Trophic transfer of nearshore basal resources: interpreting fatty acid and stable isotope biomarkers

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Abstract

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Benthic heterotrophs living in aphotic aquatic habitats rely upon subsidies of detrital energy, in the form of complex organic molecules, which are synthesized by macrophytes and phytoplankton in the photic zone. Identifying the relative importance of different basal energy resources to consumers is critical for understanding ecosystem function and trophic connectivity in aquatic habitats. Partitioning the contribution of differing detrital resources to deep nearshore subtidal consumers is impractical using solely observational techniques such as surveys of macrophyte biomass or gut content analyses. Biochemical markers such as fatty acids (FA) and multiple stable isotopes (MSI; collectively FAMSI) can help resolve which sources are actually important in consumers, but several key uncertainties in the assumptions of the approach limit its utility. In this dissertation, I used a combination of observational and

experimental approaches to determine what heterotrophs are eating using a FAMSI signature analyses. In Chapter 2, I tested whether different marine macrophyte taxa have distinct FA signatures. I found that algal FA signatures are closely linked to phylogeny; regardless of collection region, families, orders and phyla differ strongly from one another. In Chapter 3, I compared FAMSI signatures of a diverse group of conspecific consumers across photic and aphotic depths to evaluate the hypothesis that consumer biomarkers would reflect evidence of basal resource subsidies from the shallow photic zone. A diverse assemblage of organisms had FAMSI signatures that differed among depths. In an algal aging experiment, I found support for a microbe-induced diagenesis hypothesis to explain the biochemical differences found in the field. In Chapter 4 I used a fast-growing herbivorous isopod in a laboratory feeding trial to test the hypothesis that the FAMSI signatures of diets are assimilated predictably into consumer tissue. This experimental work took advantage of the earlier discovered patterns from Chapter 2 and evaluated whether the fractionation of FAMSI signatures between diets and consumers was diet-specific. Each of these three chapters represents an important step in advancing the FAMSI biomarker field beyond qualitative observational studies that are based largely on untested assumptions.

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CHAPTER 1- TRACKING BASAL RESOURCES INTO NEARSHORE FOOD WEBS BY FOLLOWING THE BIOMARKERS

Introduction

Heterotrophs living in aquatic environments below the photic zone depend upon inputs of detrital energy generated by a diverse array of primary producers. Regardless of the source, energy fixed into usable molecules by primary producers constrains secondary trophic production (Power 1992) and is processed through the food chain by interrelated and cyclical networks of microbes, herbivores, detritivores, scavengers, and predators (Hunter and Price 1992). The range of conditions that are suitable for such production to occur varies widely and organisms have exploited nearly every niche possible on earth; an example being the recent discovery of the generally saline aquatic unicellular alga *Dunaliella* growing on spider webs in the twilight openings of caves in the high altitude Atacama desert (Azúa-Bustos et al. 2010). There are nonetheless limitations to the conditions suitable for primary production, particularly in aquatic habitats where light attenuation with increasing depth constrains where photosynthesis is possible. The vast majority (~95%) of the ocean is below this critical 'photic zone' depth (Jørgensen and Boetius 2007).

Where do organisms in aphotic habitats, distant from sources of photosynthetic or chemolithotrophic primary production, get their energy? One reason this question is so fascinating is that the overall biomass of heterotrophs in aphotic habitats is not insignificant; for example Whitman et al. (1998) estimated that deep ocean sub-seafloor prokaryotic life (i.e., microbes living within the lithosphere) alone accounted for an astonishing one third of the total

biomass of all organisms (terrestrial and aquatic) on earth. Such estimates of subsurface benthic biomass have been challenged (Kallmayer et al. 2012) and significantly reduced (12-72 fold reduction; Jørgensen 2012) due to improved sampling of the seafloor across a wider variety of habitats. This is one example of how our best estimates of key patterns and processes in the deep oceans still have uncertainty spanning orders of magnitude. Benthic heterotrophs in aphotic depths are supported by the flux of particulate organic carbon (POC) originating from photosynthetic organisms (Smith et al. 1996) or from major biomass falls (e.g., whales; Smith et al. 1989). Interestingly, even at hydrothermal vents, the generation of biomass by chemoautotrophic microbes still requires photosynthetically-derived oxygen and nitrate (reviewed by Jørgensen and Boetius 2007). Hotspots of chemosynthetic production are limited in spatial extent and are very patchy and rare compared to the immense size of the ocean floor (Ramirez-Llodra 2010).

The vast majority of the benthic communities in the marine environment that do not have access to chemolithotrophic production are also in aphotic habitats. In clear tropical waters, the functional maximum depth of photosynthetic organisms can be as deep as ~200 m (Thistle 2003). However, most marine primary production occurs in temperate and arctic oceans in areas where nutrients are plentiful (due to upwelling or terrestrial discharges), and in these environments the depth limits to the vast majority of primary production are usually between 25-30 m. Deep nearshore subtidal environments (DNSE), which I define here as coastal and inland sea benthic habitats below the photic depths (~30 m), occupy a transitional zone beyond photosynthetic compensation depth but still relatively accessible for observational and experimental research. Benthic heterotrophs (whether herbivore, scavenger, or predator) living in

the DNSE depend upon inputs (subsidies) of complex, detrital organic molecules from photosynthetic organisms in shallower zones.

Marine subsidies

In recent decades, ecologists have described the flux of energy from productive to relatively resource deprived habitats, in the form of nutrients, organisms, or materials, as "spatial subsidies" (Polis et al. 1997). One of the earliest known acknowledgements of this process in the literature comes from Summerhayes and Elton (1923), who noted that on Bear Island in the high arctic, "…a large part of the food supply of animals and plants comes from the sea" (p. 232). Such observations in the early literature were an acknowledgement of the subsidy concept, but the importance of this process would likely have been opaque to ecologists at the time. Considering the transfer of photosynthesis-produced complex detrital molecules in the framework of spatial subsidy theory is valuable because there is a well-developed and informative literature about the expected processes and outcomes of these subsidies. In particular, the theory describes that the rate of the subsidy to the recipients is donor-controlled (Polis et al. 1997), meaning for example that aphotic benthic consumers cannot modulate rate of the subsidy input. Subsidy driven ecosystems therefore may not fluctuate in a typical boom-bust predator-prey oscillation, where consumers modulate or control (Power et al. 1992) prey populations. Subsidies from productive to aphotic habitats (hereafter referred to simply as spatial subsidies) are diverse in their form and function, ranging from large organism 'falls' (Smith et al. 1989), to the direct transfer and deposition of primary producers (e.g., phytoplankton, terrestrial plants, seagrasses, macroalgae; Fig. 1.1), which can support hotspots of secondary production (Vetter 1994).

An important marine spatial subsidy is the delivery of macrophyte (i.e., seagrasses and macroalgal)- derived material, from shallow subtidal photic environments (SSPE, ~0-30 m depth), to subtidal aphotic environments (~>30 m depth; Fig. 1.2). Macroalgal primary production in temperate SSPE, measured as g C fixed m^2 $year^{-1}$, rivals the most productive systems on earth (e.g., Mann 1973, Duggins 1980). Approximately 10% of this production is consumed by herbivores (Mann 1988), and the remainder is exported to subtidal (Duggins et al. 1989), intertidal (Rodriguez 2003), pelagic (Kaehler et al. 2006), and terrestrial (Polis and Hurd 1995) food webs. The importance of algal detritus as a trophic input to consumers has been demonstrated for several systems, including submarine canyons (Harrold et al. 1998, Vetter and Dayton 1998, Okey 2003), offshore soft-sediment habitats (Kim 1992), and deep rocky-reefs (Vanderklift and Wernberg 2008, Britton-Simmons et al. 2009, 2012). The first evidence of a deep-water macrophyte subsidy was the discovery of the seagrass *Thalassia* on the abyssal sea floor (>3100 m) >500 km from the nearest seagrass bed (Menzies et al. 1967). Not surprisingly, the rates of such exports appear to be a key factor in subtidal community structure, and resulting benthic invertebrate secondary production in subtidal nearshore ecosystems may vary considerably with distance from the source of production (Krumhansl and Scheibling 2012). Researchers are increasingly utilizing molecular biomarkers for tracking energy subsidies.

Why elements and molecules

There are several commonly used approaches for the study of macroalgal subsidies to DNSE. One strategy is to conduct physical in situ surveys using remotely operated vehicles (ROVs), drop cameras, SCUBA divers, or submarines to quantify standing biomass at depth (e.g., Menzies et al. 1967, Field et al. 1977, Suchanek et al. 1985, Harrold et al. 1998, Vetter and Dayton 1998, Britton-Simmons et al. 2012). A second approach is to focus on quantifying the

rate of production (in dry g C m^2 day^{-1}) at the scale of the individual algal thallus (Duggins 1980, Krumhansl and Scheibling 2011, 2012) and generate estimates of net primary production (NPP) at large scales using estimates of macrophyte standing stock (Newell et al. 1980, Reed et al. 2009). NPP is then used to further estimate the rate of export of a fraction of this production to the DNSE (e.g., Wilmers et al. 2012). A third approach is to use multiple stable isotope (MSI) ratios of C, N, or S (Duggins et al. 1989, Kaehler et al. 2000) and fatty acids (FA; Kharlamenko et al. 1995, Meziane and Tsuchiya 2000, Copeman et al. 2009, Kelly and Scheibling 2012) as molecular biomarkers for tracing different basal producer groups specifically and for tracking food web interactions generally (Fig 1.3, Table 1.1). Increasingly researchers are pairing both biomarker (collectively referred to as FAMSI here) approaches (e.g., Canuel et al. 1995, Rooker et al. 2006, Turner and Rooker 2006, Budge et al. 2008) to take advantage of the strengths of each method. The primary strength of a biomarker approach for trophic questions is that it provides a way to track what sources are actually consumed by heterotrophs, not just what is available (standing stock surveys) or how much is produced (NPP estimates).

FA have distinct chemical structures, and it is possible to routinely identify >50 FA within a given marine organism (Iverson 2009). A common approach for the study of autotrophic subsidies is to evaluate consumer tissues for the presence and abundance of basal resource-specific 'FA biomarkers' (e.g., Parrish et al. 2000, Dalsgaard et al. 2003, Copeman et al. 2009). A long list of individual or 'classes' of FA have been identified as indicative biomarkers for diatoms, dinoflagellates, terrestrial plants, macroalgae, seagrasses, bacteria, etc. (Budge et al. 2001, Kelly and Scheibling 2012). Furthermore, certain ω3 and ω6 essential fatty acids (EFA; Table 1.1) are important for the growth, survival, and reproduction in a wide range of aquatic organisms (Brett and Muller-Navarra 1997), but are only generated in significant amounts by

aquatic algae (Gladyshev et al. 2009), making them potentially useful as biomarkers. Several studies have focused on the contribution of phytoplankton-derived EFA to aquatic food webs (e.g., Kainz et al. 2004, Ravet et al. 2010), but the importance of macroalgal-originated EFA in nearshore ecosystems has generally been overlooked. This is particularly surprising due to the high productivity (Mann 1973) and importance of benthic algae to nearshore fish and invertebrate communities (Steneck et al. 2002). The extent and importance of macroalgal derived EFA to subtidal food webs is an area in need of further investigation.

Multiple stable isotope (MSI; $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) analysis is an additional valuable biomarker tool for ecologists (Fry 2006). Carbon isotope ratios (mean ±SD) are assumed to change very little (0.4±1.3‰; Post 2002) between trophic levels, and can be used to track different autotrophs. In the nearshore northeastern Pacific Ocean, for example, $\delta^{13}C$ ratio of phytoplankton is generally more deplete (e.g., -20.4 to <-25‰; Miller at al. 2013, Fry 1996, respectively) compared to Laminarian kelps and seagrasses (-15.9‰ and -10.9‰ respectively; Dethier et al. 2013). When the range of $\delta^{13}C$ signatures fixed by primary producers is sufficiently large and unique among the different producers, these rations can be traced into consumer tissues (Duggins et al. 1989). Nitrogen isotope ratios are used for estimating the number of trophic levels between a consumer and its diet because consumers are enriched in $\delta^{15}N$ ratios by an average value of +3.4±1.0‰ between trophic levels (Cabana and Rasmussen 1996; Post 2002). Recent research has acknowledged that 'simple' fractionation may vary considerably across consumer taxa (Woodland et al. 2012). The sulfur isotope ratio is not known to change substantively between trophic levels, and can indicate whether the sulfur came from oceanic or terrestrial sources, making it useful as an indicator of geographical origin (e.g., drainage or basin) of organic material (Peterson and Fry 1987; Fry 2006).

Not so elementary after all

Using ratios of stable isotopes and the abundance of diverse total extracts of fatty acids from the tissues of animals offers great promise for helping to sort out the importance of disparate basal resources to consumers. However, both methods are limited by our incomplete understanding of how distinct biomarker signatures are in the primary producers and how biomarkers are altered by diagenesis, bacterial processing, and food web trophic transfer. For example, a potential complicating factor for the use of FA in identifying trophic interactions arises because invertebrates can, to varying degrees, modify and bioconvert new FA molecules via desaturase and elongase enzymes (Dalsgaard et al. 2003). Animals cannot synthesize long-chain essential fatty acids (EFAs) de novo, but may be able to bioconvert them through modification of other shorter chain (C_{18}) ω3 and ω6 FA (Table 1.1). Most published research using FA has not investigated the actual modification of lipid composition relative to diet except in a few organisms (e.g., freshwater zooplankton, Brett et al. 2006), even though researchers are aware that consumers can modify the FA that they obtain from their diets (Dalsgaard et al. 2003). Knowing the actual FA "fractionation" (preferential catabolism and retention, as well as desaturation and chain-elongation) is critical when attempting to infer diet from consumer lipid profiles. Thus, the use of FA as dietary tracers for invertebrate consumers requires controlled feeding experiments (Kelly et al. 2008) to determine a consumer's tendency to modify the biomarker profiles being used.

There are several other important uncertainties regarding biomarker trophic transfer and the methods commonly used for evaluating biomarkers in food webs that need further investigation. There is limited empirical evidence demonstrating either consumer fractionation or tissue turnover time for MSI biomarkers from diets into consumer tissues (see Gannes et al.

1997, del Rio et al. 2009). For both MSI and FA, inference about turnover time in any given organism is likely to differ with the assay tissue (e.g., muscle, digestive gland; Napolitano and Ackman 1993), yet little is known about tissue turnover times for the vast majority of animals studied. For example, it is generally assumed that MSI signatures of muscle tissue from wild animals represent an integration of diet for time periods ranging from weeks to months (Fry 2006), but rarely have these assumptions been tested with robust experiments. Biomarker approaches generally assume that all important food sources are identified, that variation is adequately characterized, and represented in mixing models. Finally, in order to partition contribution of primary producers to consumers using mixing models, biochemical signatures of the basal resources need to have distinct FAMSI signatures.

