel	1.00	1
	purple urchin	0.37	2
	green urchin	0.14	3
	red urchin	0.00	4
	cucumber	0.00	4

Shannon diversity values for *Pycnopodia* diet ranged from 0 (only a single prey item consumed) to 1.17 (Figure 4.6). The expected Shannon value if all prey were consumed in the proportion in which they were offered (0.2) is 1.61. Measures of evenness ranged from 0 to 0.77 (Figure 4.6), where an evenness value of 1 would indicate that all prey were consumed in the same quantity.

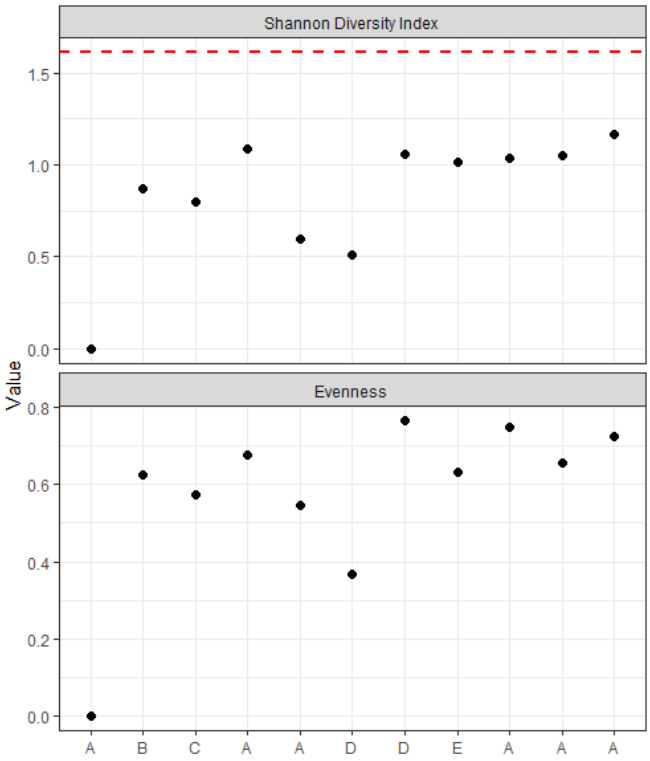


Fig 4.6: Shannon diversity and evenness of *Pycnopodia* diet in cafeteria feeding experiments. Column labels are the same as in Figure 2. Dotted red line in Shannon diversity panel indicates expected value if all prey were consumed in the proportion in which they were offered.

Pycnopodia did not demonstrate consistent prey preferences dependent upon where they were collected from (PERMANOVA, PERM = 9999, DF = 4, SS = 0.952, F = 1.117, p = 0.3598).

4. DISCUSSION

This study evaluated the natural prey spectrum as well as prey selection of the sunflower sea star *Pycnopodia* to better asses its role as a generalist predator in intertidal and shallow subtidal environments across its North Pacific range, and to explicitly address the assumptions and knowledge gaps surrounding *Pycnopodia* feeding. Their natural prey spectrum in a meta-analysis of published literature showed bivalves and urchins to be the most common prey, although diet was variable. These findings were supported through a cafeteria experiment that revealed great variability in prey selection on the level of individual *Pycnopodia*, both in quantity of prey consumed and identity of those prey. Despite the variability, *Pycnopodia* did exhibit specific preference for particular types of prey, particularly mussels and some sea urchin species. Combined, these results confirm *Pycnopodia* to be a generalist predator, but the fact that some prey were preferentially consumed, especially sea urchins, could have implications for *Pycnopodia*'s role in trophic cascades in kelp forest systems.

Pycnopodia's preference for urchins and bivalves in both the meta-analysis and cafeteria experiments is particularly interesting in the context of possible effects on ecosystem function, as these prey fulfill very different roles. Urchins are grazers that can denude kelp forests creating alternate stable state urchin barrens (Filbee-Dexter & Scheibling 2016) and bivalves are filter feeders important in pelagic-benthic coupling and—dependent upon bivalve species—can also be a foundation species (i.e., mussels; Seed 1969, Newell 2004). The ecological role of these prey can also shift across latitude and seascape conditions. For example, although purple urchins have been directly implicated in kelp forest removal across California (Bonaviri et al. 2017, Rogers-Bennett & Catton 2019, Eisaguirre et al. 2020), other species such as red urchins and green urchins are identified as the primary kelp grazers at higher latitudes (Schultz et al. 2016,

Burt et al. 2018). Likewise, although red urchins are the dominant urchin species in the San Juan Archipelago of Washington State, they are not observed to create urchin barrens as they are able to subsist on drift algae provided by strong tidal currents (Lowe et al. 2015). Considering that the range of *Pycnopodia* and urchin species linked to kelp forest loss stretches from Baja to the Aleutians (1000's of km), and across multiple seascape conditions from wave-swept coastal environments to tidal-current dominated inland seas, we must explicitly consider the effect of scale and how ecological roles shift through space (Byrne et al. 2019).

One of the environmental conditions that varies across *Pycnopodia*'s geographic range is temperature. Temperature gradients can influence the strength of predator control in marine communities, and to mediate trophic interactions and behaviors (Byrne et al. 2019, Ashton et al. 2022). The geographic range of *Pycnopodia* crosses a temperature gradient from the relatively warmer waters of the California Current in the south (~17 °C; Mauzole et al. 2020) to the cooler waters of the Alaska Current in the north (~9 °C; Bograd et al. 2005, Hickey & Royer 2010). Our meta-analysis, while spanning some oceanographic regimes over the 24° of latitude, did not allow us to test for the effects of temperature on observed *Pycnopodia* feeding across their range because of low replication and missing spatial coverage. Very little urchin consumption, however, was observed at the very southern-most end of the dataset (Pacific Grove, CA). *Pycnopodia* predation on urchins is mediated in-part by a temperature refuge in which urchins are able to persist at higher temperatures beyond those tolerated by *Pycnopodia* (>14 °C; Bonaviri et al. 2017). As marine heatwaves increase across parts of *Pycnopodia*'s range (25 day mean duration, + 0.8 °C above 99^{th} local percentiles; Frölicher et al. 2018), we can expect the size and frequency of this urchin temperature refuge to increase and the metabolic and behavioral responses of *Pycnopodia* to change locally based on exposure to these warmer waters. This could

provide additional release from predation for grazing urchins across a wide swath of the North American West Coast, adding additional grazing pressure to already temperature-stressed kelp forests (Starko et al. 2022). In contrast, warmer temperatures have been shown to increase prey consumption by sea stars through increased movement, resulting in more prey encounters, shorter handling times, and impaired prey escape responses (Barbeau & Scheibling 1994). This could suggest that *Pycnopodia* consumption of urchins will increase as long as the environmental temperatures are within *Pycnopodia*'s thermal tolerance window, and then decrease as they approach and exceed that limit. However, it is unknown if this optimum 'thermal window' would persist across large enough areas for sustained periods of time, as marine heatwaves even at the most northern end of *Pycnopodia*'s range begin to reach their thermal tolerance (>14 °C; Bonaviri et al. 2017, Bograd et al. 2010). As all of the data in our meta-analysis were collected from 1968-1983, the recent doubling in marine heatwave intensity and frequency (1982-2016; Frölicher et al. 2018) and any associated feeding preference changes would likely not be reflected in those observations. Any conclusions regarding the strength of *Pycnopodia* predation pressure in the future would need to explicitly incorporate the effects of temperature. Therefore, exactly where and how *Pycnopodia*'s specific performance curve intersects with the urchin temperature refuge is still unclear and requires further study.

The nutritional value of resources can be an important factor in consumer choices (Cruz-Rivera & Hay 2000) but needs to be balanced with the cost of acquiring that prey, according to the optimal foraging theory (Pyke et al. 1977). The metabolic cost of handling and consuming urchins by *Pycnopodia* in the laboratory has been shown to be greater compared to the handling and consumption of clams (McGaw & Twitchit 2011), and this metabolic trade-off could be an explanation for *Pycnopodia*'s preference for mussels in our cafeteria trials. The nutritional

content of mussels (the most preferred prey in our cafeteria experiment) tends to be higher only when considering protein content of tissues (Dare & Edwards 1975), but urchins (here: purple and green, the second most preferred prey) tend to be more nutrient rich with respect to carbohydrates and lipids (gonad tissue; Liyana-Pathirana et al. 2002). Lipids in particular are energy-dense macronutrients that provide calories, as well as fatty acids, many of which are essential to cellular function (Budge et al. 2006). These essential fatty acids can only be obtained by consumers through consumption, and thus may provide added incentive in prey choice when considering what a predator chooses to eat. It has been shown that selective consumption of prey —both prey identity, and body parts of prey—based on macronutrient content is a widespread phenomenon (Kohl et al. 2015). As mussels were the most preferred prey in our cafeteria trials, it could suggest that *Pycnopodia* prefer protein-rich prey items over lipid-rich ones. In addition, it has been demonstrated that *Pycnopodia* is unable to discern between purple sea urchins that have a high gonad index versus a low gonad index (a relative measure of lipid content; Galloway et al. 2023). Therefore, it is possible that either: protein content rather than lipid content is a stronger driver of *Pycnopodia* feeding preferences or; that trade-offs associated with capturing a lipid-rich prey (i.e., urchins) is high and other factors including prey defenses and prey escape behaviors may be stronger determinants of *Pycnopodia* choices. Given spatial and temporal variability of invertebrate prey associated with kelp forests (e.g., Lamy et al. 2018), prey defenses and escape behaviors could be prominently influence *Pycnopodia* prey choices if a nutritious and less costly to handle prey (e.g., bivalves) is less abundant.