In my dissertation, I have used a combination of observational and experimental approaches to advance our understanding for several critical assumptions to the FAMSI biomarker approach. The ultimate goal of this work is to quantify the importance of different basal resources to consumers in the DNSE using rigorous quantitative application of FAMSI biomarker data. Each of the following chapters is now either published, in revision, or in review at peer-reviewed journals (see Acknowledgments and Collaborators by Chapter for lists of co-authors and collaborators). The first critical assumption for using biomarkers for tracking basal production through food webs is that the primary producer groups are sufficiently different from each other so that consumers eating these sources would be expected to differ. In Chapter 2, I gathered a broad range of macrophyte taxa and asked what the taxonomic 'resolution' was of the algal FA signatures (Galloway et al. 2012). In addition to the food web implications, the broad and strategic sampling of a diverse algal assemblage provided an previously unprecedented opportunity to consider algal FA signatures within the context of algal phylogeny and

systematics. In Chapter 3, I compared FAMSI signatures of a diverse group of conspecific consumers across photic and aphotic depths to evaluate the hypothesis that consumer biomarkers would provide evidence for spatial subsidies (Galloway et al. *in press*). I used an algal aging experiment (e.g., Sosik 2012) to evaluate a post-hoc hypothesis that consistent biochemical differences between conspecifics were due to detrital diagenesis. In Chapter 4 I conducted a controlled feeding trial in the lab with an herbivorous isopod to test the hypothesis that diet FAMSI signatures are assimilated consistently and predictably into consumer tissues (Galloway et al. *in review*). This research also enabled me to evaluate whether the fractionation between diets and consumers of FAMSI signatures was diet-specific. Each of these three chapters represents an important step in advancing the FAMSI biomarker field beyond qualitative conclusions based on untested assumptions.

This research is a fundamental component of a larger ongoing project aimed at tracing which sources of basal production are supporting subtidal consumers (Fig. 1.1) in the inland sea DNSE of the Salish Sea in the northeastern Pacific Ocean. I co-authored several additional papers during my work on the algal subsidy project in addition to my dissertation chapters. In Britton-Simmons et al. (2012), we used and ROV to survey drift macrophyte abundance in the DNSE of San Juan Channel. We found that detached, drift algal material and red urchins (*Strongylocentrotus franciscanus*) were common across all depths surveyed. In follow up research, we used SCUBA surveys to show that red urchins below the macroalgal zone (deeper than 20 m) move very little, and that there are clear consequences to this sedentary behavior to benthic invertebrate community composition (Lowe et al. *in review*). In Galloway et al. (2012; Chapter 2), we found order and family level resolution of FA signatures of macrophytes; a follow up project assessed the seasonal and geographic variation of FAMSI biomarkers in

macrophytes and the potential implications of such variation on a Bayesian mixing model analysis (Dethier et al. 2013). In an additional collaboration, (Taipale et al. *in review*) we found strong phylogenetic differentiation in the FA signatures of an extensive assemblage of freshwater microalgae.

Literature cited

Azua-Bustos, A., C. Gonzalez-Silva, L. Salas, R. E. Palma, and R. Vicuna. 2010. A novel subaerial *Dunaliella* species growing on cave spiderwebs in the Atacama Desert. Extremophiles **14**:443-452.

Brett, M. T. and D. C. Muller-Navarra. 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biology **38**:483-499.

Brett, M. T., D. C. Muller-Navarra, A. P. Ballantyne, J. L. Ravet, and C. R. Goldman. 2006. *Daphnia* fatty acid composition reflects that of their diet. Limnology and Oceanography **51**:2428-2437.

Britton-Simmons, K. H., G. Foley, and D. Okamoto. 2009. Spatial subsidy in the subtidal zone: utilization of drift algae by a deep subtidal sea urchin. Aquatic Biology **5**:233-243.

Britton-Simmons, K. H., A. L. Rhoades, R. E. Pacunski, A. W. E. Galloway, A. T. Lowe, E. A. Sosik, M. N. Dethier, and D. O. Duggins. 2012. Habitat and bathymetry influence the landscape-scale distribution and abundance of drift macrophytes and associated invertebrates. Limnology and Oceanography **57**:176-184.

Budge, S. M., C. C. Parrish, and C. H. McKenzie. 2001. Fatty acid composition of phytoplankton, settling particulate matter and sediments at a sheltered bivalve aquaculture site. Marine Chemistry **76**:285-303.

Budge, S. M., M. J. Wooller, A. M. Springer, S. J. Iverson, C. P. McRoy, and G. J. Divoky. 2008. Tracing carbon flow in an arctic marine food web using fatty acid-stable isotope analysis. Oecologia **157**:117-129.

Cabana, G., and J. B. Rasmussen. 1996. Comparison of aquatic food chains use in nitrogen isotopes. Procedings of the National Academy of Sciences of the United States of America **93**:10844-10847.

Canuel, E. A., J. E. Cloern, D. B. Ringelberg, J. B. Guckert, and G. H. Rau. 1995. Molecular and isotopic tracers used to examine sources of organic matter and its incorporation into the food webs of San Francisco Bay. Limnology and Oceanography **40**:67-81.

Copeman, L. A., C. C. Parrish, R. S. Gregory, R. E. Jamieson, J. Wells, and M. J. Whiticar. 2009. Fatty acid biomarkers in coldwater eelgrass meadows: elevated terrestrial input to the food web of age-0 Atlantic cod *Gadus morhua*. Marine Ecology Progress Series **386**:237-251.

Dalsgaard, J., M. St. John, G. Kattner, D. C. Muller-Navarra, and W. Hagen. 2003. Fatty acid trophic markers in the pelagic marine food environment. Advances in Marine Biology **46**:225-340.

del Rio, C. M., N. Wolf, S. A. Carleton, and L. Z. Gannes. 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biological Reviews **84**:91-111.

Dethier, M. N., E. A. Sosik, A. W. E. Galloway, D. O. Duggins, and C. A. Simenstad. 2013. Addressing assumptions: variation in stable isotopes and fatty acids in marine macrophytes can confound conclusions of food web studies. Marine Ecology Progress Series **478**:1-14.

Duggins, D. O. 1980. Kelp beds and sea otters: an experimental approach. Ecology **61**:447-453.

Duggins, D. O., C. A. Simenstad, and J. A. Estes. 1989. Magnification of secondary production by kelp detritus in coastal marine ecosystems. Science **245**:170-173.

Field, J. G., N. G. Jarman, G. S. Dieckmann, C. L. Griffiths, B. Velimirov, and P. Zoutendyk. 1977. Sun, waves, seaweed and lobsters: the dynamics of a west coast kelp bed. South African Journal of Science **73**:7-10.

Fry, B. 2006. Stable isotope ecology. Springer.

Fry, B. 1996. $^{13}C/^{12}C$ fractionation by marine diatoms. Marine Ecology Progess Series **134**:283-294.

Galloway, A. W. E., K. H. Britton-Simmons, D. O. Duggins, P. W. Gabrielson, and M. T. Brett. 2012. Fatty acid signatures differentiate marine macrophytes at ordinal and family ranks. Journal of Phycology **48**:956-965.

Galloway, A. W. E., A. T. Lowe, E. A. Sosik, J. S. Yeung, and D. O. Duggins. *in press*. Fatty acid and stable isotope biomarkers suggest microbe-induced differences in benthic food webs between depths. Limnology and Oceanography.

Galloway, A. W. E., M. E. Eisenlord, M. N. Dethier, and M. T. Brett.. Diet-specific fractionation of fatty acids and $\delta^{15}N$ in an herbivorous marine isopod. *in review*, March 2013.

Gannes, L. Z., D. M. Obrien, and C. M. del Rio. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology **78**:1271-1276.

Gladyshev, M. I., M. T. Arts, and N. N. Sushchik. 2009. Preliminary estimates of the export of omega-3 highly unsaturated fatty acids (EPA + DHA) from aquatic to terrestrial ecosystems. Pages 179-209 *in* M. T. Arts, M. T. Brett, and M. Kainz, editors. Lipids in Aquatic Ecosystems. Springer, New York.

Harrold, C., K. Light, and S. Lisin. 1998. Organic enrichment of submarine-canyon and continental-shelf benthic communities by macroalgal drift imported from nearshore kelp forests. Limnology and Oceanography **43**:669-678.

Hunter, M. D. and P. W. Price. 1992. Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. Ecology **73**:724-732.

Iverson, S. J. 2009. Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination.*in* M. T. Arts, M. T. Brett, and M. J. Kainz, editors. Lipids in Aquatic Ecosystems. Springer, New York.

Jorgensen, B. B. and A. Boetius. 2007. Feast and famine - microbial life in the deep-sea bed. Nature Reviews Microbiology **5**:770-781.

Jorgensen, B. B. 2012. Shrinking majority of the deep biosphere. Proceedings of the National Academy of Sciences **109**:15976-15977.

Kaehler, S., E. A. Pakhomov, and C. D. McQuaid. 2000. Trophic structure of the marine food web at the Prince Edward Islands (Southern Ocean) determined by delta C-13 and delta N-15 analysis. Marine Ecology Progress Series **208**:13-20.

Kaehler, S., E. A. Pakhomov, R. M. Kalin, and S. Davis. 2006. Trophic importance of kelp-derived suspended particulate matter in a through-flow sub-Antarctic system. Marine Ecology Progress Series **316**:17-22.

Kainz, M., M. T. Arts, and A. Mazumder. 2004. Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. Limnology and Oceanography **49**:1784-1793.

Kallmeyer, J., R. Pockalny, R. R. Adhikari, D. C. Smith, and S. D'Hondt. 2012. Global distribution of microbial abundance and biomass in subseafloor sediment. Proceedings of the National Academy of Sciences of the United States of America **109**:16213-16216.

Kelly, J. R., R. E. Scheibling, S. J. Iverson, and P. Gagnon. 2008. Fatty acid profiles in the gonads of the sea urchin *Strongylocentrotus droebachiensis* on natural algal diets. Marine Ecology Progress Series **373**:1-9.

Kelly, J. R. and R. E. Scheibling. 2012. Fatty acids as dietary tracers in benthic food webs. Marine Ecology Progress Series **446**:1-22.

Kharlamenko, V. I., N. V. Zhukova, S. V. Khotimchenko, V. I. Svetashev, and G. M. Kamenev. 1995. Fatty-acids as markers of food sources in a shallow-water hydrothermal ecosystem (Kraternaya Bight, Yankich Island, Kurile Islands). Marine Ecology Progress Series **120**:231-241.

Kim, S. L. 1992. The role of drift kelp in the population ecology of a *Diopatra ornata* Moore (Polychaeta: Onuphidae) ecotone. Journal of Experimental Marine Biology and Ecology **156**:253-272.

Krumhansl, K. A. and R. E. Scheibling. 2011. Detrital production in Nova Scotian kelp beds: patterns and processes. Marine Ecology Progress Series **421**:67-82.

Krumhansl, K. A. and R. E. Scheibling. 2012. Production and fate of kelp detritus. Marine Ecology Progress Series **467**:281-302.

Lowe, A. T., R. Whippo, A. W. E. Galloway, K. H. Britton-Simmons, M. N. Dethier. *in review*, March 2013. Sedentary urchins influence subtidal benthic community composition below the macrophyte zone.

Mann, K. H. 1973. Seaweeds: their productivity and strategy for growth. Science **182**:975-981.

Mann, K. H. 1988. Production and use of detritus in various freshwater, estuarine, and coastal marine environments. Limnology and Oceanography **33**:910-930.

Menzies, R. J., J. S. Zaneveld, and R. M. Pratt. 1967. Transported turtle grass as a source of ogranic enrichment of abyssal sediments off North Carolina. Deep-Sea Research **14**:111-112.

Meziane, T. and M. Tsuchiya. 2000. Fatty acids as tracers of organic matter in the sediment and food web of a mangrove/intertidal flat ecosystem, Okinawa, Japan. Marine Ecology Progress Series **200**:49-57.

Miller, R. J., H. M. Page, and M. A. Brzezinski. 2013. $\delta^{13}C$ and $\delta^{15}N$ of particulate organic matter in the Santa Barbara Channel: drivers and implications for trophic inference. Marine Ecology Progress Series **474**:53-66.

Napolitano, G. E. and R. G. Ackman. 1993. Fatty acid dynamics in sea scallops *Placopecten magellanicus* (Gmelin, 1791) from Georges Bank, Nova Scotia. Journal of Shellfish Research **12**:267-277.

Newell, R. C., M. I. Lucas, B. Velimirov, and L. J. Seiderer. 1980. Quantitative significance of dissolved organic losses following fragmentation of kelp (*Ecklonia maxima* and *Laminaria pallida*). Marine Ecology Progress Series **2**:45-59.

Okey, T. A. 2003. Macrobenthic colonist guilds and renegades in Monterey Canyon (USA) drift algae: partitioning multidimensions. Ecological Monographs **73**:415-440.

Parrish, C. C., T. A. Abrajano, S. M. Budge, R. J. Helleur, E. D. Hudson, K. Pulchan, and C. Ramos. 2000. Lipid and phenolic biomarkers in marine ecosystems: analysis and applications. Pages 193-222 *in* P. Wangersky, editor. The handbook of environmental chemistry. Springer, Berlin.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics **18**:293-320.

Polis, G. A. and S. D. Hurd. 1995. Extraordinarily high spider densities on islands - flow of energy from the marine to terrestrial food webs and the absence of predation. Proceedings of the National Academy of Sciences of the United States of America **92**:4382-4386.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Towards an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annual Review of Ecology and Systematics **28**:289-316.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology **83**:703-718.

Power, M. E. 1992. Top-down and bottom-up forces in food webs: do plants have primacy? Ecology **73**:733-746.

Ramirez-Llodra, E., A. Brandt, R. Danovaro, B. De Mol, E. Escobar, C. R. German, L. A. Levin, P. M. Arbizu, L. Menot, P. Buhl-Mortensen, B. E. Narayanaswamy, C. R. Smith, D. P. Tittensor, P. A. Tyler, A. Vanreusel, and M. Vecchione. 2010. Deep, diverse and definitely different: unique attributes of the world's largest ecosystem. Biogeosciences **7**:2851-2899.

Ravet, J. L., M. T. Brett, and G. B. Arhonditsis. 2010. The effects of seston lipids on zooplankton fatty acid composition in Lake Washington, Washington, USA. Ecology **91**:180-190.

Reed, D. C., A. Rassweiler, and K. Arkema. 2009. Density derived estimates of standing crop and net primary production in the giant kelp *Macrocystis pyrifera*. Marine Biology **156**:2077-2083.

Rodriguez, S. R. 2003. Consumption of drift kelp by intertidal populations of the sea urchin *Tetrapygus niger* on the central Chilean coast: possible consequences at different ecological levels. Marine Ecology Progress Series **251**:141-151.

Rooker, J. R., J. P. Turner, and S. A. Holt. 2006. Trophic ecology of *Sargassum*-associated fishes in the Gulf of Mexico determined from stable isotopes and fatty acids. Marine Ecology Progress Series **313**:249-259.

Smith, C. R., D. J. Hoover, S. E. Doan, R. H. Pope, D. J. Demaster, F. C. Dobbs, and M. A. Altabet. 1996. Phytodetritus at the abyssal seafloor across 10 degrees of latitude in the central equatorial Pacific. Deep-Sea Research Part Ii-Topical Studies in Oceanography **43**:1309-1338.

Smith, C. R., H. Kukert, R. A. Wheatcroft, P. A. Jumars, and J. W. Deming. 1989. Vent fauna on whale remains. Nature **341**:27-28.

Sosik, E. A. 2012. Isotopic evidence of microbial pathways in macroalgal-detritus based coastal food webs. Master's thesis. University of Washington, Seattle.

Steneck, R. S., M. H. Graham, B. J. Bourque, D. Corbett, J. M. Erlandson, J. A. Estes, and M. J. Tegner. 2002. Kelp forest ecosystems: biodiversity, stability, resilience and future. Environmental Conservation **29**:436-459.

Suchanek, T. H., S. L. Williams, J. C. Ogden, D. K. Hubbard, and I. P. Gill. 1985. Utilization of shallow-water seagrass detritus by Caribbean deep-sea macrofauna: ^{13}C evidence. Deep-Sea Research **32**:201-214.

Summerhayes, V. S. and C. S. Elton. 1923. Contributions to the ecology of Spitsbergen and Bear Island. Journal of Ecology **11**:216-286.

Taipale, S. J., E. Peltomaa, U. Strandberg, A. W. E. Galloway, A. Ojala, M. T. Brett. Novel analysis of fatty acid biomarkers of freshwater algae. *in review*, April 2013.

Thistle, D. 2003. The deep-sea floor: an overview. Pages 5-39 *in* P. A. Tyler, editor. Ecosytems of the World, Vol. 28, Ecosystems of the Deep Oceans. Elsevier, Amsterdam.

Turner, J. P. and J. R. Rooker. 2006. Fatty acid composition of flora and fauna associated with *Sargassum* mats in the Gulf of Mexico. Marine Biology **149**:1025-1036.

Vanderklift, M. A. and T. Wernberg. 2008. Detached kelps from distant sources are a food subsidy for sea urchins. Oecologia **157**:327-335.

Vetter, E. W. 1994. Hotspots of benthic production. Nature **372**:47-47.

Vetter, E. W. and P. K. Dayton. 1998. Macrofaunal communities within and adjacent to a detritus-rich submarine canyon system. Deep-Sea Research **45**:25-54.

Whitman, W. B., D. C. Coleman, and W. J. Wiebe. 1998. Prokaryotes: The unseen majority. Proceedings of the National Academy of Sciences of the United States of America **95**:6578-65.

Wilmers, C. C., J. A. Estes, M. Edwards, K. L. Laidre, and B. Konar. 2012. Do trophic cascades affect the storage and flux of atmospheric carbon? An analysis of sea otters and kelp forests. Frontiers in Ecology and the Environment **10**:409-415.

Woodland, R. J., P. Magnan, H. Glemet, M. A. Rodriguez, and G. Cabana. 2012. Variability and directionality of temporal changes in $\delta^{13}C$ and $\delta^{15}N$ of aquatic invertebrate primary consumers. Oecologia **169**:199-209.

Table 1.1. Common abbreviations used in this dissertation.

Abbreviation	Type/Name	Description or example of role
FA	Fatty acids	Generally from total lipid extractions
SAFA	Saturated FA	No double bonds in FA molecule
MUFA	Monounsaturated FA	One double bond in FA molecule
PUFA	Polyunsaturated FA	≥ two double bonds in FA molecule
HUFA	Highly unsaturated FA	≥ C_{20} and ≥ 3 double bonds in FA molecule
EFA	"Essential" FA	Context dependent; generally ω3 or ω6, animals cannot synthesize *de novo*
ω3	Omega-3 FA	Double bond 3 C back from methyl end
ω6	Omega-6 FA	Double bond 6 C back from methyl end
LIN (18:2ω6)	Linoleic acid	C_{18} ω6; precursor to GLA and ARA
GLA (18:3ω6)	γ-Linoleic acid	C_{18} ω6; precursor to ARA
ALA (18:3ω3)	α-Linoleic acid	C_{18} ω3; precursor to SDA, EPA and DHA
SDA (18:4ω3)	Stearidonic acid	C_{18} ω3; precursor to EPA and DHA
ARA (20:4ω6)	Arachidonic acid	C_{20}ω6
EPA (20:5ω3)	Eicosapentaenoic acid	C_{20}ω3; precursor to DHA
DHA (22:6ω3)	Docosahexaenoic acid	C_{20}ω3
FAME	FA methyl esters	Transesterified FA (methanol) for analysis
GC	Gas chromatography	Analytical approach used to measure FAME
GC-FID	Flame ionization detection	Identifies FAME based upon standards and timing of peak elution
GC-MS	Mass-spectrometry	Identified FAME based upon ion fragmentation when burned
SI	Stable isotopes	Ratios of heavy to light isotopes for C, N, S
$\delta^{13}C$	C isotope ratio	Generally used as a 'source' tracker
$\delta^{15}N$	N isotope ratio	Generally used as a tracer of trophic position
$\delta^{34}S$	S isotope ratio	Generally used as a geographic source tracker
MSI	Multiple SI	Refers to the use of ≥ two SI
FAMSI	FA+MSI	Refers to the combined use of FA and MSI
PERMANOVA	Permutational MANOVA	Multivariate analysis of variance
PERMDISP	Permutational test of multivariate dispersion	Test of multivariate dispersion from centroid (compare to homogeneity of variance)
NMDS	Non-metric multidimensional scaling	Visualization plot for evaluating multivariate similarity of samples (non-parametric)
PCA	Principal Components Analysis	Visualization plot for evaluating multivariate similarity of samples (parametric)
SJA	San Juan Archipelago	Study region
SKP	Skipjack Island	Core study site, lower salinity, Boundary Passage
PIL	Pillar	Core study site, higher salinity, Haro Strait
PTC	Point Caution	Core study site, intermediate salinity, San Juan Channel

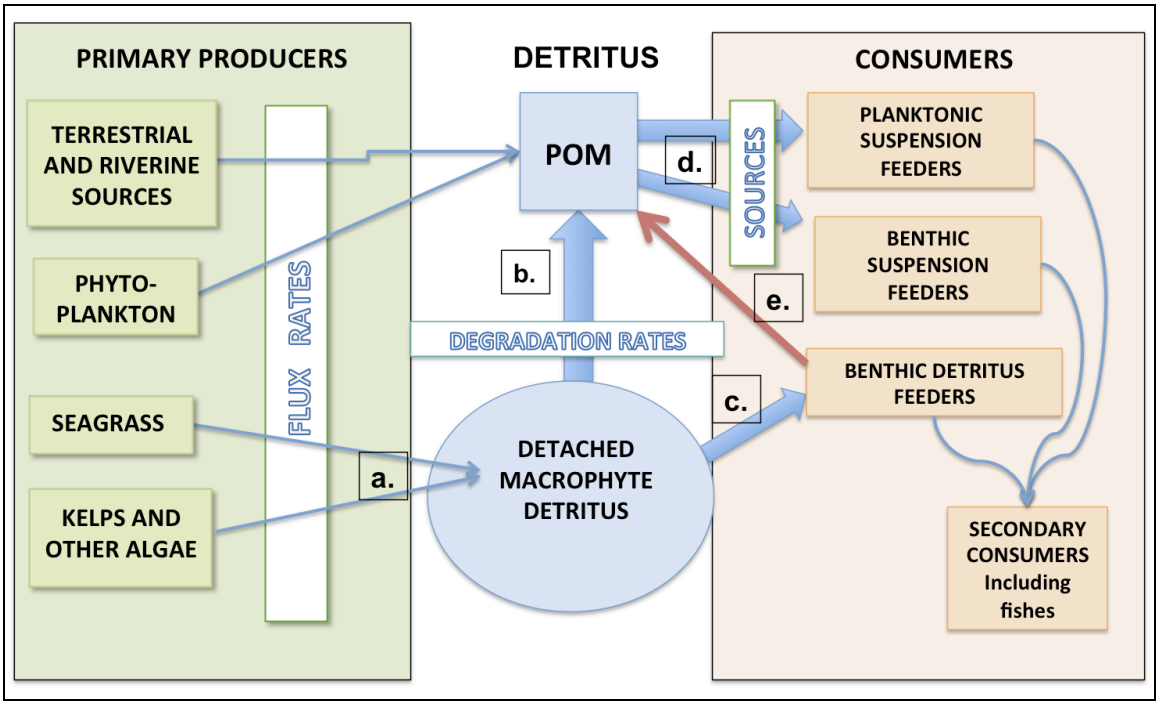

Fig. 1.1. Conceptual diagram of photosynthetically synthesized energy flow through a nearshore marine food web. The diagram shows how energy from primary producers goes through an intermediate detrital pathway before uptake by heterotrophic consumers. The research in this disseratation focuses on characterizing fatty acid (FA) signatures of macrophytes (a) in Chapter 2; in Chapter 3 biomarker signatures (FA and stable isotopes; FAMSI) of wild conspecifics in all 4 consumer categories shown in the diagram were compared between photic and aphotic depths, and an algal aging experiment evaluated changes in FA signatures during degradation (b-c). In Chapter 4 distinct algal diets were fed to herbivorous consumers (c) in a controlled feeding trial designed to measure FAMSI fractionation from diet to consumer. Image copywrited (2013) by Megan Dethier, used by permission.

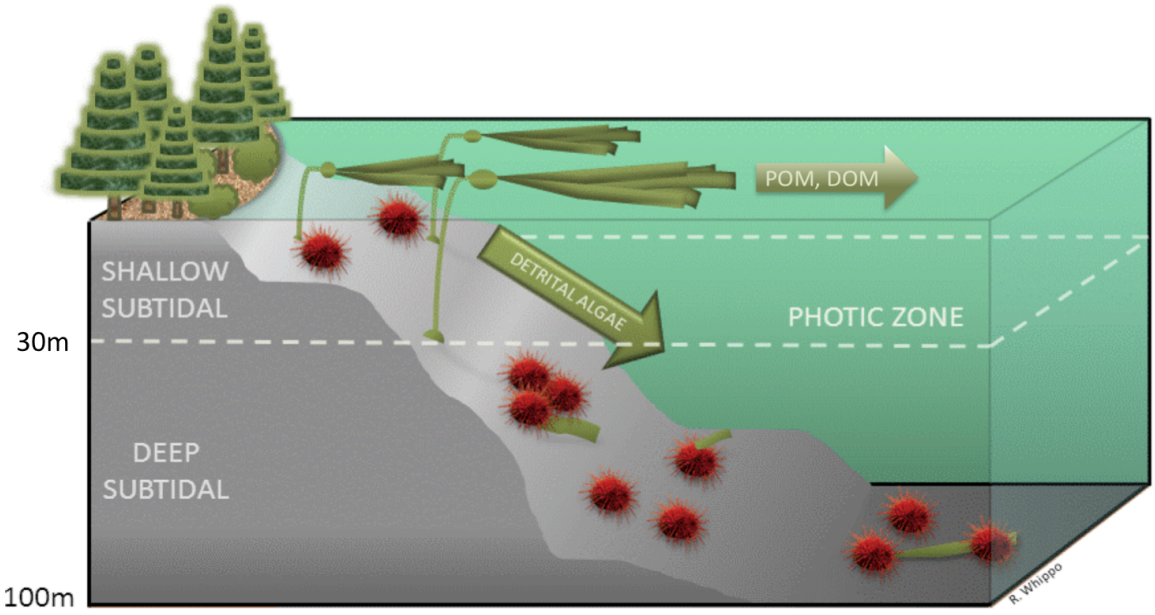

Fig. 1.2. Schematic of macroalgal subsidy from shallow subtidal photic zone (30 m) to consumers in deep nearshore aphotic habitats. Image copywrited (2011) by Ross Whippo, used by permission.

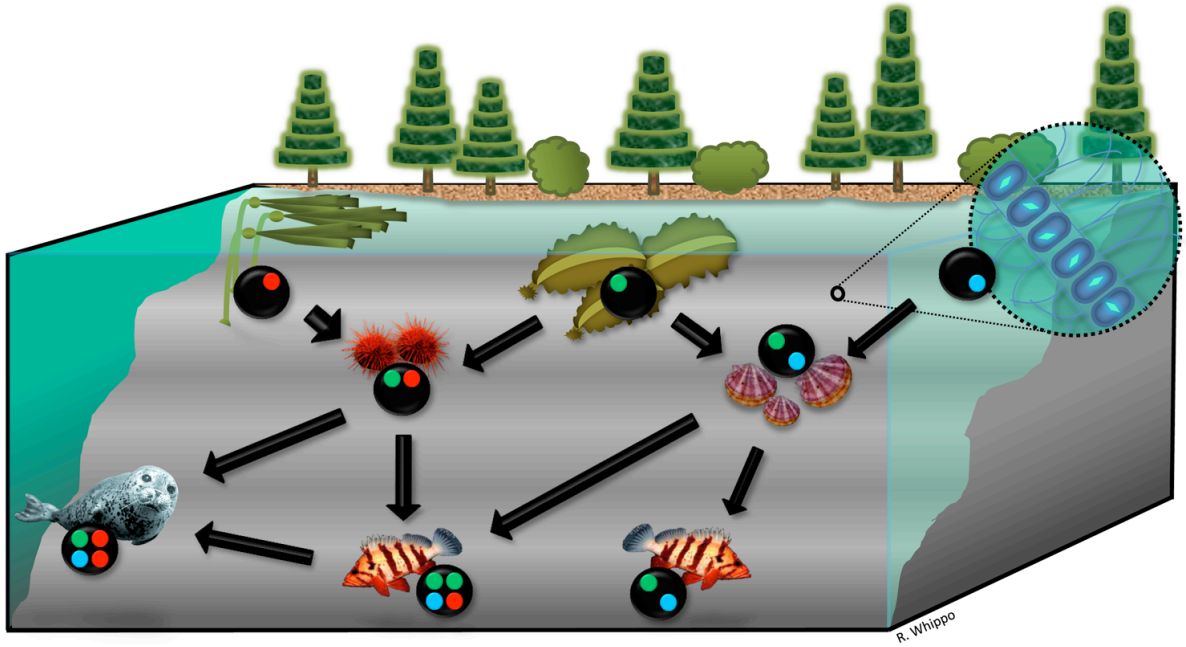

Fig. 1.3. Conceptual schematic of fatty acid biomarker transfer through a nearshore marine food web. Each of the three primary producers in this simple model have a different 'indicator' FA unique to themselves (e.g., red and green in the 2 brown algae, and blue in the diatom). In a static consumer that doesn't alter (e.g., biosynthesize) these marker FA, the presence of the basal producer marker FA in their tissues indicates the producers that contributed to the consumer tissues. Image copywrited (2011) by Ross Whippo, used by permission.