Prey characteristics such as spine length and test size did not seem to affect *Pycnopodia* preferences when considering green and purple urchins, which were preferred nearly identically across cafeteria trials despite the longer spines and thicker test of purple urchins. Spine length,

however, likely was a factor when considering red urchins, as red urchins possess much longer, thicker spines than purple or green urchins (e.g., Tegner & Dayton 1981), and only the smaller red urchin individuals were consumed in cafeteria trials. Other urchin predators selectively feed on purple over red urchins, and the shorter spine length in purple urchins was the primary factor when selections were made (Tegner & Levin 1983).

In our cafeteria experiment all prey were offered with no opportunities to employ predator escape tactics as they were contained in a single enclosure with no refuge provided. Sea cucumbers were observed to react strongly to physical contact with *Pycnopodia* and would often quickly move away. This relatively quick escape response could partially explain the low-consumption rate of sea cucumbers by *Pycnopodia* in these trials. In the wild, sheltering behavior by sea urchin species, whether under rocks (Nichols et al. 2015) or under larger conspecifics (Nishizaki & Ackerman 2007), are the most-commonly employed escape strategies and they likely influence *Pycnopodia* predation behavior when attempting to feed on them. As sessile organisms, mussels use byssal attachment and shell strength as a primary deterrent to predation, as well as settling among conspecifics to reduce chances of predation. The first two strategies were not enough to provide sufficient protection from *Pycnopodia* in feeding trials, and the third strategy was not possible in the experimental setting. The chiton species offered in our experiment was also not fast enough to 'run away' from *Pycnopodia*, which are some of the fastest crawling sea stars (Montgomery 2014); however, chitons were likely able to avoid predation through their ability to adhere firmly to the bottom of the enclosure with a muscular foot. Feeding scars were apparent on several of the chitons over the course of the experiment, indicating that the *Pycnopodia* attempted to consume them, but was unable to remove them from

the substrate or penetrate their leathery protective mantle tissue completely covering the dorsal side in this species.

Two additional sources of observed variation in the meta-analysis of *Pycnopodia* prey preferences could be explained by 1) the habituation of *Pycnopodia* to prey items commonly found in their habitat of origin (i.e., urchins in kelp forests, bivalves in sand flats, etc.); or 2) abundance and identity of predatory competitors that may shift *Pycnopodia* preferences to particular prey types. It has been suggested that echinoderms are capable of associative conditioning and habituation (Freas & Cheng 2022). We did not see differences in prey selection based on site of collection in our experiment; however, all but one *Pycnopodia* came from hard-bottom substrate, which did not provide the statistical power to differentiate between substrate types. It is also important to note that our *Pycnopodia* were fed a maintenance diet of mussels and this could potentially be a source of diet habituation, however, whether *Pycnopodia* actually habituate to diets and the time it would take for them to habituate is unknown and a topic of future study. Predatory competitors may also change feeding preferences in *Pycnopodia* through intra- and inter-specific interactions (Gaymer et al. 2002). Sea otters, spiny lobsters, and sheephead are all present in the southern end of *Pycnopodia*'s range, and are known to be purple urchin predators (Eisaguirre et al. 2020). Likewise, sea otter populations have been recovering across much of their range, which overlaps with *Pycnopodia*'s range, though otter distribution is patchy (Bodkin 2015). Even if there are competitive interactions that drive prey selection in *Pycnopodia*, those sorts of phenomena are notoriously difficult to quantify and observed prey-switching may simply be a response to the abundance of prey in a particular habitat (Vallina et al. 2014) rather than competitive interactions.

As *Pycnopodia* populations begin to recover after their decline during sea star wasting, understanding how their trophic interactions affect foundation species such as kelp is critical to conserving these habitats. This is especially true as *Pycnopodia* are also found in association with other foundations species like seagrass (Gravem et al. 2021), and may more generally play important roles in maintaining ecosystem health. Based on our study we cannot conclude about *Pycnopodia* feeding preferences based on habitat type (i.e., hard bottom versus soft bottom), though this is an important consideration when determining the ecological 'reach' of *Pycnopodia* in nearshore environments, especially in light of their preference for bivalves. This study provides an additional piece of the puzzle by synthesizing our knowledge about *Pycnopodia* feeding preferences in the wild, and quantifying relative preferences for common prey species that they would encounter *in situ.* Further studies building on these results include integrating the effects of climate change, especially considering the predicted increase in marine heatwaves (Frölicher et al. 2018), on *Pycnopodia*'s feeding behavior, determining their feeding preferences across more diverse suites of prey, and parsing out their relative role as a predator and scavenger.

BRIDGE

Chapter IV quantified the feeding preferences of the generalist predator *Pycnopodia helianthoides*. In the next chapter I consider how non-consumptive effects of *Pycnopodia* can change the grazing and foraging behavior of one of their preferred prey items, the purple sea urchin *Strongylocentrotus purpuratus*.

CHAPTER V

THE SUNFLOWER SEA STAR *PYCNOPODIA HELIANTHOIDES* REDUCES SEA URCHIN GRAZING BEHAVIOR THROUGH NON-CONSUMPTIVE EFFECTS

The study contains previously unpublished coauthored material and was conceived by myself with conceptual support from coauthors Aaron Galloway and Sarah Gravem. I carried out all field collections with assistance from Aaron Galloway. Lab experiments were conducted by myself. Video data extraction was done by coauthor Ethan Porter-Hughes. Statistical analyses were developed by myself with assistance from Sarah Gravem. I wrote the manuscript with editorial input from all authors.

1. INTRODUCTION

Predator-prey interactions are among the strongest drivers shaping ecosystem processes, with often far-reaching consequences for community stability and resilience across spatial scales (Paine 1966, Preisser et al. 2005, Kissling & Schleuning 2015). This is particularly true of strongly interacting species that affect associated community diversity and production through trophic cascades, in which one species indirectly controls another (Ives et al. 2005, Byrnes et al. 2006). While these interactions are frequently mediated in the form of lethal or consumptive density-mediated indirect interactions (DMII), it is also possible for non-lethal phenotypic or behavioral trait-mediated indirect interactions (TMII) driven by the predators to influence ecological outcomes (Preisser et al. 2005, Harding & Scheibling 2015).

TMII in species relationships often act through the alteration of traits of the prey species

involved, including changes to life history or trophic roles in the ecosystem (Bolker et al. 2003). These non-consumptive effects (NCE) then scale up to the ecosystem level as an analog to the classic trophic cascade based on consumptive predation pressure (e.g., Ripple et al. 2016). Frequently, TMII acts via NCEs through the modification of a grazing prey species' feeding behavior, relieving the grazers' food source from grazing pressure (Haggerty et al. 2018, Gravem & Morgan 2019). The so-called 'ecology of fear' can have dramatic effects on ecosystem productivity, changing foraging times and grazing rates of important consumers in a suite of habitats (Brown et al. 1999).

In marine ecosystems, NCEs have been implicated in a range of predator-prey interactions (Werner & Peacor 2003). Predatory cues propagate through these systems via various pathways such as visual, auditory, or chemosensory pathways. Chemosensory cues proliferate as water flow carries the 'cue' of the predator through the water and can change the behavior of organisms across the seafloor as they encounter it (Kats & Dill 1998). As such, a single predator may have a 'larger' effect (i.e., multiple prey species responding) via this non-consumptive interaction than compared to consumption of a single prey item. Chemosensory cues can act as NCEs and may trigger various physiological and behavioral responses in the prey. Physiological responses may include metabolic changes (Hawlena & Schmitz 2010), reduced fecundity (Bourdeau et al. 2016), and impaired growth (Werner & Peacor 2003), while behavioral changes can include reduced foraging time (Werner & Peacor 2003), increased crypsis (Scheibling & Hamm 1991), and more frequent escape response (Freeman 2006). All these effects can be experienced individually, or simultaneously, and can have serious consequences for the prey species. The frequency of encounters with a predator cue may also produce various responses in the potential prey. Continuous versus irregular exposure to a

predator cue could result in a prolonged high-stress state on one hand, or gradual habituation on the other (Van Dievel et al. 2016, Dehaudt et al. 2019). In addition, the preexisting physiological state of an organism can modulate the specific response to a predator. This is seen, for example, when a hungry prey species is willing to take more risk of predation than a fed one to obtain food (Vadas et al. 1994).