CHAPTER 2 – FATTY ACID SIGNATURES DIFFERENTIATE MARINE MACROPHYTES AT ORDINAL AND FAMILY RANKS

Abstract

Primary productivity by plants and algae is the fundamental source of energy in virtually all food webs. Furthermore, photosynthetic organisms are the sole source for ω3 and ω6 essential fatty acids (EFA) to upper trophic levels. Because animals cannot synthesize EFA, these molecules may be useful as trophic markers for tracking sources of primary production through food webs if different primary producer groups have different EFA signatures. We tested the hypothesis that different marine macrophyte groups have distinct fatty acid (FA) signatures by conducting a phylogenetic survey of 40 marine macrophytes (seaweeds and seagrasses) representing 36 families, 21 orders, and four phyla in the San Juan Archipelago, WA, USA. We used multivariate statistics to show that FA composition differed significantly ($P<0.001$) among phyla, orders, and families using 44 FA and a subset of seven EFA ($P<0.001$). A second analysis of published EFA data of 123 additional macrophytes confirmed that this pattern was robust on a global scale ($P<0.001$). This phylogenetic differentiation of macrophyte taxa shows a clear relationship between macrophyte phylogeny and FA content and strongly suggests that FA signature analyses can offer a viable approach to clarifying fundamental questions about the contribution of different basal resources to food webs. Moreover, these results imply that taxa with commercially valuable EFA signatures will likely share such characteristics with other closely related taxa that have not yet been evaluated for FA content.

Introduction

Photosynthetic organisms are the source of virtually all energy in food webs. Upper trophic level consumers are constrained by this production (e.g., Power 1992), but for many systems the relative importance of different sources of production to consumer communities is debated and poorly resolved (e.g., see Pace et al. 2004, Brett et al. 2009). In nearshore marine and aquatic environments, sources of primary production may be autochthonous (e.g., macrophytes [macroalgae and seagrasses], single-celled phytoplankton [diatoms, dinoflagellates]), or allochthonous, e.g., (terrestrial plants). Assessing the relative importance of these distinct basal resources in marine food webs has been a complex problem because direct observation or gut content analysis is not possible for many primary consumers. The use of stable isotopes (SI; Duggins et al. 1989, Kaehler et al. 2000, Page et al. 2008) and fatty acids (FA; Budge et al. 2008, Richoux and Froneman 2008, Copeman et al. 2009) as biomarkers in this regard has shown promise but these approaches assume that all important food sources are identified, adequately characterized, and represented in mixing models. Moreover, FA and SI can only be used when all relevant primary production sources have distinct signatures, which often not the case with SI. Here, we investigated the phylogenetic differentiation of FA content in the four major macrophyte phyla found worldwide in nearshore marine habitats, to evaluate the potential of FA signature analysis for clarifying fundamental questions about energy sources in food webs.

FA are necessary constituents of the tissues of all living organisms. Because FA have distinct chemical structures, it is possible to routinely identify up to 70 FA within a given organism (Iverson 2009). The identities and quantities of FA in a given sample constitute the FA 'signature'. Of particular interest are the essential FA (EFA), generally defined as ω3 and ω6 FA

families, which animals are unable to synthesize (Bell and Tocher 2009), and as such are potentially conservative molecular biomarkers. In addition, EFA are especially useful in a food web context because they are important for physiological processes (Sargent et al. 1999, Muller-Navarra 2008), including survival, growth, and reproduction in a wide range of aquatic species (Brett and Muller-Navarra 1997), but are only synthesized in biologically relevant amounts by plants and algae (Gladyshev et al. 2009). Previous research on the role of algae as a supply of EFA has focused on the contribution of EFA by phytoplankton to aquatic food webs (Kainz et al. 2004, Ravet et al., 2010).

The role of nearshore macrophytes as a subsidy (e.g., Polis et al. 1997) source of EFA for marine food webs is unknown. This is particularly surprising due to the high productivity (Mann 1973, Duarte and Cebrian 1996) and known importance of benthic algae for nearshore invertebrate assemblages (e.g., Dunton and Schell 1987). As little as 10% of this production is believed to be directly consumed by herbivores as standing stock (Mann 1988). The vast majority of this energy is exported as a spatial subsidy to subtidal (Duggins et al. 1989), intertidal (Rodriguez 2003), pelagic (Kaehler et al. 2006), and terrestrial (Polis and Hurd 1995) food webs. The patterns of FA composition of marine macrophytes may be used to further explain the role of macrophytes as a source of EFA to higher trophic levels and to increase the resolution of marine food web models if these patterns are conserved in consumers. The transfer of EFA synthesized by plants and algae to food webs should be of fundamental interest to resource managers, as these primary producers are the ultimate source of ω3 and ω6 EFA in higher trophic level consumers, such as fish in the marine environment, which are sought after for human consumption.

Northeast (NE) Pacific nearshore marine macrophyte communities contain a very diverse mix of species (>640 taxa) representing four phyla (Gabrielson et al. 2006). The FA composition of <10% of these species is known. Algae encompass a wide diversity of organisms, often only distantly related to each other (Stengel et al. 2011), which are known to exhibit an astounding array of FA (Harwood and Guschina 2009), even among closely related taxa. Lang et al. (2011) recently demonstrated a significant phylogenetic signal in the FA composition of cultured microalgae, and several studies have reported on the FA content of marine macrophytes in different parts of the world (Khotimchenko 1998, Graeve et al. 2002, Khotimchenko et al. 2002, Hanson et al. 2010, Kumari et al. 2010). However, whether marine macrophytes segregate taxonomically with respect to their FA composition has not been demonstrated explicitly for a diverse assemblage of taxa.

We conducted a broad survey of the FA content of 40 NE Pacific marine macrophyte taxa representing 36 families, in 21 orders, across four phyla (Table 2.1; seagrasses, Anthophyta; brown algae, Ochrophyta; green algae, Chlorophyta; red algae, Rhodophyta) in the San Juan Archipelago (SJA), NE Pacific, to evaluate the taxonomic resolution of macrophytes as basal resources in a food web context. We compared this analysis with an evaluation of published macrophyte FA data (1994-2010) from an additional 123 independently collected taxa from 36 families, in 21 orders (Table 2.2) across the same four phyla in all major oceans of the world. Specifically, we asked: 1) Does macrophyte FA composition differ among phylogenetic categories of phylum, order, or family level using 44 FA in the SJA dataset? 2) Is the same taxonomic resolution achieved with the SJA dataset using only a subset of seven EFA? 3) Are locally observed patterns consistent with published global macrophyte EFA data?

Methods

NE Pacific macrophytes

Selection of taxa:

Our goal was to compare FA signatures from the four major marine macrophyte phyla present worldwide in nearshore waters. We selected species to maximize taxonomic diversity by creating a list of 80 macrophyte species that we expected to find during a May sampling period (see below) in the SJA. We removed species from the list that were difficult to identify due to lack of reliable morphological/anatomical traits (Gabrielson et al. 2006). The list was filtered to maximize the number of orders. Once an order was represented by one taxon, we selected multiple taxa within that order: 1) if each species was from a different family; 2) if the different species could be found in different habitats (e.g., intertidal vs. subtidal) and 3) in the case of Laminariales (kelps), we included eight species because of their biomass dominance in the drift (Britton-Simmons et al. 2009) in the SJA. Recent molecular work has shown that the green algal species commonly referred to as *Ulva lactuca* in the NE Pacific and other temperate marine waters in the northern and southern hemisphere has been misidentified (O'Kelly et al. 2010). We therefore extracted DNA from two specimens we had identified as *U. lactuca* and amplified the *rbc*L gene following the methods of O'Kelly et al. (2010). The sequences obtained match those from temperate-zone specimens that have been assigned to "*U. lactuca.*" We refer to this entity as *Ulva sp.* in Tables 2.1 and 2.2 until a valid name for this species has been assigned. Finally, the brown alga *Syringoderma abyssicola* was added opportunistically after we encountered it in the field because it represented a rare order in the local flora.

Sample collection:

Previous work has documented variation in macrophyte FA signature by season (Nelson et al. 2002), within-thallus location (Khotimchenko and Kulikova 2000), across different light and temperature regimes and with depth (Becker et al. 2010). Whether such variation is biologically significant in food webs is currently unexplored. To minimize variation due to these factors, we constrained specimen collection to a 3-week window (21 May-10 June, 2010) for all but two species that were not found until 16 June and 16 August 2010. Moreover, when possible we collected only sporophytes for taxa with heteromorphic life histories. An exception was made for the red alga *Opuntiella californica* because the gametophyte is a conspicuous upright and the sporophyte an uncommon subtidal crust. As the potential for within-species site/location variability was unknown, we constrained sample collection to five locations in the SJA within a 15 km radius. For any given species we tried to collect all specimens at one site. The vast majority of specimens were collected at three sites: Point Caution, San Juan Channel (61% of all taxa; 48.56° N, -123.01° W), Skipjack Island, Boundary Pass (17%; 48.73° N, -123.03° W), and Andrews Bay, Haro Strait (15%; 48.55° N, -123.17° W).

We collected five replicate specimens (>2 m apart) of each species from its median depth distribution (using SCUBA for subtidal species). Specimens were stored in flow-through sea tables (<8 hours) until cleaned and frozen (-20° C). Our 40 species represented a diverse array of thallus morphologies. To constrain our sampling to functionally comparable areas, we focused our sampling in the center of the vegetative "blade" (or comparable portion) of each thallus. Meristematic, reproductive, and stipe/holdfast tissues were avoided. We cleaned specimens by brushing gently with a toothbrush under filtered seawater before collecting ~2 g wet weight from each replicate. Small thallus size of some taxa required pooling multiple thalli into one replicate.

In these cases, we still collected the material to be pooled from locations >2 m apart in the field. We selected only tissue that was healthy and not fouled by encrusting epibionts.

Fatty acid extraction:

We extracted FA from three replicates within seven months of collection and retained the remaining samples as vouchers. We lyophilized samples for 48 h, ground the dry material into a powder, and extracted lipids following Brett et al. (2009). Briefly, 10 mg of dry material was suspended in a 4:2:1 chloroform/methanol/water mixture, sonificated, vortexed, and centrifuged before removing the organic layer. This procedure was repeated three times, the organic extracts were then pooled, and evaporated to dryness under nitrogen. Samples were then transesterified in a 1:2 toluene/1% sulfuric acid in methanol mixture for 16 h in a 50° C water bath. After cooling, 2% $KHCO_3$ and hexane:diethyl ether (with BHT 0.01%) was added, and after vortexing and centrifugation, the upper phase was removed. A second addition of hexane:diethyl ether and subsequent extraction was pooled with the first. Solvent was then evaporated off the derivitized FA methyl esters (FAME) and re-suspended in 1.5 mL of hexane prior to GC analysis. FAME were analyzed with an HP 6958 gas chromatograph (GC) equipped with an auto sampler and flame-ionization detector using an Agilent DB-23 column (30 m, 0.25 mm diam., 0.15 um film), and 37-component FAME standards mix (Supelco™, Bellefonte, PA; Taipale et al. 2011) with a total run time of 85 minutes. We cross-verified FAME identification in our chromatograms by running a subset of our samples through a GC at a lab that had previously verified the FAME found in our samples using GCMS. This procedure ultimately identified a total of 44 unique FA. Individual FA were expressed as a percentage of total FA mass.

Global taxa data gathering

We gathered EFA data from the literature published between 1994-2010 (Fleurence et al. 1994, Khotimchenko 1998, Graeve et al. 2002, Khotimchenko et al. 2002, Kelly et al. 2008, Richoux and Froneman 2008, Allan et al. 2010, Hanson et al. 2010, Kumari et al. 2010). One challenge with published FA data is that researchers may interpret certain peaks as representing different FA, depending upon the analysis method used (e.g., GC-flame ionization detection vs. GCMS), and report quantitative results for only several to >60 FA. In addition, when mining literature data, it is not often possible to control for factors such as season and depth across studies. Finally, incorrect species identification and contamination from unwanted, associated microscopic epi- and endo-phytic taxa is a complicating factor for macroalgae in particular. For this reason we felt justified in selecting data that were collected and evaluated in a manner as consistent as possible with our approach. This dataset was not assumed to be a comprehensive list of all published macrophyte FA data, but rather a broad subsample of taxa from many regions sufficient for our research question. The global taxa list, sources, phylogenetic grouping variables, and sampling regions are presented in Table 2.2. Due to the plasticity of names in the algal literature, we searched in AlgaeBase (Guiry and Guiry 2011) at genus rank for each species from the literature and aligned each entity with its current order and family name. All literature FA values were analyzed with the *a priori* hypothesis that results of the global data analysis would not differ from what was observed in the SJA. From each paper, we used only the seven EFA (see below) that were previously used in the SJA analysis.

Data analysis

We used PERMANOVA (Anderson 2001) to evaluate differences among groups with multivariate FA datasets for both the SJA and global datasets. Taxonomic factors for both

analyses were nested (e.g., family was nested with order, which was nested with phylum). We used PERMANOVA to test for the significance of the factors order and 10 ocean basin sub-regions (Arctic, Southern, N Indian, SE Indian, SW Indian, NE Pacific, NW Pacific, NE Atlantic, NW Atlantic, and SE Atlantic) and their interaction in the published global dataset. Because of assumed relationships among the taxa with regards to taxonomic ranking, factors were treated as fixed in analyses. PERMANOVA is a non-parametric analog to MANOVA where statistical significance is determined by repeated (n=9999) permutations of the raw Euclidian distance matrix to generate null distributions for comparing with observed values. We calculated percent variance in the PERMANOVA table by dividing the variance component estimated for each factor by the sum of all variance components to quantify the relative magnitude of effects (Hanson et al. 2010). PERMANOVA does not require multivariate normality, so the results reported here are from running the analyses on untransformed FA data. However, because the routine can be sensitive to differences in-group dispersions (described below), we arcsine-transformed ($x'=\sin^{-1}\sqrt{x}$) the FA datasets and confirmed that the results of the analyses were not sensitive to a lack of transformation.

To investigate the FA variation of different lineages, we used PERMDISP (Anderson 2006) to test the null hypothesis of no differences among the dispersions of macrophyte FA signatures using grouping variables phylum and order in both the SJA full 44 FA dataset (40 taxa) and the combined SJA and global EFA dataset. In this pooled analysis only, one reported FA signature (selected randomly) was used to represent a taxon that multiple sources had evaluated in different regions so that more commonly evaluated taxa would not receive more weight in the analysis than rarely evaluated taxa. In addition, we later verified that the results of this analysis were not dependent upon the taxa selected. We used a post-hoc pairwise

PERMDISP of orders to investigate relative differences in FA dispersion between orders in the combined EFA dataset, including only those orders with at least 3 independent taxa. Results of this analysis were summarized by plotting the mean distances of the group centroid (± 1 SE) value as calculated from the Euclidean distance matrix. We used Principal Components Analysis (PCA) ordinations of Euclidean resemblance matrices of non-transformed percent FA composition data for multivariate data visualization. Arcsine-transformations to the raw FA data did not affect visual interpretation of the PCA results. PCA plots were accompanied by eigenvector plots showing FA, which correlated (Pearson >0.4) with the first two principal components (PCs). The seven ω3 and ω6 EFA used in the reduced analyses were selected *a-priori* and included: 18:2ω6 [LIN], 18:3ω6, 18:3ω3 [ALA], 18:4ω3 [SDA], 20:4ω6 [ARA], 20:5ω3 [EPA], and 22:6ω3 [DHA]. Due to the large number of FA variables and taxa studied, we limited our attempt to summarize individual FA means and focus instead on evaluation of our research questions, and provided the entire SJA FA dataset as a supplementary table (see below). However, we did summarize the 5 abundant EFA for the SJA dataset at the phylum level. Two EFA, 18:3ω6 and DHA, were not included in this *post-hoc* summary because they each accounted for a grand mean of less than 1% of total EFA in the SJA taxa. All analyses were performed using PRIMER v. 6.0 and PERMANOVA+ add on (PRIMER-E Ltd, Plymouth, UK).