The recent population collapse of the generalist invertebrate mesopredator *Pycnopodia helianthoides* (hereafter *Pycnopodia*) due to sea star wasting disease has been implicated as a major contributing factor to the proliferation of the grazing purple urchin, *Strongylocentrotus purpuratus*, after its release from *Pycnopodia* predation across much of the West Coast of North America (Burt et al. 2018, Harvell et al. 2019, Hamilton et al. 2021, Galloway et al. 2023). This effect has been most pronounced along the central and southern end of the range of both *Pycnopodia* and purple urchins along the Oregon and California coasts, although similar patterns have been observed between *Pycnopodia* and red urchins (*Mesocentrotus franciscanus*) and green urchins (*Strongylocentrotus droebachiensis*) farther northward into British Columbia (Schultz et al. 2016, Burt et al. 2018, Rogers-Bennett & Catton 2019).

Pycnopodia is a common predator of a variety of sea urchins, including *S. purpuratus* (Mauzey et al. 1968, Herrlinger 1983, Shivji et al. 1983), which has a less effective defense strategy against *Pycnopodia* predation than other urchin species (Moitoza & Phillips 1979). NCEs from *Pycnopodia* chemical cues may add to the response of urchins in addition to removal via direct consumption (Freeman 2006). *Pycnopodia* is only one among a suite of potential predators for purple urchins, although the geographic extent and identity of urchin predators change across their range. The spiny lobster (*Panulirus interruptus*) and California sheephead (*Semicossyphus pulcher*) are constrained to the southern end of the purple urchin's range (Tegner

& Dayton 1981), while the wolf eel (*Anarrhichthys ocellatus;* Marliave 1987) and sea otter (*Enhydra lutris*; Smith et al. 2021) are found in low densities or are not uniformly distributed across seascape. As such, the coincident population decline of *Pycnopodia* and concurrent increase in urchin populations is thought to be a causative relationship, though additional factors including warming water temperatures and increased urchin recruitment are also implicated in the recent urchin population surge (Burt et al. 2018, Rogers-Bennett & Catton 2019).

The decline of kelp forests by up to >90% across the central North American West coast is attributed, in part, to a predation release on grazing urchins and has been suggested to be a putative trophic cascade (Schultz et al. 2016, Rogers-Bennett & Catton 2019). The resulting mosaic of kelp forests and urchin barrens–which are considered an alternate stable state–are marked by decreased primary productivity, low food web complexity, and a reduction in economically important species such as abalone (Filbee-Dexter & Scheibling 2014, Schultz et al. 2016, Rogers-Bennett & Catton 2019). Urchins within these urchin barrens can maintain this alternate stable state when the reduced kelp biomass leads to starvation, and constant grazing inhibits the regrowth of kelp and other macroalgae (Estes & Duggins 1995, Filbee-Dexter & Scheibling 2014, Spindel et al. 2021, Dolinar & Edwards 2021).

Although direct consumptive effects of *Pycnopodia* on urchins is an important pathway of grazer control (Bonaviri et al. 2017, Galloway et al. 2023), behavioral responses by urchins to predation cues suggest that NCEs may also play a key role in suppressing sea urchin grazing behavior (Moitoza & Phillips 1979, Bernstein et al. 1981, Duggins 1983, Lee et al. 2016). In addition, higher concentrations of urchins in urchin barrens can increase their hunger-state and their response to food and predator cues, resulting in potentially complex behaviors (Parnell et al. 2017). Yet, the effect of NCEs between *Pycnopodia* and sea urchin behavior and grazing rates

has not been explicitly quantified. Understanding how and to what degree *Pycnopodia* may alter sea urchin grazing can provide important insights into their role in maintaining healthy kelp forest ecosystems through TMII. To address these knowledge gaps, we here asked the questions: 1) Do NCEs between *Pycnopodia* and *S. purpuratus* alter the feeding rates of the urchins (*feeding experiment*)?, and 2) Does the hunger-state of *S. purpuratus* change their response to food in the presence of a predator cue (*movement experiment*)?

2. METHODS

2.1 Feeding experiment organism collections and care

Purple sea urchins (*S. purpuratus*) were collected at Lopez Island (48.4558, -122.9383) in the San Juan Archipelago of Washington State on September 11, 2020 (71 urchins) through a permitting arrangement between the Friday Harbor Labs and Washington State (Washington State House Bill 68, R.C.W.28.77.230, 1969 Revision R.C.W.28B.20.320). Collections were made by hand on SCUBA between 3-8 m below mean lower low water (MLLW) in bull kelp (*Nereocystis luetkeana*) stands on bedrock. Urchins were then held for 2 days in flow-through sea water tanks (60 cm x 90 cm x 30 cm, WHD) prior to experimentation beginning on September 13, 2020. Urchins were shaded from direct sunlight and fed *N. luetkeana* blade tissue *ad libitum* while in holding.

Pycnopodia were collected at Goose Island in the San Juan Archipelago (48.4575°, -122.9544°) on July 29, 2020 at a depth of 6 m below MLLW through the same permitting as above. Two *Pycnopodia* were collected to be used in all feeding experiments because of the overall low abundance of this species after the sea star wasting decline and to minimize possible local, ecological effects of removing an endangered species. *Pycnopodia* were transported

following lab and handling protocols described in Hodin et al. (2021) in covered 5-gallon buckets filled with seawater and placed in shaded flow-through seawater tanks for 6 weeks (120 cm × 40 cm × 45 cm WHD) prior to experimentation. They were fed a maintenance diet of 2 mussels (*Mytilus* spp.) every other day prior to experimentation, and in-between trials during experimentation following lab and handling protocols described in Hodin et al. (2021).

2.2 Feeding experiment setup

To determine if there are non-consumptive effects of *Pycnopodia* on sea urchin feeding rates, five replicate feeding trials were run between September 13 – October 2, 2020, each lasting from 51-69 h using a total of two *Pycnopodia* to generate chemical cues and 71 *S. purpuratus*. We used only two individual *Pycnopodia* across multiple trials for the reasons of minimizing ecological impacts listed above. All urchins were used in the experiment only once to ensure independence of replicates. Sea urchin test diameters were measured with hand calipers to the nearest millimeter, and urchins were assigned to two experimental groups (*Pycnopodia* exposure or control). Experimental setup, including organism arrangement, tanks, and plumbing, were modified after the initial Trial 1 to reduce stress on the *Pycnopodia*, but the effective predator cue treatments remained consistent between trials, so they were analyzed as replicate experiments. For Trial 1, five urchins were placed in each of three tanks (circular: 50 cm depth x 104 cm diameter); one of these tanks contained a caged *Pycnopodia* so that chemical cues were released into the tank but no physical interaction with the urchins was possible. The other two tanks served as controls without a *Pycnopodia*. For Trails 2-5, seven urchins were placed into each of two tanks (circular: 30 cm depth x 104 cm diameter). One of these tanks received a predator cue via effluent from a header tank containing *Pycnopodia* (Figure 1A) to the urchin

tank while the other served as a control and received plain seawater (no cue). Urchins within a tank in all treatments were individually contained within buckets perforated with multiple small holes on the sides and top of each container (<1 cm diameter each) to maintain water flow within each, while preventing interactions among urchins in the tank (Figure 1A). Water flow rate into experimental tanks was 3.89 ± 0.46 liters/minute, water temperature during the experiment was 10.0 ± 0.16 °C, salinity 30.54 ± 0.81, as measured by the Friday Harbor Labs Ocean Observatory, and ambient light was an 11:13 h light:dark cycle.

Each urchin in the individual tank buckets was fed 12 standardized circular pieces of kelp 'confetti' (21 mm diameter) created by using a cork punch on vegetative *Nereocystis luetkeana* (hereafter kelp) blade tissue. A subsample of kelp confetti was weighed at the beginning and end of the experiment to estimate biomass of kelp consumed, and a subset of trials (n = 4) contained additional control confetti held separately from urchins that were weighed before and after each trial to detect changes in confetti weights that were not related to urchin feeding. Urchins in each experimental unit were fed kelp confetti *ad libitum* and their consumption rates tracked by counting number of intact confetti pieces and estimating proportion of partially-consumed pieces present at three time points per day (08:00, 14:00, 20:00) for up to three days. Kelp confetti had a mean biomass of 0.341 ± 0.030 g per disc (n = 49), and control confetti weights were not statistically different before and after trials (linear mixed-effects model [lme], df = 92, t = 0.45, p = 0.65) and so were not used as a correction factor when considering biomass loss. Any consumed confetti were replaced with fresh confetti maintaining a total of ~12 pieces per urchin. Trial 1 had to be stopped after 51 hours, while all remaining trials were run for 69 hours.