Results

San Juan Archipelago taxa

Using the full suite of all 44 routinely identified FA, we found that that macrophyte FA composition differed significantly among phylogenetic grouping variables of phylum, order, and family (PERMANOVA, P=0.0001; Table 2.3, Fig. 2.1) for the 40 taxa evaluated. A second

analysis of the SJA taxa using only seven ω3 and ω6 EFA (see Methods) yielded comparable results with the phylogenetic grouping variables of phylum, order, and family still explaining significant variation among species (PERMANOVA, P=0.0001; Table 2.3, Fig. 2.2). The comparisons across phyla explained the largest portion of the variability (Table 2.2), 36.5% in the 44 FA analysis and 40.5% in the EFA analysis. The order and family ranks explained progressively less variation than the phylum (Table 2.3), with the residual (i.e., within taxon) variances accounting for 13.9% and 13.2%, respectively.

The mean ± SD values of FA percent composition (three replicates per taxon except for *Syringoderma* where n=1) for all 44 FA quantified in this analysis are presented in Appendix A. Multivariate data from the analyses of the 44 FA and 7 EFA datasets are visualized using PCA in Figs. 2.1a and 2.2. PCA eigenvector plots (Figs. 2.1b, 2.2) show the trajectory of the correlations (Pearson, >0.4) of the FA variables to PCs 1 and 2. The multivariate dispersions of the FA variability (44 FA) of the SJA phyla and orders were not equal (PERMDISP; Phyla: $F_{3, 2}$=20.319, P=0.001; Orders: $F_{20, 97}$=13.652, P=0.001). A summary plot of five abundant EFA (see Methods) in the SJA taxa (Fig. 2.3) shows the mean percent composition (± 1 SD) and discriminating potential of EFA for differentiating macrophyte phyla. The red algae were particularly characterized by the EPA, ARA (highly variable means of ~11% and ~17% of total FA, respectively), 14:1ω5, and the saturated FA 16:0 and 18:0. The brown algae exhibited a relatively even distribution of EFA (see Fig. 2.3), sharing an abundance of EPA and ARA (~13% and 12%, respectively) with the red algae, and were primarily separated from this group by a relative lack of 16:0 and 18:0 (Fig. 2.1b). Green algae had relatively consistent abundance of LIN, ALA, and SDA (means of ~5%, 17%, and 7%, respectively). The Ulvales primarily drove this pattern in SDA in the green algae. Seagrasses were consistently segregated from other taxa

by a concurrent and consistent abundance of LIN and ALA (means of ~7% and 46% of total FA, respectively). The saturated FA 20:0 and 24:0 were not abundant (0-2% of total FA) in the seagrasses, brown and green algae, but were never found in red algae and thus were also useful in discriminating these groups (Fig 2.1b).

Published global taxa

Because taxonomic resolution at the order and family ranks was achieved using only EFA in the SJA dataset, we then extracted published values of these same EFA from marine macrophyte studies worldwide (123 macrophyte taxa, 21 orders, and 36 families in 10 distinct ocean 'regions'; Table 2.2). We found the same taxonomic resolution in the global macrophyte data (not including any of the SJA taxa) using only the seven EFA (PERMANOVA, P=0.0001, Table 2.3, Fig. 2.4). The FA most important for differentiating the global EFA dataset were ARA, EPA, LIN, and ALA, shown in the trajectory of the correlations (Pearson, >0.4) of the FA variables to PCs 1 and 2 as overlays in Fig. 2.4. These patterns were consistent with the SJA EFA dataset (Figs. 2.2, 2.4).

Combined SJA and global taxa

Using the combined SJA and global EFA datasets, (n=163 taxa) we found a significant interaction between taxonomic order and ocean basin (PERMANOVA, P=0.0097, Table 2.4), indicating that macrophyte EFA content within orders also depended upon geographic location. The multivariate dispersions of the variability of phyla and orders of the combined SJA and global EFA datasets were not equal (PERMDISP; Phyla: $F_{3, 117}$=7.768, P=0.001; Orders: $F_{25, 95}$=3.725, P=0.005). A summary plot of the pairwise comparisons of the multivariate dispersions of phylogenetic order (Fig. 2.5) shows the mean distance to group centroid (± 1 SE)

in Euclidean space for all orders, demonstrating that some orders, particularly in the red algae, had much higher variation in FA signatures than others.

Discussion

We have shown that there is a substantial and clear taxonomic signal in macrophyte FA composition (Figs. 2.1, 2.2, and 2.4). This taxonomic pattern was robust when the analysis was constrained to include only the seven EFA (Table 2.2, Fig. 2.4), which are only manufactured by plants and algae and are of key importance to animals in all food webs. Moreover, the taxonomic resolution of macrophyte EFA described here was consistent across two separate data sets (40 SJA taxa and 123 global taxa) collected in different regions using different methods. Since 1972, a link between FA composition and marine macrophyte groups has been hypothesized (Jamieson and Reid 1972) and more recently discussed (Graeve et al. 2002), but only in this decade have researchers attempted to evaluate the these differences using statistical tests (e.g., Hanson et al. 2010, Kumari et al. 2010). However, implications from previous analyses have been limited by a relatively small number of taxa (eight macroalgal species and three seagrasses) of limited taxonomic breadth (no green algae) in the former (Hanson et al. 2010) and repeated univariate tests in the latter (Kumari et al. 2010). Our results offer strong multivariate statistical support for the previously hypothesized link between macrophyte phylogeny and FA content.

The differences in EFA composition among the three macroalgal groups (red, green and brown algae) is perhaps not so surprising, as the evolutionary lineages of reds and greens diverged hundreds of millions of years ago and brown algae are unrelated to that lineage (Keeling et al. 2005). However, green algae and seagrasses are in the same lineage with the same suite of photosynthetic pigments and similar biochemical pathways, yet their FA also were divergent. The most likely explanation is that marine green algae have occupied that habitat for

hundreds of millions of years, whereas seagrasses are recent colonizers of the marine environment. Both lineages have closely related freshwater and terrestrial members that warrant further study to determine if the correlation we demonstrated is more widespread. Differences in FA signatures of microalgae between phylogenetic phyla, classes, and even within genera have recently been demonstrated for a large collection of cultured microalgae (Lang et al. 2011). Our taxa list did not allow for statistically meaningful within genera comparisons for the macrophytes.

EFA of the red algae were clearly more variable relative to the brown algae (Figs. 2.2-5). The brown and red algae in both datasets exhibit substantial variation in thallus morphology, yet morphological characters often do not reflect taxonomic similarity. Compare for example the brown algal orders Ectocarpales, which have generally 'simple' thallus morphology and isomorphic life histories, with Laminariales, which have large complex thalli and heteromorphic life histories. Despite these morphological differences, these are recognized as sister clades (Phillips et al. 2008), and their FA were very similar (Fig. 2.1). The large dispersion in the EFA of the well sampled orders of Gigartinales and Ceremiales (red algae) relative to the Laminariales (Fig. 2.5) may be related to the evolutionary history of the two lineages (Graeve et al. 2002). DNA evidence strongly supports that the red algae obtained their plastids through an ancient primary endosymbiotic event, whereas the lineage that includes brown algae obtained their plastids through a primary as well as a secondary endosymbiosis (Keeling 2004). The mechanism for the greater variety of the EFA found in Gigartinales, Corallinales, and Gracilariales compared to other red algal orders is currently unresolved and merits further investigation.

Our FA analyses have important implications for food web research. Presently, it is commonplace for researchers to cite data from outside their own study system when defining 'FA biomarkers' without investigating the validity of those biomarkers for a particular question or region. In addition, it is not uncommon that one FA will be cited as a biomarker for an entire taxonomic group (e.g., 20:4ω6 [ARA] as a brown algal FA biomarker; Hanson et al. 2010). While we would not dispute that ARA is a common constituent and important FA in brown algae, we found this FA in similar amounts in some red algae (Fig. 2.3). Whereas the FA signatures of the macrophyte orders and families are clearly distinct, we also found that the factors oceanic region and order showed a significant interaction ($P<0.01$, Table 2.4). This result may be in due to variation in the sampling season for the global taxa, or increased degree of FA unsaturation reported in algae in polar regions as compared to temperate analogs (e.g., Graeve et al. 2002). Although it is unclear what is causing the interaction of order and ocean region, this result supports the importance of evaluating potential primary producer FA biomarkers from the study system in question (Dalsgaard et al. 2003). Moreover, our analyses suggest that multiple FA, rather than individual FA, should be used concurrently as trophic markers.

FA clearly offer a higher taxonomic resolution for differentiating sources of primary production to food webs than what has been demonstrated using multiple SI (MSI) analysis (e.g., see Hanson et al. 2010). Recent work by Marconi et al. (2011) found wide heterogeneity in both $\delta^{13}C$ and $\delta^{15}N$ signatures for a comprehensive list of 85 macrophyte taxa in 4 phyla. Hanson et al. (2010) showed taxonomic differentiation at the phylum and species levels for $\delta^{13}C$ but not for $\delta^{15}N$ for a macrophyte assemblage, however, the implications of these findings to food web studies is obfuscated by the limited number of taxa surveyed. As researchers increase the number of elements considered in SI analyses (e.g., sulfur; Connolly et al. 2004), resolution power for

this approach may increase. We have shown that FA signatures of macrophyte groups differ to at least the rank of family, whereas SI signatures of macrophyte groups have been demonstrated to differ only to the rank of phylum. Additional research is needed to investigate whether MSI (i.e., >3 SI), applied to phylogenetically broad taxa lists, can provide the family level taxonomic resolution for marine macrophytes demonstrated here.

Researchers are increasingly utilizing a combination of MSI and FA biomarkers for evaluating questions about the relative contribution of basal resources to food webs (e.g., Turner and Rooker 2006, Budge et al. 2008, El-Sabaawi et al. 2010), and whereas the combination of both approaches may increase taxonomic resolution, it may not be feasible to use both approaches when faced with limited resources. In our experience, the raw materials costs of SI and FA analyses are relatively similar, but the FA extraction and quantification process is more time consuming and requires additional equipment and training. This added expense is may be reasonable if the research question requires a fine level of taxonomic resolution (e.g., family or ordinal) of primary producers. However, both methods currently suffer from a lack of experimental evidence demonstrating the predicted fractionation in SI (e.g., Gannes et al. 1997, del Rio et al. 2009, but see Wehi and Hicks 2010) and transfer of FA biomarkers up the food chain in controlled feeding trials (but see Hall et al. 2006, Kelly et al. 2008, Kelly et al. 2009). Such experimental feeding trials are clearly the most important future direction for MSI and FA trophic ecology and represent a crucial next step in evaluating whether the taxonomic differences in FA signature of primary producers actually transfers to upper trophic level consumers in predictable ways.

That phylum, order, and family level taxonomic resolution were elucidated using only a suite of seven ‘essential’ ω3 and ω6 EFA will be of particular interest to ecologists who wish to

use FA signature analysis for tracing primary producer contributions to food webs. Although the content of these EFA can be modified by animals through FA chain modification, animals do not have desaturase enzymes necessary to insert double bonds at the ω3 and ω6 positions of FA in order to synthesize these molecules *de novo* (Dalsgaard et al. 2003). It is important to account for the abilities of the consumers under study to elongate or retroconvert these EFA in controlled feeding trials (Hall et al. 2006, Kelly et al. 2009) before making quantitative assessments of various sources to consumer diets. Such feeding trials can take advantage of the phylogenetic rank differences in macrophyte FA signatures shown here to evaluate the degree to which FA remain intact in the herbivore or are converted to other FA. The response in consumer FA signatures to diets of different FA signatures is poorly understood or completely unknown for most consumer taxa. Despite this uncertainty, it is not uncommon for researchers to use published algae FA biomarkers as evidence that consumers are foraging on specific primary producers in the field (Richoux and Froneman 2008, Allan et al. 2010, El-Sabaawi et al. 2010). It may ultimately be possible to model consumer diets using quantitative FA signature analysis (QFASA; Iverson 2004, Iverson 2009), however, such modeling requires a comprehensive dataset of FA signatures of all of the important/potential prey items and also requires knowledge about the consumers' ability to synthesize/modify FA (i.e., to account for potentially bioactive FA molecules in the consumer). Furthermore, such quantitative modeling will require additional evaluation of the potential importance of seasonal and geographic variation in primary producer biochemical signals. Here we argue that researchers must first test the fundamental assumption that the FA composition of a diverse array of possible macrophyte basal resources actually differ before attempting to quantitatively or qualitatively model the transfer of these resources to consumers. Until now, this assumption has not been evaluated. This work further advances the

goal of utilizing a quantitative approach like QFASA for an herbivorous consumer because it has helped identify the FA signatures of a wide range of potential consumers 'prey' items.

The four major marine macrophyte groups have distinct signatures of FA and even of EFA. Because EFA are limiting in many aquatic ecosystems (Brett and Muller-Navarra 1997, Litzow et al. 2006) and are synthesized only by plants and algae, they may function as conservative trophic tracers. The phylogenetic differentiation in EFA content of marine macrophytes offers finer taxonomic resolution (e.g., order and family ranks), than what has previously been demonstrated for SI (phylum rank) and FA. The fact that different macrophyte groups have distinct EFA signatures is also important in the context of ecosystem services, as large amounts of detached, drift macrophyte biomass is transported to deep subtidal (e.g., Britton-Simmons et al. 2012) and intertidal (e.g., Bustamante et al. 1995) habitats and is utilized for energy by consumers. The patterns of macrophyte EFA content are of particular interest in this context because animals rely upon the service of primary producers to synthesize and provide these molecules to animals that cannot generate ω3 and ω6 EFA *de novo*. In addition, the phylogenetic signal present in macrophyte FA signatures has important implications for commercial interests that seek to find and isolate valuable bioactive compounds such as EFA from the natural environment. The results presented here show support for the hypothesis that taxa with potentially economically desirable FA signatures will share those characteristics with other closely related taxa that have not yet been evaluated for FA content. This taxonomic resolution may be used to address fundamental ecological questions about the relative importance of different basal resources to herbivorous consumers and marine food webs if controlled feeding trials can demonstrate predictable transfer of biomarker FA from distinct producers to primary consumers.