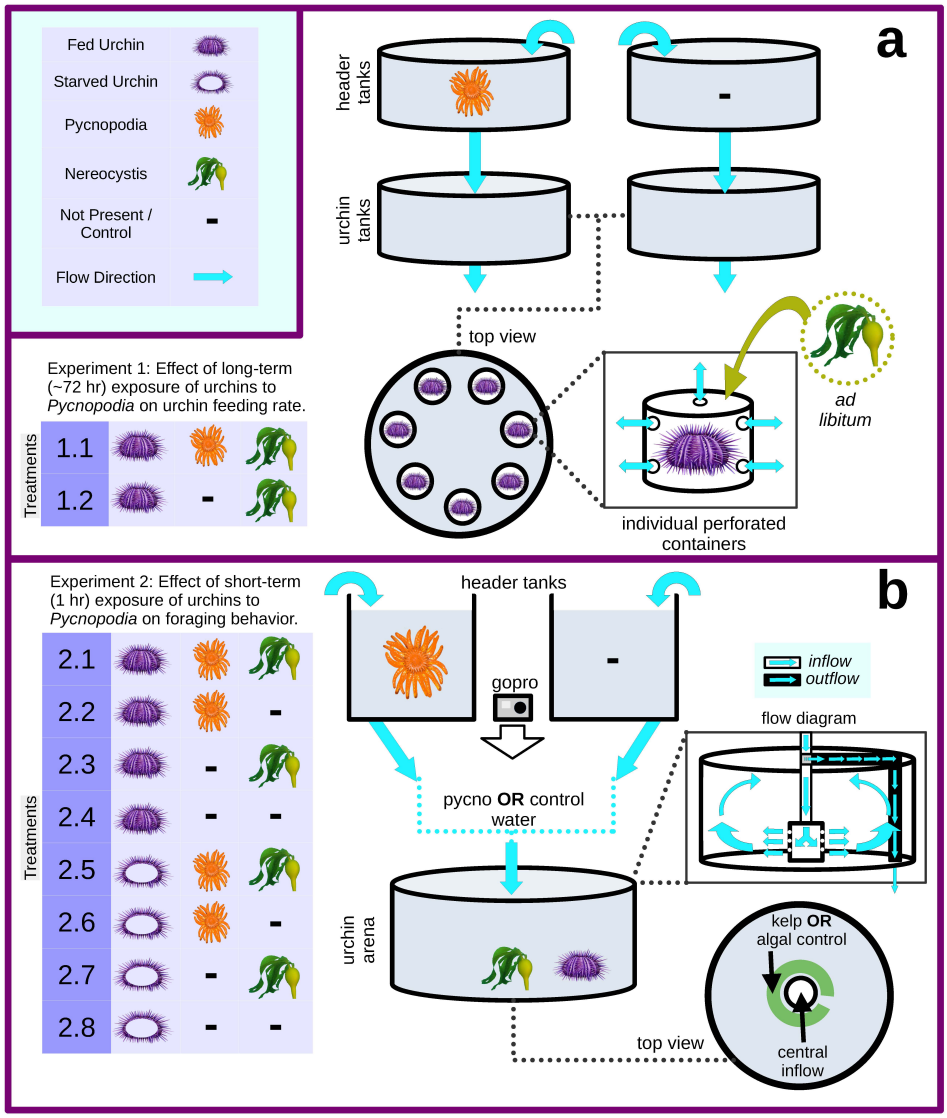


Figure 5.1: Setup for urchin feeding (A) and urchin movement (B) experiments. All treatment combinations are shown for each experiment in tables. The urchin grazing behavior setup depicts the 'fed urchin with algae' treatment, and the flow diagram shows general flow created by water inflow located at the center-bottom of the arena, and outflow at the center-top. Two identical 'urchin arenas' were used for the grazing behavior experiment to allow for tank cleaning between trials, but only one is shown here for clarity.

2.3 Movement experiment organism collections and care

S. purpuratus were collected at Lopez Island (48.4558°, -122.9383°) in the San Juan Archipelago of Washington State on June 11, 2021 (49 urchins) under FHL permitting (see above). Collections were made by hand using the same protocols as above, and urchins then held

in flow-through sea water tanks (circular: 30 cm depth x 104 cm diameter) before being moved into the experimental tanks (rectangular: 60 cm x 90 cm x 30 cm WHD).

A single *Pycnopodia* was collected under the FHL docks (48.54532, -123.01192) on July 3, 2021 via SCUBA at a depth of 4 m below MLLW under FHL permitting. Only a single *Pycnopodia* was used as a source of predator-cue for this experiment to minimize effects of collection on wild populations of this endangered species. The sea star was transported in a shaded 5-gallon bucket filled with seawater and then held in a partially shaded flow-through seawater tank (120 cm × 40 cm × 45 cm WHD) prior to experimentation.

2.4 Movement experiment setup

S. purpuratus (n = 49) were measured for diameter using calipers and separated haphazardly into two groups: ‘fed’ and ‘starved'. ‘Fed’ urchins were fed kelp, *ad libitum*, and starved urchins were not fed for seven weeks while in holding. This time frame has been shown to be sufficient to result in different gonadal conditions among urchin feeding groups (Galloway et al. 2023). At the end of the starvation period all urchins were used in individual trials, placing starved (n = 25) and fed urchins (n = 24) separately in one of two identical circular aquarium ‘arenas’ (30 cm depth x 104 cm diameter), respectively, in which individual urchin movement and behavior was tracked over one hour. There were a total of eight treatments in a factorial design crossing urchin treatment (fed vs. starved), *Pycnopodia* cue (cue vs. no cue), and kelp treatment (kelp vs. kelp replica; Figure 1B). The *Pycnopodia* cue was delivered as effluent from a header tank containing one *Pycnopodia* (120 cm × 40 cm × 45 cm WHD) and ‘control’ no-cue water from a header tank with no *Pycnopodia (*same dimensions). In kelp-present trials, a 55 cm x 4 cm kelp strip was wrapped around an inverted perforated sampling cup through which the

header source water flowed into the experimental arena. In no-kelp control treatments a kelp replica (55 cm x 4 cm plastic sheeting material) was wrapped around the entry point of the header water source. In all cases, the centrally placed header *Pycnopodia* cue inflow and kelp/replica location is referred to as the 'cue source'.

All inflow of water during each 1-h trial came from the header tank. Water temperature (12.9 ± 1.19 °C), and light flux (1922 ± 1166 lux) were measured using a HOBO TidbiT v2 Data Logger (Onset, Bourne, MA, USA), and salinity (31.1 ± 1.46) was measured using a hand refractometer. Experimental flow rates were standardized to 2.5 L/min. Patterns of flow diffusion within each experimental unit were visualized intermittently between trials using fluorescein dye injected at the outflow of the header tank to confirm constant flow and even distribution of the cue. Each treatment was run at least six times with naive urchins for all eight possible combinations, and one trial was rerun due to a malfunctioning camera, for a total of 49 individual trials. Urchins were observed in 1-min intervals for each 1-h trial for the following target behaviors: movement [not moving or moving]; interacting with central cue [foraging, not foraging; foraging was defined as at least one tube foot touching the cue source (kelp or kelp replica)]. Quantity of kelp consumed (in trials with kelp) was calculated as reduction in surface area of experimental kelp strip from known dimensions after the trial was concluded using photos taken with a scale bar and processed in ImageJ (Schneider et al. 2012). Experimental arenas were drained, rinsed with fresh water, and scrubbed by hand between all trials to eliminate cross contamination of chemosensory cues.

Trials were also recorded with GoPro cameras (HERO4 Silver; San Mateo, CA, USA) with a medium FOV setting. Videos of each trial were processed in the video analysis software Tracker (Brown et al. 2021) using the AutoTracking function. The path of each urchin was

extracted as x and y coordinates in 3.3-sec increments and calibrated to a bullseye of known dimensions underneath the urchin. The bullseye was correlated with a center of mass function in Tracker to maximize accuracy. Total distance traveled was calculated for each urchin using adehabitatLT (Calenge 2006).

2.5 Statistical analysis

Data were summarized, quality controlled, and visualized using R (R Core Team 2022) in RStudio (RStudio Team 2022) with the tidyverse, adehabitatLT, and viridis packages (Calenge 2006, Wickham et al. 2019, Garnier et al. 2021). Statistical analyses were performed in R with the lme4 package (Bates et al. 2015) and in JMP v17.0.0 (SAS Institute Inc., Cary, NC, USA).

2.5.1 Feeding experiments – Differences in mean amount of kelp consumed per urchin per hour between treatments over the course of each trial were tested with a linear mixed-effects model (lme). The response variable of mean kelp consumed per hour was log+1 transformed to address variance heteroscedasticity. Fixed effects included: treatment, hour since beginning of the trial, and their interactions. Random effects included: urchin identity nested within treatment and trial tank, and trial tank nested within treatment, to account for any variation not attributed to the fixed effects.

2.5.2 Movement experiment – Urchin movement behaviors were averaged over 5-min increments to detect any changes in behavior over the course of each individual trial. Behaviors were assigned binary classifications for each urchin, with 0 = not moving or not foraging, and 1 = moving or foraging to give proportion of time performing each behavior within each increment.