Literature Cited

Allan, E. L., S. T. Ambrose, N. B. Richoux, and P. W. Froneman. 2010. Determining spatial changes in the diet of nearshore suspension-feeders along the South African coastline: stable isotope and fatty acid signatures. Estuarine, Coastal and Shelf Science **87**:463-471.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology **26**:32-46.

Anderson, M. J. 2006. Distance-based tests for homogeneity of multivariate dispersions. Biometrics **62**:245-253.

Becker, S., M. Graeve, and K. Bischof. 2010. Photosynthesis and lipid composition of the Antarctic endemic rhodophyte *Palmaria decipiens*: effects of changing light and temperature levels. Polar Biology **33**:945-955.

Bell, M. V. and D. R. Tocher. 2009. Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: general pathways and new directions. Pages 211-236 *in* M. T. Arts, M. T. Brett, and M. Kainz, editors. Lipids in aquatic ecosystems.

Brett, M. T. and D. C. Muller-Navarra. 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biology **38**:483-499.

Brett, M. T., M. J. Kainz, S. J. Taipale, and H. Seshan. 2009. Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. Proceedings of the National Academy of Sciences of the United States of America **106**:21197-21201.

Britton-Simmons, K. H., G. Foley, and D. Okamoto. 2009. Spatial subsidy in the subtidal zone: utilization of drift algae by a deep subtidal sea urchin. Aquatic Biology **5**:233-243.

Britton-Simmons, K. H., A. L. Rhoades, R. E. Pacunski, A. W. E. Galloway, A. T. Lowe, E. A. Sosik, M. N. Dethier, and D. O. Duggins. 2012. Habitat and bathymetry influence the

landscape-scale distribution and abundance of drift macrophytes and associated invertebrates. Limnology and Oceanography **57**:176-184.

Budge, S. M., M. J. Wooller, A. M. Springer, S. J. Iverson, C. P. McRoy, and G. J. Divoky. 2008. Tracing carbon flow in an arctic marine food web using fatty acid-stable isotope analysis. Oecologia **157**:117-129.

Bustamante, R. H., G. M. Branch, and S. Eekhout. 1995. Maintenance of an exceptional intertidal grazer biomass in South-Africa - subsidy by subtidal kelps. Ecology **76**:2314-2329.

Connolly, R. M., M. A. Guest, A. J. Melville, and J. M. Oakes. 2004. Sulfur stable isotopes separate producers in marine food-web analysis. Oecologia **138**:161-167.

Copeman, L. A., C. C. Parrish, R. S. Gregory, R. E. Jamieson, J. Wells, and M. J. Whiticar. 2009. Fatty acid biomarkers in coldwater eelgrass meadows: elevated terrestrial input to the food web of age-0 Atlantic cod *Gadus morhua*. Marine Ecology Progress Series **386**:237-251.

Dalsgaard, J., M. St. John, G. Kattner, D. C. Muller-Navarra, and W. Hagen. 2003. Fatty acid trophic markers in the pelagic marine food environment. Advances in Marine Biology **46**:225-340.

del Rio, C. M., N. Wolf, S. A. Carleton, and L. Z. Gannes. 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biological Reviews **84**:91-111.

Duarte, C. M. and J. Cebrian. 1996. The fate of marine autotrophic production. Limnology and Oceanography **41**:1758-1766.

Duggins, D. O., C. A. Simenstad, and J. A. Estes. 1989. Magnification of secondary production by kelp detritus in coastal marine ecosystems. Science **245**:170-173.

Dunton, K. H. and D. M. Schell. 1987. Dependence of consumers on macroalgal (*Laminaria solidungula*) carbon in an arctic kelp community: $\delta^{13}C$ evidence. Marine Biology **93**:615-625.

El-Sabaawi, R., A. R. Sastri, J. F. Dower, and A. Mazumder. 2010. Deciphering the seasonal cycle of copepod trophic dynamics in the Strait of Georgia, Canada, using stable isotopes and fatty acids. Estuaries and Coasts **33**:738-752.

Fleurence, J., G. Gutbier, S. Mabeau, and C. Leray. 1994. Fatty-acids from 11 marine macroalgae of the French Brittany coast. Journal of Applied Phycology **6**:527-532.

Gabrielson, P. W., T. B. Widdowson, and S. C. Lindstrom. 2006. Keys to the seaweeds and seagrasses of Southeast Alaska, British Columbia, Washington and Oregon. PhycoID, Hillsborough, NC.

Gannes, L. Z., D. M. Obrien, and C. M. delRio. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology **78**:1271-1276.

Gladyshev, M. I., M. T. Arts, and N. N. Sushchik. 2009. Preliminary estimates of the export of omega-3 highly unsaturated fatty acids (EPA + DHA) from aquatic to terrestrial ecosystems. Pages 179-209 *in* M. T. Arts, M. T. Brett, and M. Kainz, editors. Lipids in Aquatic Ecosystems. Springer, New York.

Graeve, M., G. Kattner, C. Wiencke, and U. Karsten. 2002. Fatty acid composition of Arctic and Antarctic macroalgae: indicator of phylogenetic and trophic relationships. Marine Ecology-Progress Series **231**:67-74.

Guiry, M. D. and G. M. Guiry. 2011. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. National University of Ireland, Galway. Available at: http://www.algaebase.org/ (last accessed 20 March 2013).

Hall, D., S. Y. Lee, and T. Meziane. 2006. Fatty acids as trophic tracers in an experimental estuarine food chain: tracer transfer. Journal of Experimental Marine Biology and Ecology **336**:42-53.

Hanson, C. E., G. A. Hyndes, and S. F. Wang. 2010. Differentiation of benthic marine primary producers using stable isotopes and fatty acids: implications to food web studies. Aquatic Botany **93**:114-122.

Harwood, J. L. and I. A. Guschina. 2009. The versatility of algae and their lipid metabolism. Biochimie **91**:679-684.

Iverson, S. J., C. Field, W. D. Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. Ecological Monographs **74**:211-235.

Iverson, S. J. 2009. Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination.*in* M. T. Arts, M. T. Brett, and M. J. Kainz, editors. Lipids in Aquatic Ecosystems. Springer, New York.

Jamieson, G. R. and E. H. Reid. 1972. Component fatty acids of some marine algal lipids. Phytochemistry **11**:1423-1432.

Kaehler, S., E. A. Pakhomov, R. M. Kalin, and S. Davis. 2006. Trophic importance of kelp-derived suspended particulate matter in a through-flow sub-Antarctic system. Marine Ecology-Progress Series **316**:17-22.

Kaehler, S., E. A. Pakhomov, and C. D. McQuaid. 2000. Trophic structure of the marine food web at the Prince Edward Islands (Southern Ocean) determined by $\delta^{13}C$ and $\delta^{15}N$ analysis. Marine Ecology Progress Series **208**:13-20.

Kainz, M., M. T. Arts, and A. Mazumder. 2004. Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. Limnology and Oceanography **49**:1784-1793.

Keeling, P. J. 2004. Diversity and evolutionary history of plastids and their hosts. American Journal of Botany **91**:1481-1493.

Keeling, P. J., G. Burger, D. G. Durnford, B. F. Lang, R. W. Lee, R. E. Pearlman, A. J. Roger, and M. W. Gray. 2005. The tree of eukaryotes. Trends in Ecology & Evolution **20**:670-676.

Kelly, J. R., R. E. Scheibling, S. J. Iverson, and P. Gagnon. 2008. Fatty acid profiles in the gonads of the sea urchin *Strongylocentrotus droebachiensis* on natural algal diets. Marine Ecology Progress Series **373**:1-9.

Kelly, J. R., R. E. Scheibling, and S. J. Iverson. 2009. Fatty acids as tracers for native and invasive macroalgae in an experimental food web. Marine Ecology Progress Series **391**:53-63.

Khotimchenko, S. V. 1998. Fatty acids of brown algae from the Russian Far East. Phytochemistry **49**:2363-2369.

Khotimchenko, S. V. and I. V. Kulikova. 2000. Lipids of different parts of the lamina of *Laminaria japonica* Aresch. Botanica Marina **43**:87-91.

Khotimchenko, S. V., V. E. Vaskovsky, and T. V. Titlyanova. 2002. Fatty acids of marine algae from the Pacific coast of North California. Botanica Marina **45**:17-22.

Kumari, P., M. Kumar, V. Gupta, C. R. K. Reddy, and B. Jha. 2010. Tropical marine macroalgae as potential sources of nutritionally important PUFAs. Food Chemistry **120**:749-757.

Lang, I. K., L. Hodac, T. Friedl, and I. Feussner. 2011. Fatty acid profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection. BMC Plant Biology **11**:124.

Litzow, M. A., K. M. Bailey, F. G. Prahl, and R. Heintz. 2006. Climate regime shifts and reorganization of fish communities: the essential fatty acid limitation hypothesis. Marine Ecology Progress Series **315**:1-11.

Mann, K. H. 1973. Seaweeds: their productivity and strategy for growth. Science **182**:975-981.

Mann, K. H. 1988. Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystems. Limnology and Oceanography **33**:910-930.

Marconi, M., M. Giordano, and J. A. Raven. 2011. Impact of taxonomy, geography, and depth on $\delta^{13}C$ and $\delta^{15}N$ variation in a large collection of macroalgae. Journal of Phycology **47**:1023-1035.

Muller-Navarra, D. C. 2008. Food web paradigms: the biochemical view on trophic interactions. International Review of Hydrobiology **93**:489-505.

Nelson, M. M., C. F. Phleger, and P. D. Nichols. 2002. Seasonal lipid composition in macroalgae of the Northeastern Pacific Ocean. Botanica Marina **45**:58-65.

O'Kelly, C. J., A. Kurihara, T. C. Shipley, and A. R. Sherwood. 2010. Molecular assessment of *Ulva* spp. (Ulvophyceae, Chlorophyta) in the Hawaiian Islands. Journal of Phycology **46**:728-735.

Pace, M. L., J. J. Cole, S. R. Carpenter, J. F. Kitchell, J. R. Hodgson, M. C. Van de Bogert, D. L. Bade, E. S. Kritzberg, and D. Bastviken. 2004. Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature **427**:240-243.

Page, H. M., D. C. Reed, M. A. Brzezinski, J. M. Melack, and J. E. Dugan. 2008. Assessing the importance of land and marine sources of organic matter to kelp forest food webs. Marine Ecology Progress Series **360**:47-62.

Phillips, N., R. Burrowes, F. Rousseau, B. de Reviers, and G. W. Saunders. 2008. Resolving evolutionary relationships among the brown algae using chloroplast and nuclear genes. Journal of Phycology **44**:394-405.

Polis, G. A. and S. D. Hurd. 1995. Extraordinarily high spider densities on islands: flow of energy from the marine to terrestrial food webs in the absence of predation. Proceedings of the National Acadamy of Sciences **92**:4382-4386.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Towards an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annual Review of Ecology and Systematics **28**:289-316.

Power, M. E. 1992. Top-down and bottom-up forces in food webs: do plants have primacy? Ecology **73**:733-746.

Ravet, J. L., M. T. Brett, and G. B. Arhonditsis. 2010. The effects of seston lipids on zooplankton fatty acid composition in Lake Washington, Washington, USA. Ecology **91**:180-190.

Richoux, N. B. and P. W. Froneman. 2008. Trophic ecology of dominant zooplankton and macrofauna in a temperate, oligotrophic South African estuary: a fatty acid approach. Marine Ecology Progress Series **357**:121-137.

Rodriguez, S. R. 2003. Consumption of drift kelp by intertidal populations of the sea urchin *Tetrapygus niger* on the central Chilean coast: possible consequences at different ecological levels. Marine Ecology Progress Series **251**:141-151.

Sargent, J. R., G. Bell, L. McEvoy, D. R. Tocher, and A. Estevez. 1999. Recent developments in the essential fatty acid nutrition in fish. Aquaculture **177**:191-199.

Stengel, D. B., S. Connan, and Z. A. Popper. 2011. Algal chemodiversity and bioactivity: sources of natural variability and implications for commercial application. Biotechnology Advances **29**:483-501.

Taipale, S. J., M. J. Kainz, and M. T. Brett. 2011. Diet-switching experiments show rapid accumulation and preferential retention of highly unsaturated fatty acids in *Daphnia*. Oikos **120**:1674-1682.

Turner, J. P. and J. R. Rooker. 2006. Fatty acid composition of flora and fauna associated with *Sargassum* mats in the Gulf of Mexico. Marine Biology **149**:1025-1036.

Wehi, P. M. and B. J. Hicks. 2010. Isotopic fractionation in a large herbivorous insect, the Auckland tree weta. Journal of Insect Physiology **56**:1877-1882.

Table 2.1. 40 NE Pacific marine macrophyte taxa studied in the San Juan Archipelago, USA

Phylum	Order	Family	*Genus species*	Depth[a]	ID#[b]
Anthophyta	Alismatales	Zosteraceae	*Phyllospadix scouleri*	-3	1
			Zostera marina	-5	2
Chlorophyta	Bryopsidales	Codiaceae	*Codium fragile*	-2	3
	Cladophorales	Cladophoraceae	*Cladophora columbiana*	+2	4
	Prasiolales	Prasiolaceae	*Prasiola meridionalis*	+3	5
	Ulvales	Ulvaceae	*Ulva intestinalis*	+10	6
			Ulva sp.[c]	+2	7
Ochrophyta	Desmarestiales	Desmarestiaceae	*Desmarestia munda*	-6	8
	Dictyotales	Dictyotaceae	*Dictyota binghamiae*	-4	9
	Ectocarpales	Chordariaceae	*Soranthera ulvoidea*	0	10
		Scytosiphonaceae	*Scytosiphon lomentaria*	0	11
	Fucales	Fucaceae	*Fucus distichus*	+2	12
		Sargassaceae	*Sargassum muticum*	-4	13
	Laminariales	Alariaceae	*Alaria marginata*	0	14
		Costariaceae	*Agarum fimbriatum*	-8	15
			Costaria costata	-5	16
		Laminariaceae	*Nereocystis luetkeana*	-5	17
			Saccharina latissima	-6	18
			Saccharina sessilis	-2	19
			Saccharina subsimplex	-6	20
		Lessoniaceae	*Egregia menziesii*	-3	21
	Ralfsiales	Heterochordariaceae	*Analipus japonicus*	-3	22
	Syringodermatales	Syringodermataceae	*Syringoderma abyssicola*	-10	23
Rhodophyta	Bonnemaisoniales	Bonnemaisoniaceae	*Bonnemaisonia californica*	-3	24
	Ceramiales	Dasyaceae	*Rhodoptilum plumosum*	-6	25
		Delesseriaceae	*Polyneura latissima*	-3	26
		Rhodomelaceae	*Neorhodomela larix*	+1	27
			Osmundea spectabilis	-5	28
	Corallinales	Corallinaceae	*Calliarthron tuberculosum*	-3	29
	Erythropeltidales	Erythrotrichiaceae	*Smithora naiadum*	-4	30
	Gigartinales	Dumontiaceae	*Constantinea subulifera*	-2	31
		Endocladiaceae	*Endocladia muricata*	+2	32
		Furcellariaceae	*Opuntiella californica*	-6	33
		Gigartinaceae	*Chondracanthus exasperatus*	-6	34
			Mazzaella splendens	0	35
		Kallymeniaceae	*Callophyllis flabellulata*	-5	36
	Halymeniales	Halymeniaceae	*Prionitis sternbergii*	+2	37
	Palmariales	Palmariaceae	*Halosaccion glandiforme*	+4	38
	Plocamiales	Plocamiaceae	*Plocamium pacificum*	-6	39
	Rhodymeniales	Rhodymeniaceae	*Sparlingia pertusa*	-6	40

[a]Depth collected from in the field (m) relative to datum.
[b]Taxa ID # corresponds to numbering in PCA in Figure 2.1a.
[c]This entity is generally referred to as *Ulva lactuca* in the NE Pacific but was recently shown to be a tropical taxon (O'Kelly et al. 2010) and was therefore treated as *Ulva* sp. until a valid name is assigned.