Both proportion of time moving, and proportion of time foraging were tested with lme. Fixed effects for both responses included: urchin status (fed, starved), *Pycnopodia* cue (present, absent), kelp cue (present, absent), time increment (5-60 in 5-min steps), and the full interaction of all these fixed effects. Random effects included trial arena nested within urchin status, *Pycnopodia* cue, and kelp cue, to account for any variation not attributed to the fixed effects. Feeding on kelp, when it occurred, was tested with a linear model.

Total distance traveled by urchins during each trial were tested with lme. Fixed and random effects were the same as above; however, time increment was not included as the resolution of the data was only on the level of a per-trial total.

3. RESULTS

3.1 Feeding experiment

Urchins exposed to a waterborne *Pycnopodia* cue consumed 53% less kelp per hour on average (0.127 ± 0.104 g/h) than urchins not exposed to a *Pycnopodia* cue (0.067 ± .047g/h) across all trials (lme, DFden = 8.64, t = 2.64, p = 0.0279; Figure 5.2A, Table 5.1). There was a significant interaction between cue treatments and hours since the beginning of the trial (lme, DFDen = 538.9, t Ratio = -4.96, $p < 0.001$), reflecting differing changes in consumption rates from the beginning to the end of each trial based on treatment; however, consumption was highly variable across all treatments. Generally, urchins exposed to a *Pycnopodia* cue increased their rate of consumption over time (3 h: 0.039 ± 0.075 g/h; 69 h: 0.084 ± 0.061 g/h) while urchins not exposed to a *Pycnopodia* cue decreased their rate of consumption (3 h: 0.191 ± 0.197 g/h; 69 h: 0.111 ± 0.105 g/h). Consumption rates became indistinguishable after 27 h (Figure 5.2B-C).

Mean hourly consumption of kelp at 27 h was 0.055 ± 0.089 g/h (*Pycnopodia* cue) and 0.128 ± 0.112 g/h (no *Pycnopodia* cue; lme, DF = 69, t = -3.017, p = 0.00357), and at 33 hr was 0.095 ± 0.105 g/h (*Pycnopodia* cue) and 0.139 ± 0.129 g/h (no *Pycnopodia* cue; lme, DF = 69, t = -1.574, p = 0.12).

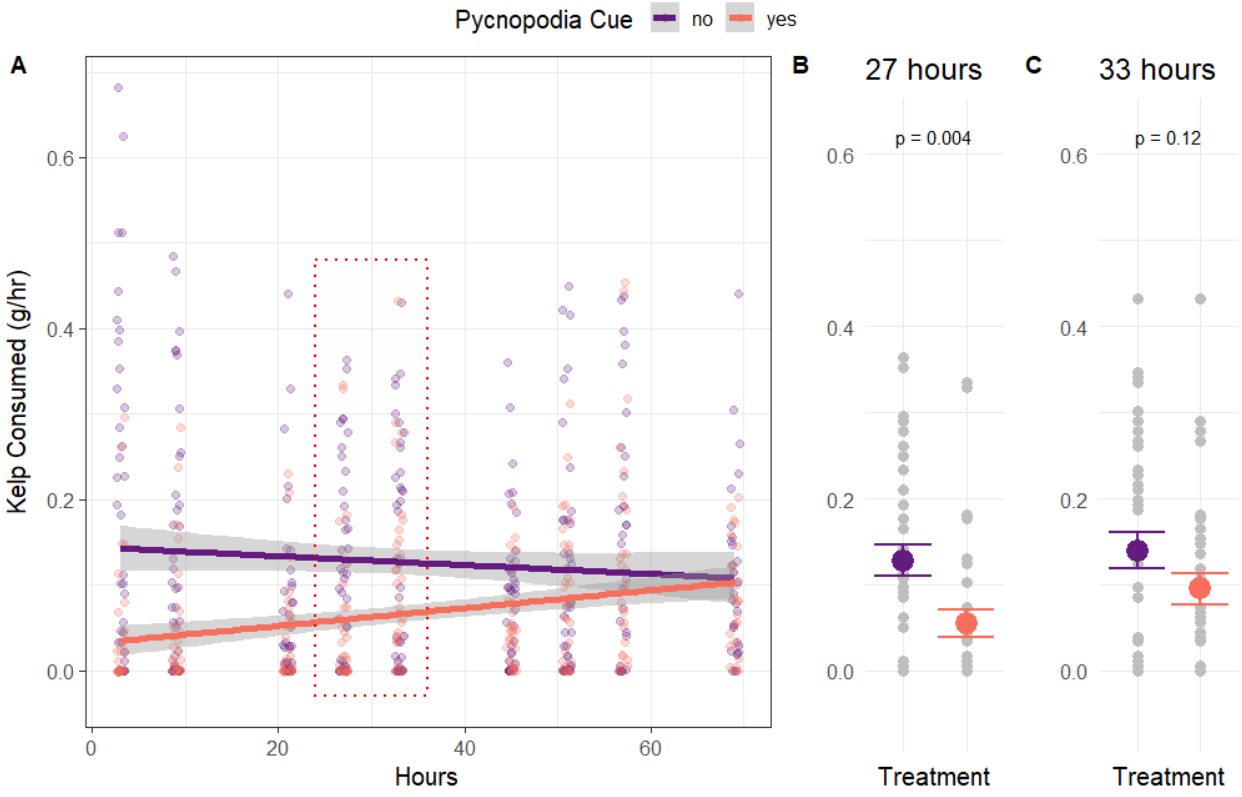


Figure 5.2: Hourly consumption of kelp by urchins that were exposed (light orange) and not exposed (dark purple) to a *Pycnopodia* cue across the 69 hour experimental period (A). Regression lines show the best fit and 95% confidence interval for each group. Differences in hourly feeding rate (dotted red box) are shown at 27 hours (B) and 33 hours (C), the time at which feeding rates become indistinguishable between the two treatments.

Table 5.1: Mixed-effects model of the effect of a *Pycnopodia* cue on urchin feeding.

Effect of Pycnopodia Cue on Urchin Feeding Rates					
Predictor	*Estimate*	*Std Error*	*DFDen*	*t Ratio*	*Prob>|t|*
(Intercept)	0.0342	0.0046	13.6100	7.4500	**<.0001**
Pycnopodia Cue	0.0108	0.0041	8.6440	2.6400	**0.0279**
Hour of Experiment	0.0001	0.0001	538.9000	1.4500	0.1465
Pycnopodia Cue * Hour of Experiment	-0.0003	0.0001	538.9000	-4.9600	**<.0001**
Random Effect	*Var Component*	*Std Error*	*95% Lower*	*95% Upper*	*Wald p-Value*
Urchin ID / Pycnopodia Cue / TrialTank	0.0008	0.0002	0.0005	0.0011	**<.0001**
TrialTank / Pycnopodia Cue	0.0000	0.0001	-0.0001	0.0002	0.6362
Residual	0.0009	0.0001	0.0008	0.0011	
Total	0.0018	0.0002	0.0015	0.0022	

3.2 Movement experiment

Across all treatments, starved urchins spent an average of 59.8 ± 49.0% of the experiment moving within the arena, while fed urchins spent an average of 86.7 ± 34.0% of the experiment moving (Figure 5.3A). Urchin status (fed, starved) was the primary single fixed factor to drive differences in time spent moving across all treatments (lme, DFDen = 41, t Ratio = 4.83, $p < 0.0001$; Table 5.2). Various interactions between urchin status and other effects were also statistically important, notably urchin status and algal treatment (lme, DFDen = 41, t Ratio = -2.20, p= 0.0334), and urchin status and time (lme, DFDen = 531, t Ratio = 3.14, p = 0.0018). *Pycnopodia* cue was only significant in an interaction with urchin status and time (lme, DFDen = 531, t Ratio = 2.05, p = 0.0407).

Time spent foraging varied widely across treatments with starved urchins spending an average of 32.1 ± 46.7% of their time foraging, while fed urchins spent only 9.2 ± 29.0% of their time foraging (Figure 5.3B). Starved urchins in the kelp-present treatment spent much more time

foraging than other groups, regardless of *Pycnopodia* cue (56.7 ± 49.6). Single fixed effects driving observed patterns of foraging included urchin status (lme, DFDen = 41, t Ratio = -2.89, p = 0.0062), algal treatment (lme, DFDen = 41, t Ratio = -3.67, p = 0.0007), and time (lme, DFDen = 531, t Ratio = -2.74, p = 0.0063; Table 2). Various interactions were observed between effects, though *Pycnopodia* cue was not included in any of those interactions.

Figure 5.3: Proportional time spent by starved (light teal) and fed (dark orange) urchins across all treatment combinations in the movement experiment for the proportional time moving (A) and proportional time foraging (B). Large circles are overall means per group per treatment, and transparent small circles are 5-minute increment averages for individual urchins (12 points per urchin = 60-min trial). Vertical dashed line indicates mean proportional time spent doing each behavior for all urchins in the behavioral control treatment (no Pycno, no Kelp). Total distance traveled (C) in each of the four treatments by starved (light teal) and fed (dark orange) urchins. Large circles indicated group mean for urchin conditions (starved, fed), and smaller points indicated values for individual urchins within each treatment. The total quantity of kelp consumed in kelp treatments (D) were not different between *Pycnopodia* cue treatments between starved urchins. Fed urchins never consumed kelp in any treatment.

Table 5.2: Mixed-effects model of the effect of a *Pycnopodia* cue, urchin status (starved, fed), and algal treatment (kelp offered, not offered) on the movement, foraging, and distance traveled of a sea urchin.

Effect of Pycnopodia Cue on Urchin Movement

Predictor	*Estimate*	*Std Error*	*DFDen*	*t Ratio*	*Prob>|t|*
(Intercept)	0.7391	0.0330	86.94	22.37	**<.0001**
Urchin Status	0.1315	0.0273	41	4.83	**<.0001**
Pycnopodia Cue	-0.0114	0.0273	41	-0.42	0.6778
Urchin Status * Pycnopodia Cue	0.0184	0.0273	41	0.67	0.5046
Algal Treatment	0.0420	0.0273	41	1.54	0.1314
Urchin Status * Algal Treatment	-0.0600	0.0273	41	-2.20	**0.0334**
Pycnopodia Cue * Algal Treatment	0.0190	0.0273	41	0.70	0.4887
Urchin Status * Pycnopodia Cue * Algal Treatment	0.0115	0.0273	41	0.42	0.6751
Time	-0.0001	0.0006	531	-0.21	0.8302
Urchin Status * Time	0.0018	0.0006	531	3.14	**0.0018**
Pycnopodia Cue * Time	0.0002	0.0006	531	0.36	0.716
Urchin Status * Pycnopodia Cue * Time	0.0012	0.0006	531	2.05	**0.0407**
Algal Treatment * Time	0.0010	0.0006	531	1.67	0.0963
Urchin Status * Algal Treatment * Time	-0.0018	0.0006	531	-3.15	**0.0017**
Pycnopodia Cue * Algal Treatment * Time	0.0005	0.0006	531	0.88	0.3768
Urchin Status * Pycnopodia Cue * Algal Treatment * Time	-0.0006	0.0006	531	-1.13	0.2598
Random Effect	*Var Component*	*Std Error*	*95% Lower*	*95% Upper*	*Wald p-Value*
TrialTank / Urchin Status / Pycnopodia Cue / Algal Treatment	0.0315	0.0080	0.0158	0.0473	**<.0001**
Residual	0.0576	0.0035	0.0512	0.0652	
Total	0.0891	0.0087	0.0743	0.1088	

Effect of Pycnopodia Cue on Urchin Foraging

Predictor	*Estimate*	*Std Error*	*DFDen*	*t Ratio*	*Prob>|t|*
(Intercept)	0.2474	0.0415	57.67	5.96	**<.0001**
Urchin Status	-0.1099	0.0381	41	-2.89	**0.0062**
Pycnopodia Cue	-0.0144	0.0381	41	-0.38	0.7065
Urchin Status * Pycnopodia Cue	0.0096	0.0381	41	0.25	0.8027
Algal Treatment	-0.1397	0.0381	41	-3.67	**0.0007**
Urchin Status * Algal Treatment	0.1182	0.0381	41	3.11	**0.0034**
Pycnopodia Cue * Algal Treatment	0.0394	0.0381	41	1.04	0.3063
Urchin Status * Pycnopodia Cue * Algal Treatment	0.0196	0.0381	41	0.51	0.6095
Time	-0.0014	0.0005	531	-2.74	**0.0063**
Urchin Status * Time	-0.0021	0.0005	531	-4.21	**<.0001**
Pycnopodia Cue * Time	0.0002	0.0005	531	0.36	0.7188
Urchin Status * Pycnopodia Cue * Time	-0.0006	0.0005	531	-1.15	0.2495
Algal Treatment * Time	-0.0015	0.0005	531	-2.93	**0.0035**
Urchin Status * Algal Treatment * Time	0.0020	0.0005	531	4.02	**<.0001**
Pycnopodia Cue * Algal Treatment * Time	-0.0003	0.0005	531	-0.68	0.4956
Urchin Status * Pycnopodia Cue * Algal Treatment * Time	-0.0007	0.0005	531	-1.44	0.1495
Random Effect	*Var Component*	*Std Error*	*95% Lower*	*95% Upper*	*Wald p-Value*
TrialArena / Urchin Status / Pycnopodia Cue / Algal Treatment	0.0671	0.0156	0.0364	0.0977	**<.0001**
Residual	0.0450	0.0028	0.0400	0.0509	
Total	0.1120	0.0158	0.0865	0.1509	

Effect of Pycnopodia Cue on Urchin Distance Traveled

Predictor	*Estimate*	*Std Error*	*DFDen*	*t Ratio*	*Prob>|t|*
(Intercept)	60.1777	2.8242	4.355	21.31	**<.0001**
Urchin Status	10.9064	2.8242	4.355	3.86	**0.0154**
Pycnopodia Cue	-3.0424	2.8242	4.355	-1.08	0.3374
Urchin Status * Pycnopodia Cue	5.7438	2.8242	4.355	2.03	0.1060
Algal Treatment	2.5141	2.8242	4.355	0.89	0.4198
Urchin Status * Algal Treatment	3.9976	2.8242	4.355	1.42	0.2243
Pycnopodia Cue * Algal Treatment	-3.6239	2.8242	4.355	-1.28	0.2635
Urchin Status * Pycnopodia Cue * Algal Treatment	-1.3749	2.8242	4.355	-0.49	0.6499
Random Effect	*Var Component*	*Std Error*	*95% Lower*	*95% Upper*	*Wald p-Value*
TrialArena / Urchin Status / Pycnopodia Cue / Algal Treatment	22.7157	71.0509	-116.5414	161.9728	0.7492
Residual	264.5786	65.3478	171.9103	459.3453	
Total	287.2943	77.3684	180.3669	528.2971	

The total distance traveled by urchins was different between starved (576 ± 225 cm) and fed (842 ± 203) urchins (lme, DFDen = 4.355, t Ratio = 3.86, p = 0.0154; Figure 5.3C, Table

5.2), and no other fixed effect other than urchin state contributed to those differences.

Only starved urchins were ever observed to consume the kelp in trials that contained the cue, and though they tended to consume more when the *Pycnopodia* cue was present (mean = 0.74 ± 0.03 g) compared to when it was not (mean = 0.46 ± 0.39 g), this difference was not statistically significant (Figure 5.3D).

4. DISCUSSION

We found that waterborne cues of the sunflower sea star *Pycnopodia* decreased short-term feeding rates (~27 h) of the purple sea urchin *S. purpuratus*, as well as changed urchin movement patterns, through non-consumptive effects (NCEs). In one-hour behavior trials, urchin feeding behavior was further modified by the starvation state of the urchins, with starved urchins moving less than fed urchins, primarily to feed on kelp regardless of predator cue presence or absence. Urchins in a metabolic state similar to those in barrens, which had been starved for seven weeks, would feed on kelp, regardless of a *Pycnopodia* cue. This suggests that starved urchins in urchin barrens may continue to feed on kelp even as *Pycnopodia* populations begin to rebound, which may inhibit the role of NCEs on the recovery of kelp forest habitats from an urchin barren state. The current proliferation of purple urchins across the West Coast has coincided with kelp declines across much of the same area and direct consumption of kelp by urchins, suppression of kelp juveniles, and stressors directly associated with climate change including warming waters have also been implicated in this decline (Pfister et al. 2018, Rogers-Bennett & Catton 2019, Beas-Luna et al. 2020, McPherson et al. 2021). The amelioration of a single-origin stressor in the form of over-grazing by urchins, though only one of several multiple stressors, could lower the tipping point for alternate stable states of former kelp forests.

Pycnopodia is an important member of a diverse range of benthic marine ecosystems, and has been identified as a key mesopredator in kelp forest communities (Schultz et al. 2016, Burt et al. 2018). As *Pycnopodia* begin to recover from sea star wasting disease, they may mitigate the impact of urchins that are overpopulated in places where kelp has been extirpated (Galloway et al. 2023). Our study showed that there is a measurable and significant non-consumptive effect of *Pycnopodia* on sea urchin feeding rates and behavior that may benefit kelp, particularly on short time-scales; these NCEs may have important consequences for kelp ecosystems. As *Pycnopodia* population densities begin to rebound, a larger proportion of grazing sea urchins in subtidal communities will be subject to both direct consumptive and non-consumptive interactions, potentially reducing grazing rates on a population-wide and a per-capita basis. Although the single mass of the average *Nereocystis* individual is ~1200 g (Stekoll et al. 2006), well beyond the feeding capacity of even a dense aggregation of urchins, it has been found that even small biomass removal, particularly from the stipe of canopy kelps can reduce kelp resistance to waves and currents, detaching the entire kelp plant and, thus, removing it from the system (Duggins et al. 2001). The release of urchins from the direct effects of *Pycnopodia* predation is identified as one causal link leading to kelp forest decline, as *Pycnopodia* may consume up to 0.68 ± 0.33 urchins per day (Galloway et al. 2023). Our work demonstrates that, in addition to this direct consumptive effect, the waterborne cues of nearby *Pycnopodia* likely exert a non-consumptive effect on urchins by suppressing grazing rates over 50%. Even if *Pycnopodia* do not directly consume purple urchins at such high rates, as what has been found in the lab, the non-consumptive effects documented here represents an important reduction in urchin feeding and a potentially important TMII, in which *Pycnopodia* benefit kelp by suppressing urchin grazing.