Table 2.2. 123 global macrophyte taxa, collection ocean region, and source (see text for references).

Phylum	Order	Family	*Genus species*	Ocean	Source
Anthophyta	Alismatales	Cymodoceaceae	*Amphibolis griffithii*	SE Indian	1
		Hydrocharitaceae	*Halophila ovalis*	SE Indian	1
		Posidoniaceae	*Posidonia sinuosa*	SE Indian	1
		Zosteraceae	*Zostera capensis*	SW Indian	2
Chlorophyta	Bryopsidales	Bryopsidaceae	*Lambia antarctica*	Southern	3
		Caulerpaceae	*Caulerpa racemosa*	N Indian	4
			Caulerpa veravalensis	N Indian	4
			Caulerpa filiformis	SE Atlantic	5
		Codiaceae	*Codium extricatum*	SE Atlantic	5
			Codium sp.	SW Indian	2
	Cladophorales	Cladophoraceae	*Chaetomorpha linum*	NE Pacific	6
	Prasiolales	Prasiolaceae	*Prasiola crispa*	Arctic	3
	Ulvales	Ulvaceae	*Ulva tubulosa*	N Indian	4
			Ulva linza	N Indian	4
			Ulva fasciata	N Indian	4
			Ulva rigida	N Indian	4
			Ulva reticulata	N Indian	4
			Ulva lactuca	N Indian	4
			Ulva sp.	N Indian	4
			Ulva lactuca	NE Pacific	6
			Enteromorpha compressa	NE Pacific	6
			Ulva rotunda	NE Atlantic	7
			Enteromorpha intestinalis	NE Atlantic	7
			Ulva sp.	SE Atlantic	5
Ochrophyta	Desmarestiales	Desmarestiaceae	*Desmarestia muelleri*	Southern	3
			Desmarestia antarctica	Southern	3
			Desmarestia viridis	NW Atlantic	8
			Desmarestia ligulata	NW Pacific	9
	Dictyotales	Dictyotaceae	*Padina tetrastomatica*	N Indian	4
			Spatoglossum asperum	N Indian	4
			Stoechospermum marginatum	N Indian	4
	Ectocarpales	Chordariaceae	*Leathesia difformis*	NW Pacific	9
			Spaerotreichia divaricata	NW Pacific	9
			Puntaria plantaginea	NW Pacific	9
			Dictyosiphon foeniculaceus	NW Pacific	9
	Fucales	Fucaceae	*Fucus distichus*	NE Pacific	6
			Fucus vesiculosus	NE Atlantic	7
			Fucus evanescens	NW Pacific	9
			Pelvetia wrightii	NW Pacific	9
		Sargassaceae	*Cystoseira indica*	N Indian	4
			Sargassum tenerrimum	N Indian	4
			Cystoseira osmundacea	NE Pacific	6
			Halidrys siliquosa	NE Atlantic	7
		Sargassaceae	*Sargassum sp*	SE Indian	1
			Sargassum heterophyllum	SE Atlantic	5
			Cystoseira crassipes	NW Pacific	9
			Coccophora langsdorffi	NW Pacific	9
	Laminariales	Alariaceae	*Alaria marginata*	NE Pacific	6
	Laminariales	Alariaceae	*Undaria pinnatifida*	NE Atlantic	7
			Alaria angustata	NW Pacific	9
			Undaria pinnatifida	NW Pacific	9
		Chordaceae	*Chorda filum*	NW Pacific	9
		Costariaceae	*Costaria costata*	NW Pacific	9
			Agarum cribrosum	NW Pacific	9
		Laminariaceae	*Laminaria solidungula*	Arctic	3
			Laminaria dentigera	NE Pacific	6
			Hedophyllum sessile	NE Pacific	6
			Macrocystis integrefolia	NE Pacific	6
			Postelsia palmaeformis	NE Pacific	6
			Agarum clathratum	NW Atlantic	8
			Saccharina longicruris	NW Atlantic	8
			Laminaria saccharina	NE Atlantic	7
			Laminaria digitata	NE Atlantic	7
			Laminaria bongardiana	NW Pacific	9

			Laminaria japonica	NW Pacific	9
			Laminaria cichorioides	NW Pacific	9
			Arthrothamnus kurilensis	NW Pacific	9
		Lessoniaceae	*Egregia menziesii*	NE Pacific	9
			Ecklonia radiata	SE Indian	1
	Scytosiphonales	Scytosiphonaceae	*Analipus japonicus*	NE Pacific	6
			Analipus japonicus	NW Pacific	9
			Petalonia fascia	NW Pacific	9
Rhodophyta	Acrochaetiales	Acrochaetiaceae	*Audouinella purpurea*	Southern	3
	Ahnfeltiales	Ahnfeltiaceae	*Ahnfeltia plicata*	N Indian	4
	Bangiales	Bangiaceae	*Porphyra umbilicalus*	NE Atlantic	7
	Ceramiales	Delesseriaceae	*Phycodrys rubens*	Arctic	3
			Delessaria lancifolia	Southern	3
			Myriogramme smithii	Southern	3
			Neuroglossum ligulatum	Southern	3
			Pantoneura plocamioides	Southern	3
			Cryptopleura violaceae	NE Pacific	6
		Rhodomelaceae	*Laurencia cruciata*	N Indian	4
			Laurencia papillosa	N Indian	4
			Odonthalia floccosa	NE Pacific	9
			Laurencia filiformis	SE Indian	1
			Polysiphonia sp.	SE Indian	1
		Wrangeliaceae	*Ptilota gunneri*	Arctic	3
			Georgiella confluens	Southern	3
	Corallinales	Corallinaceae	*Amphiora anceps*	N Indian	4
			Coraline sp.	NW Atlantic	8
			Amphiora anceps	SE Indian	1
			Haliptilon roseum	SE Indian	1
			Metagoniolithon stelliferum	SE Indian	1
			Corallina sp.	SE Atlantic	5
	Gelidiales	Gelidiaceae	*Gelidium pristoides*	SE Atlantic	5
			Gelidium enterobium	SE Atlantic	5
	Gigartinales	Cystocloniaceae	*Hypnea musciformis*	N Indian	4
			Hypnea esperi	N Indian	4
			Hypnea spicifera	SE Atlantic	5
	Gigartinales	Gigartinaceae	*Gigartina skottsbergii*	Southern	3
			Iridaea cordata	NE Pacific	9
			Gigartina harveyana	NE Pacific	9
			Chondrus crispus	NE Atlantic	7
		Phyllophoraceae	*Gymnogongrus turquetii*	Southern	3
		Solieriaceae	*Kappaphycus alverzii*	N Indian	4
			Sarconema filiforme	N Indian	4
	Gracilariales	Gracilariaceae	*Gracilaria debilis*	N Indian	4
			Gracilaria dura	N Indian	4
			Gracilaria furgosonii	N Indian	4
			Gracilaria verrucosa	NE Atlantic	7
			Curdea obesa	SE Indian	1
	Halymeniales	Halymeniaceae	*Grateloupia indica*	N Indian	4
			Grateloupia wattii	N Indian	4
			Prionitis lanceolata	NE Pacific	9
			Prionitis linearis	NE Pacific	9
	Palmariales	Palmariaceae	*Devaleraea ramentacea*	Arctic	3
			Palmaria decipiens	Arctic	3
			Palmaria palmata	Southern	3
			Palmaria palmata	NE Atlantic	7
	Plocamiales	Plocamiaceae	*Plocamium violaceum*	NE Pacific	9
			Plocamium corallorhiza	SE Atlantic	5
	Rhodymeniales	Fryeellaceae	*Hymenocladiopsis crustigena*	Southern	3
		Rhodymeniaceae	*Rhodymenia subantarctica*	Southern	3

Sources: [1]Hanson et al. 2010, [2]Richoux and Froneman 2008, [3]Graeve et al. 2002, [4]Kumari et al. 2010, [5]Allan et al. 2010, [6]Khotimchenko et al. 2002, [7]Fleurance et al. 1994, [8]Kelly et al. 2008, [9]Khotimchenko 1998

Table 2.3. Results of PERMANOVA analyses on three datasets testing for differences in FA percent composition of macrophyte taxa between phylogenetic grouping factors of phylum, order, and family. Dataset 1 uses all 44 identified FA in San Juan Archipelago (SJA) macrophyte taxa (n=40; Table 2.1). Dataset 2 uses only seven 'essential' ω3 and ω6 FA (EFA, see Methods) from the SJA taxa. Dataset 3 uses only the same seven EFA, gathered from macrophyte FA literature (n=123; Table 2.2). The SJA dataset includes FA data for three replicates per taxon, whereas published data are mean taxon percent FA values (with varying levels of within group replication). Analyses use Type III sums of squares, fixed effects, and use 9999 permutations (see Methods). Percent variance (% Var) is the variance component estimated for each factor divided by the sum of all variance components to quantify the relative magnitude of effects.

Dataset *Source*	*df*	*MS*	*Pseudo-F*	*P(perm)*	*% Var*
1. SJA – 44 FA					
PHY	3	9732	114.4	0.0001	36.5
ORD(PHY)	17	1392	16.4	0.0001	25.3
FAM(ORD(PHY))	11	1099	12.9	0.0001	24.2
Residual	86	85.1			13.9
Total	117				
2. SJA – 7 EFA					
PHY	3	7083	156.2	0.0001	40.5
ORD(PHY)	17	719	15.9	0.0001	23.6
FAM(ORD(PHY))	11	574	12.7	0.0001	22.7
Residual	86	45.3			13.2
Total	117				
3. Global – 7 EFA					
PHY	3	4532	27.4	0.0001	36.2
ORD(PHY)	19	670	4.1	0.0001	22.5
FAM(ORD(PHY))	16	313	1.9	0.0083	15.3
Residual	84	166			26.0
Total	122				

Table 2.4. Results of PERMANOVA testing for the significance of the factors order and ocean and their interaction in FA percent composition of 163 macrophyte taxa in 27 orders and 10 ocean sub-regions (Arctic, Southern, N Indian, SE Indian, SW Indian, NE Pacific, NW Pacific, NE Atlantic, NW Atlantic, and SE Atlantic). This test combines all SJA (Table 2.1; n=40 taxa) and the global (Table 2.2; n=123 taxa) data and considers only seven 'essential' ω3 and ω6 FA (EFA, see Methods). All analyses use Type III sums of squares, assume fixed effects, and significance is determined with 9999 permutations. Relative magnitude of the effects is expressed as % Var (see Methods).

Source	*df*	*MS*	*Pseudo-F*	*P(perm)*	*% Var*
ORDER	26	998	6.5	0.0001	37.5
OCEAN	9	419	2.8	0.0003	14.6
ORDER x OCEAN	37	237	1.6	0.0097	16.4
Residual	90	153			31.5
Total	162				

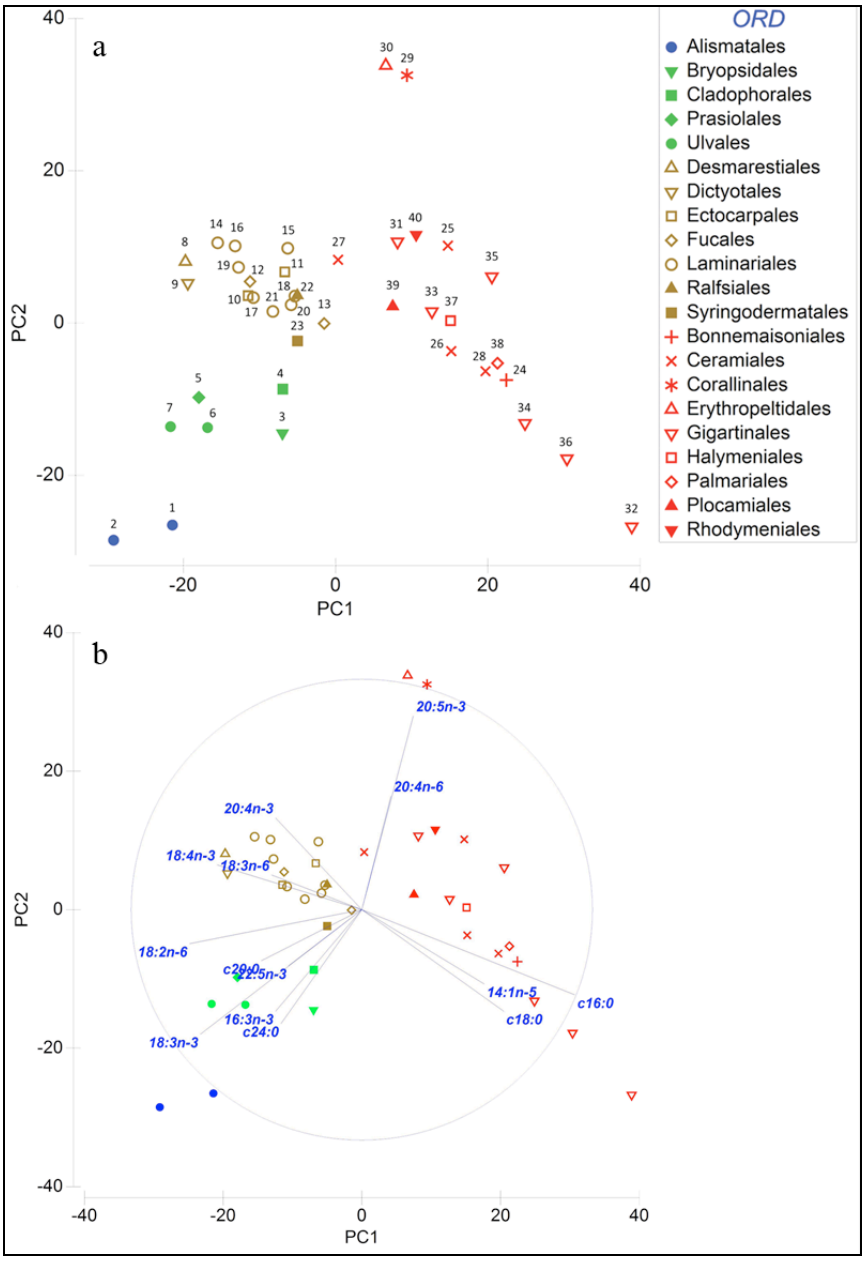

Fig. 2.1. (a) Principal Component Analysis (PCA) visualization of mean multivariate macrophyte fatty acid (FA) composition data. PCA is run on a Euclidean distance matrix of mean percent composition of all 44 regularly identified FA for 40 San Juan Archipelago (NE Pacific) macrophyte taxa from 21 orders and 36 families. Symbol colors represent different phyla (Anthophyta, blue; Chlorophyta, green; Ochrophyta, brown; Rhodophyta, red) and phylogenetic order as symbols. The first 2 of 5 PCs (plotted here) account for 65.2% of the cumulative variation and show the lowest two-dimensional solution of the dataset. Numbers above symbols correspond to the mean value of each taxon in the analysis (Table 2.1). (b) PCA eigenvector plot shows the trajectory of the correlations (Pearson, >0.4) of the FA variables to PCs 1 and 2.