This work also broadens our knowledge about urchin response to NCEs and how direct chemosensory detection of a sea star predator is sufficient to alter the urchins' behavior. Much work to date on the sea urchin response to predation cues has focused on their reaction to damaged conspecifics as an alarm cue, rather than the presence of a predator itself (Campbell et al. 2001, Spyksma et al. 2017, Belleza et al. 2021). While this is a good proxy for actively feeding predators that may break or otherwise damage urchins such as sea otters and spiny lobsters, it does not account for the potential effects of predators that are not actively feeding. When the effect of a predator cue has been tested directly on urchins, it has been done with gastropod (*Hexaplex trunculus*) and arthropod (*Panulirus interruptus*) predators (Pagès et al. 2021, Knight et al. 2022). Similar to Knight et al. (2022), we found that starved urchins tended to consume kelp whether a predator was present or not, though their study did detect a difference in consumption between predator treatments in the case of well-fed urchins, while in our movement experiment fed urchins did not consume kelp in any treatment, including those without a *Pycnopodia* cue. This difference could be a result of a number of factors, including differential response to particular predators (*Pycnopodia* versus lobster), an effect of urchin density cues in treatments (tested individually versus with conspecific cue), or a statistical effect (n = 8 in Knight et al. (2022) versus n = 71 in this study). In other studies, urchins exposed to gastropod predator cues have been shown to exhibit a more linear and fast movement escape response than urchin movement without the cue (Pagès et al. 2021). This is in contrast to our study, where we did not detect a statistical difference in total distance traveled or proportion of time spent moving in response to a *Pycnopodia* cue between fed and starved urchins, though starved urchins moved slightly less during the movement experiment compared to fed ones, and there was a slight reduction in distance traveled when fed urchins were exposed to a *Pycnopodia* cue only. This

raises the possibility that response of urchins to a predator cue is highly variable or dependent upon other factors such as conspecific density, urchin size variation, or shelter availability (Nishizaki & Ackerman 2007, Green 2012).

Predator speed and hunting style may also affect the response of urchins to a particular predator cue. Starved urchins moved less than fed urchins, regardless of *Pycnopodia* presence, though this difference was most pronounced when there was no predator cue but with kelp offered. This is most likely a hunger-response by the starved urchins to the kelp cue, which suggests that the non-consumptive waterborne *Pycnopodia* cue was not sufficient to inhibit urchin feeding. This may also be a metabolic consequence of the starved urchins as they are known to divert energy from reproduction into maintenance and need access to food to quickly restore metabolic activity and replenish somatic reserves (Smith & Garcia 2021). Both the hunger and metabolic consequences of starvation could indicate that food access despite the danger of predation is a stronger incentive than predator avoidance for starved urchins.

The fact that starved urchins increased total distance traveled in the case of a *Pycnopodia* cue compared to no predator cue could suggests predator evasion, although the overall patterns we observed were not strong enough to for unequivocal conclusions. The same is not true of the fed urchins, which, while moving greater distances than starved urchins in general, had decreased movement distances in the presence of a *Pycnopodia* cue alone. This seems to be contrary to a predator flight response evident in starved urchins, and these differences highlight the need to further study indirect effects of sea star predators on urchins.

Pycnopodia are generalist scavengers and opportunistic predators that are highly mobile within their environment, and are known to elicit escape responses by green and red sea urchins (Paul & Feder 1975, Freeman 2006, Montgomery 2014). This mobility may increase the reach of

the *Pycnopodia*'s non-consumptive effects, especially where urchins are congregated in dense aggregations. Such aggregations are most common in urchin barrens (Rowley 1989), which typically represents urchins in starved conditions (Smith & Garcia 2021, Dolinar & Edwards 2021). Considering the difference in urchin behavior in terms of feeding and movement in the presence of a *Pycnopodia* cue (see above), the effect of a predator cue would likely be different in urchin barrens where the urchins are in a relatively starved state compared to well-fed urchins in kelp habitats.

Studies that have targeted the non-consumptive interactions between *Pycnopodia* and sea urchins have found that urchin size (Freeman 2006), water temperature (Bonaviri et al. 2017), and urchin species identity (Duggins 1983) can result in different feeding or escape behaviors. Additionally, it has been found that juvenile and small urchins seek shelter by moving closer to the spine canopy of larger conspecifics (Nishizaki & Ackerman 2007). This behavior may allow urchins that have not reached a size-refuge from *Pycnopodia* predation to continue feeding, as has been seen in cryptic urchins exposed to predator cues (Green 2012). Interestingly, we did not detect a statistical effect of urchin size across any of our experiments, however, we did detect a trend in urchin size and feeding rate in the feeding experiment (Figure 5.4). As we targeted a homogeneous diameter size specifically, it's possible that the size effect was dampened, and a greater effect of size would be seen if a larger range of urchins sizes were incorporated into the experiment.

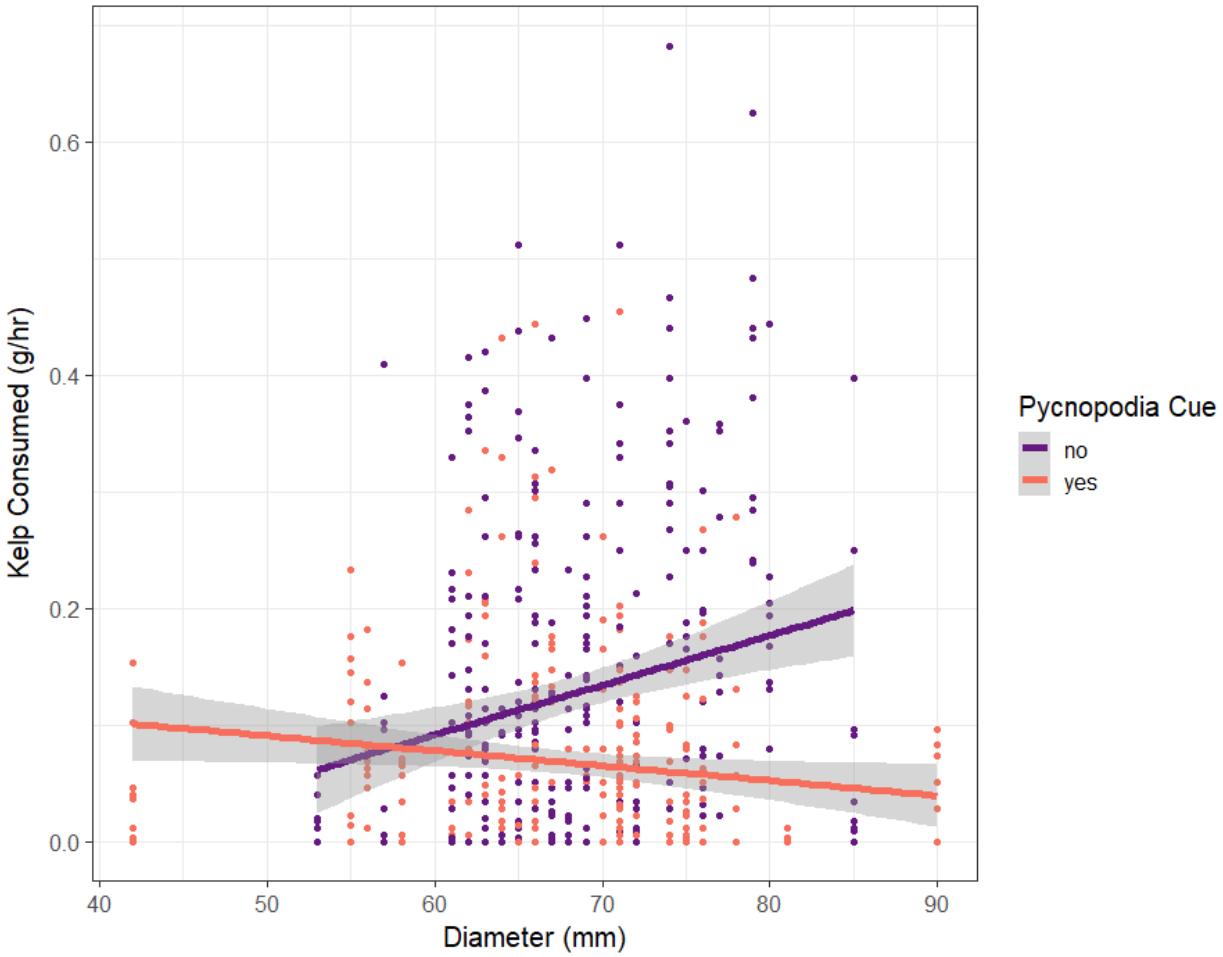


Figure 5.4: Relationship between urchin test diameter and mean quantity of kelp consumed per hour when exposed (light orange) and not exposed (dark purple) to a *Pycnopodia* cue. The trend is not statistically significant, but indicates that size may play a role in urchin feeding behaviors when exposed to a predator cue.

This study demonstrated a non-consumptive behavioral effect of *Pycnopodia* on *S. purpuratus* in laboratory conditions, but the potential ecological impact of this effect would be dependent upon *in situ* conditions and the ability of urchins to detect *Pycnopodia* cues in nature, which are subject to diffusion, wave action, and currents (Chivers et al. 2013). In general, however, the importance of water-borne chemical cues mediating ecological responses in the marine environment have been well documented (e.g., Zimmer & Butman 2000, Hadfield & Paul 2001). On the one hand, this 'dilution' of the predator cue may reduce or negate any indirect

effect that itinerant *Pycnopodia* may have on urchins. On the other hand, if the detection threshold is low enough, it may instead spread the cue over a wide area, affecting a large number of urchins, especially densely aggregated urchins in a barren. In addition, the frequency and duration of these cues may produce different responses in urchins depending on whether they become accustomed to a predator cue over either long-term, or repeated short-term exposure. In contrast, the accumulated exposure to predator cues has also been shown to "train" marine invertebrates in improving their escape response to predators (Rochette et al. 1998). It remains to be seen which long-term effects *Pycnopodia* may have on urchins, whether that be persistent cryptic behavior (Spyksma et al. 2017), habituation (found in holothuroids; Hamel et al. 2021), or something in between. Although we found short-term (3 h to two days) behavioral changes, the effects of concentration and periodicity of predator cues require more study. Future directions to expand our knowledge of how *Pycnopodia* might indirectly affect sea urchins include: 1) What is the strength of a *Pycnopodia* cue *in situ* (i.e., at what distance can an urchin detect a *Pycnopodia* in the water)?; 2) What is the starvation threshold at which a sea urchin will risk predation to acquire food?; 3) How long will a *Pycnopodia* suppress the appetite of a sated sea urchin (i.e., will a sea urchin acclimate to the *Pycnopodia* cue over time)?; 4) Does a sea urchin's size modulate the risks it will take to acquire food?; and 5) Do multi-species predator cues elicit a stronger behavioral response from sea urchins?

Scaling up the effects of individual urchins in our experiments, it is possible that the laboratory-observed ~50% reduction in feeding with *Pycnopodia* waterborne cues could have a large effect on kelp persistence. Given that *N. luetkeana* can grow up to 6% in body size a day (Kain 1987), the reduction in urchin grazing intensity could result in a considerable increase in biomass build-up of kelp in the presence of *Pycnopodia* and assist in the long-term re-

establishment of local kelp forests (Galloway et al. 2023). Once a kelp forest establishes, the delivery of drift kelp through wave and current action can provide a large proportion of urchin diet in multiple systems, inhibiting feeding on live kelp plants (Lowe et al. 2015, Kriegisch et al. 2019). This work provides key findings on the effects of non-consumptive effects between *Pycnopodia* and their *S. purpuratus* prey, and contributes new knowledge to our understanding of the indirect effects of *Pycnopodia* on kelp. The results show that re-establishment of *Pycnopodia* populations could assist in the re-establishment of currently degraded kelp forests in the Northeast Pacific based on indirect predator-prey interactions.

CHAPTER VI

CONCLUSION

This dissertation highlights the community ecology of macroalgal forests with a broad geographic, taxonomic, and methodological scope, from the scale of algal tissues, to the scale of regional marine protected areas. I have identified the unique fatty acid profiles and stable isotope content of 31 Antarctic macroalgae, provided a summary of the effects of conservation areas on algal assemblages, and provided evidence for non-consumptive effects and feeding preferences of the sunflower seastar on grazing sea urchins.

Chapter II described biomarker profiles from common Antarctic macroalgae across the WAP, and suggested that a combination of biomarkers (i.e., fatty acids and stable isotopes) can provide additional information about food webs with more taxonomic resolution than a single type of biomarker alone. In line with previous fatty acid studies, we found that most species were rich in polyunsaturated fatty acids, suggesting they could be a resource of essential fatty acids for higher consumers. Fatty acid profiles of macroalgae grouped strongly by phylogenetic divisions, while stable isotopes were able to differentiate some closely-related species based on physiological differences. While the phylogenetic differentiation driven by fatty acids had a stronger influence on distinguishing Antarctic macroalgae, the added dimension of stable isotopes can likely make the combination of the two approaches particularly powerful in the application of food web studies.

In Chapter III I used long-term data from the Oregon Marine Reserves to identify the distribution of target macroalgal species inside and outside of the marine reserves and determine

if and how they have changed through time. Additionally, I provided methodological guidance on conducting more robust surveys in the future to capture changes in the community. I found that all targeted kelp species were observed across the marine reserves, but their presence varied among sites and years. I also found that monitoring data suggested declines in macroalgal cover over time, but this was likely attributable to methodological shifts, not true biological changes. These methodological shifts were primarily a result of the difficulties associated with adapting SCUBA surveys to open ocean conditions in Oregon, thereby limiting our ability to survey prime subtidal kelp habitat. To address these methodological difficulties I suggested several changes to the monitoring program's SCUBA surveys including: using permanent transects to detect changes in brown algal densities; constraining surveys to shallower (<10 m) depths to target the photic zone where macroalgae are found; survey during the peak production season (July-August); and adding UAV surveys at select sites to improve tracking of macroalgal focal species.

Chapter IV moved from the whole community, to the role of a single important generalist predator, the sunflower sea star *Pycnopodia helianthoides*. Our goal was to summarise the feeding preferences of *Pycnopodia* through a meta-analysis of existing literature on the wild diet of *Pycnopodia* and a cafeteria-style experiment to determine the feeding preferences of *Pycnopodia* on sea urchins and a suite of common benthic rocky habitat prey species. We identified only six published datasets in the literature that quantitatively described the wild diet of *Pycnopodia*. A total of 114 prey taxa were reported across all studies, with bivalves and urchins tending to be the primary observed prey items. This agreed with our cafeteria experiments that found *Pycnopodia* tended to prefer green and purple urchins, and mussels, although the quantity of each prey type consumed was highly variable. We also found that *Pycnopodia* tended to consume smaller purple and red urchins than the mean size of those

species that were offered. These results suggested that *Pycnopodia* is a general predator but does exhibit a preference for urchins, supporting the idea that *Pycnopodia* are important in maintaining kelp forest health and resilience.

Finally, in Chapter V I explored the non-consumptive effects of *Pycnopodia* on their prey, the purple sea urchin *Strongylocentrotus purpuratus*, who is a primary kelp consumer across much of the West Coast of North America. Recent work has begun to quantify the predation rates effects (i.e., direct consumptive effects) of *Pycnopodia* on sea urchins that may lead to density-mediated indirect effects on kelp. However, the importance of non-consumptive effects on urchin behavior and the possible trait-mediated indirect effects of *Pycnopodia* on kelp have not been well understood. This left a critical gap in our knowledge about how these predators may be controlling grazer populations and, indirectly, primary production by macroalgae in nearshore habitats. This chapter tested the non-consumptive behavioral effects of *Pycnopodia* on *S. purpuratus* including grazing rates, feeding behavior, and movement of starved and fed urchins, the latter simulating urchin metabolic conditions within urchin barrens. I found that the presence of a waterborne *Pycnopodia* cue reduced the grazing rate of fed urchins by 50% over short (~24 h) time scales. In contrast, starved urchins consumed kelp and did not exhibit an escape response in the presence of a *Pycnopodia* cue. This chapter highlights a trait-mediated indirect interaction between *Pycnopodia*, *S. purpuratus* and kelp, and how the urchin response to a predator cue may differ based on urchin metabolic conditions. Moreover, this study builds our understanding of the importance of the decline of *Pycnopodia* from sea star wasting disease and the positive role of *Pycnopodia* on kelp forest health.

Macroalgal forests are critical to the health of our coastal oceans, providing countless ecosystem services, both objectively quantifiable—as I have demonstrated in this dissertation—

and abstract. I am drawn to these forests primarily by their abstract qualities, their beauty, their cultural significance, and the indescribable feeling I experience when I am diving beneath their canopy. My greatest hope is that the objectively quantifiable qualities of macroalgal forests that I have presented in this dissertation will help us preserve, support, and rejuvenate them now and for years to come, so that future generations can experience their beauty as well.

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