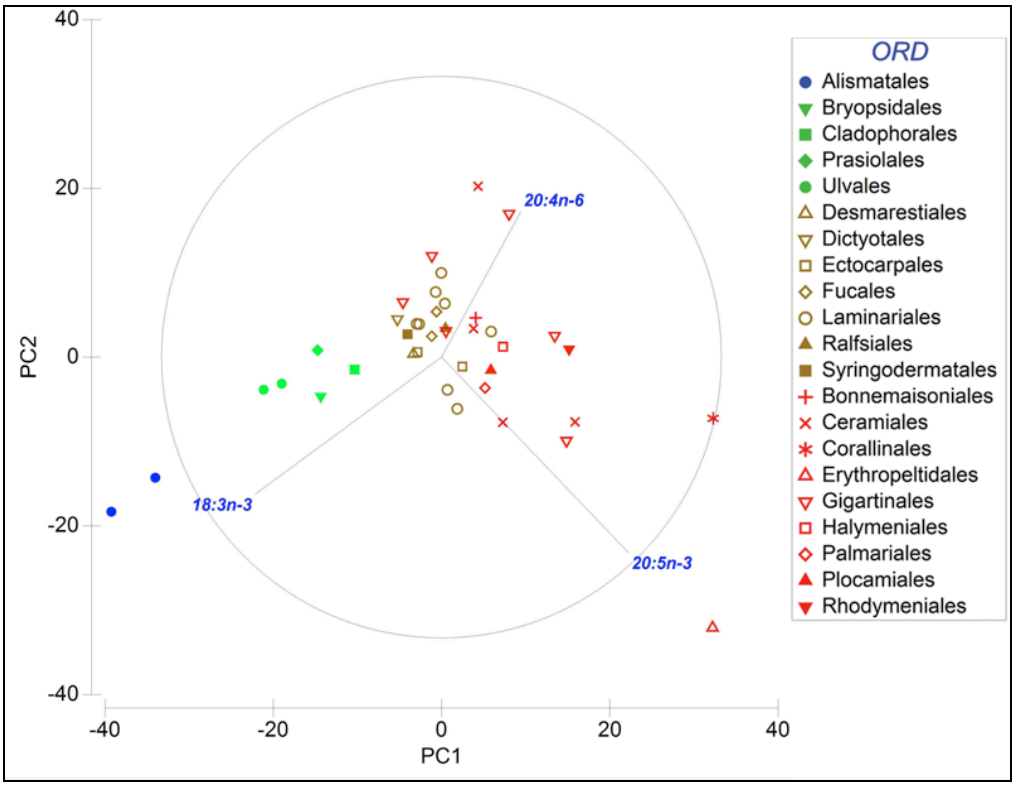

Fig. 2.2. Principal Component Analysis (PCA) visualization of mean multivariate macrophyte essential fatty acid (EFA) composition data. PCA is run on a Euclidean distance matrix of mean percent composition of seven 'essential' ω3 and ω6 FA (selected *a priori* - see Methods) for 40 San Juan Archipelago (NE Pacific) macrophyte taxa from 21 orders and 36 families (Table 2.1). Symbol colors represent different phyla (described in Fig. 2.1) and phylogenetic order as symbols. The first 2 of 5 PCs (plotted here) account for 73.0% of the cumulative variation. A PCA eigenvector overlay shows the trajectory of the correlations of three of the EFA variables (Pearson, >0.4) to PCs 1 and 2.

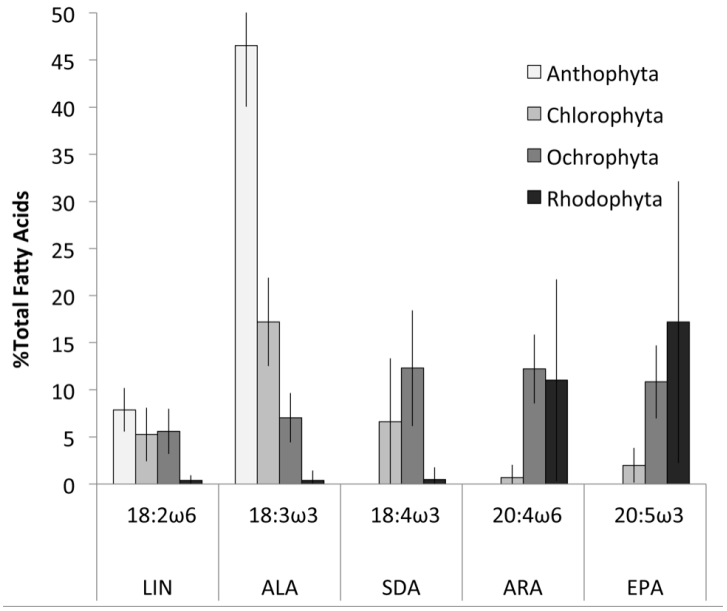

Fig. 2.3. Percent composition of five 'essential' ω3 and ω6 FA relative to the total FA in 40 macrophyte taxa evaluated from the San Juan Archipelago in the Anthophyta (empty), Chlorophyta (light gray), Ochrophyta (medium gray), and Rhodophyta (dark gray). This *post-hoc* summary plot includes only the abundant EFA for the SJA dataset at the phylum level. Two EFA, 18:3ω6 and DHA, were not included because they each accounted for a grand mean of less than 1% of total EFA in the SJA taxa. Bars are means across all taxa in each division, error bars are SD.

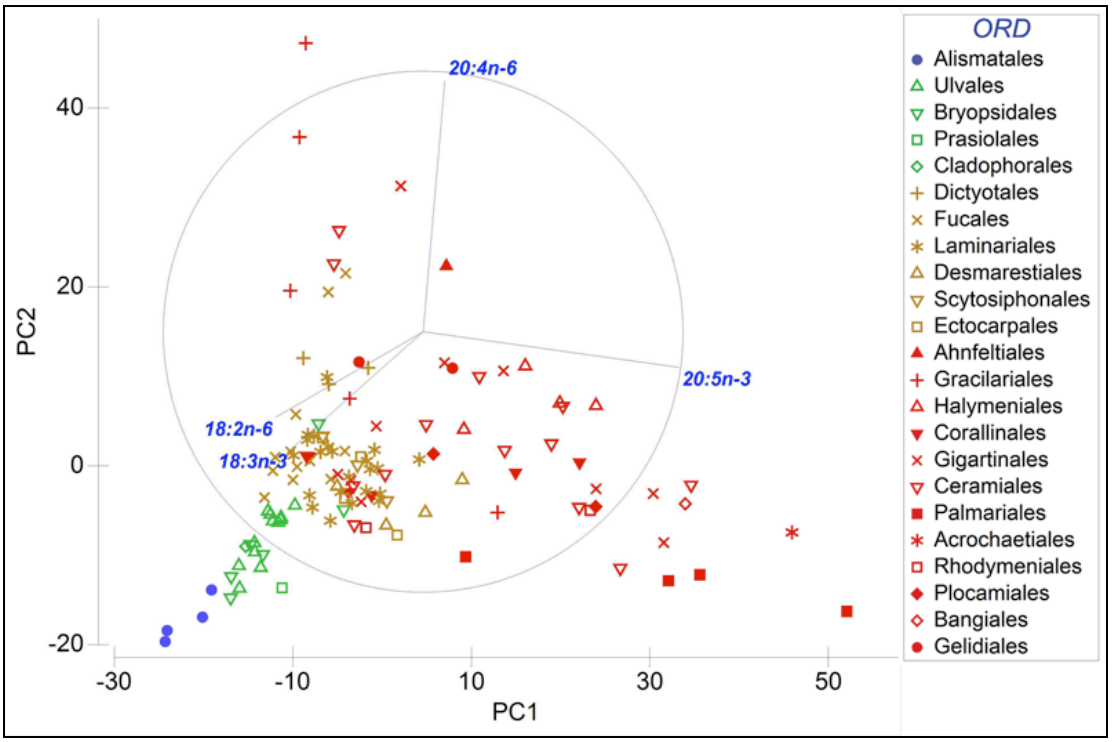

Fig. 2.4. PCA of global macrophyte FA composition data. PCA was run on a Euclidean distance matrix of the percent composition of only seven EFA reported for 123 global taxa from 21 orders and 36 families collected in 10 global ocean sub-regions (see Table 2.2). Symbol colors represent different phyla (described in Fig. 2.1) and phylogenetic order as symbols. Each plotted symbol represents EFA mean data for 1 published taxon, where taxa means in literature represent a range of independent specimens from 1->5. The first 2 of 5 PCs (plotted here) account for 77.8% of the cumulative variation. A PCA eigenvector A PCA eigenvector overlay shows the trajectory of the correlations of four of the EFA variables (Pearson, >0.4) to PCs 1 and 2.

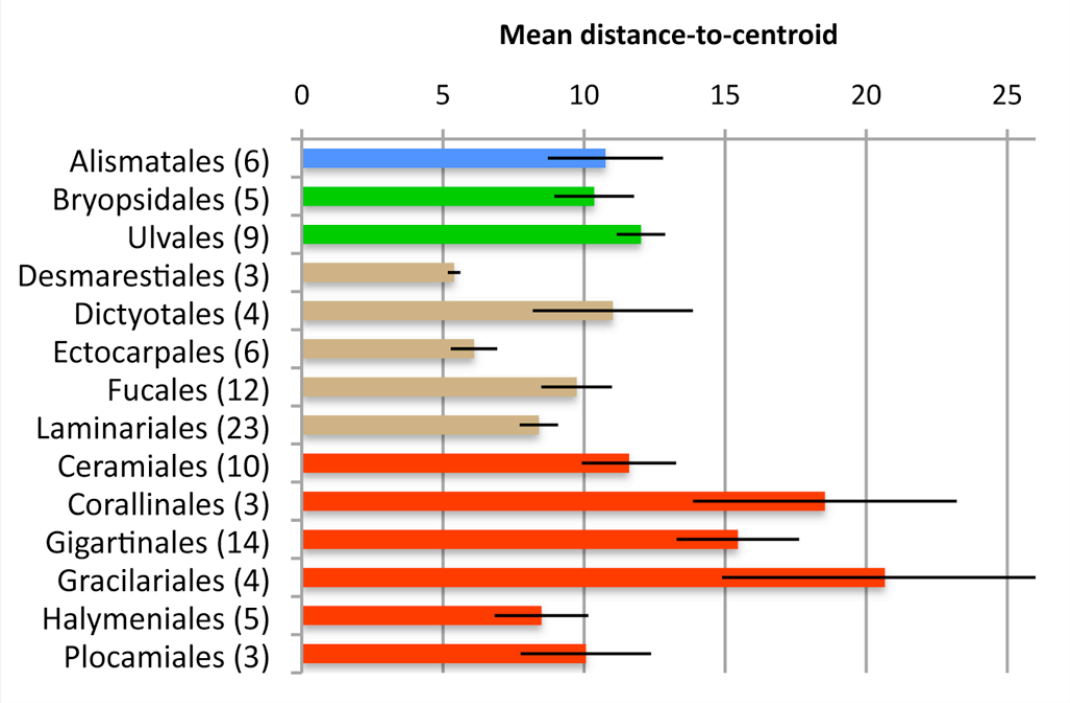

Figure 2.5. Summary plot of the post-hoc pairwise comparisons of the multivariate FA dispersions of macrophyte phylogenetic order. The mean distance to group centroid value (± 1 SE) is calculated from the Euclidean distance matrix of the seven 'essential' ω3 and ω6 FA from the combined San Juan Archipelago and global taxa list (see Methods). The associated PERMDISP analysis ($F_{25, 95}$=3.725, P=0.005) is a test of the null hypothesis of no differences among the dispersions of the FA signatures of the taxa within orders. Bar colors represent different phyla (Anthophyta, blue; Chlorophyta, green; Ochrophyta, brown; Rhodophyta, red) and individual bars are all phylogenetic orders (with sample size of unique taxa within that order in parentheses).

CHAPTER 3 – FATTY ACID AND STABLE ISOTOPE BIOMARKERS SUGGEST MICROBE-INDUCED DIFFERENCES IN BENTHIC FOOD WEBS BETWEEN DEPTHS

Abstract

Benthic marine consumers inhabiting the subphotic zone rely on subsidies of energy synthesized by macrophytes and phytoplankton in the photic zone. The effects of this energy subsidy on the trophic ecology of deep invertebrates are generally unknown. We used fatty acids (FA) and multiple stable isotopes (MSI) as trophic biomarkers to compare tissues from conspecifics of primary and secondary consumers in photic and subphotic habitats (15 and 100 m depth) at three sites in the San Juan Archipelago, Washington. FA composition differed across depths for all five species and MSI differed across depths for 6 of 7 species. We found a general pattern of enrichment in $\delta^{13}C$ from shallow to deep for all consumers. $\delta^{15}N$ was consistently enriched in deep herbivores and suspension feeders, but did not differ in predators. Total ω3 FA were lower in deep primary consumers, whereas predator ω3 FA did not differ between depths. Total bacterial marker FA were lower in deep suspension feeders but higher in deep predators. The results suggest a possible mechanism for the differences in FA and enrichment between habitats: deep consumers potentially ingest detritus that has been biochemically altered by microbes during transport. We found support for this hypothetical mechanism in an algal aging experiment. Aged algae colonized by microbes responded with increases in bacterial FA, and decreases in ω3 FA. This study highlights the power of combining FA and MSI biomarkers, and provides evidence for the importance of organic matter degradation to food web studies.

Introduction

Trophic interactions in the deep, subphotic habitats, which account for ~90% of the earth's oceans (Ramirez-Llodra et al. 2010), are poorly understood. Deep nearshore subtidal environments (DNSE), here functionally defined as coastal habitat below the photic zone (~30-500 m depth) occupy a transitional zone that is beyond the depth of net photosynthesis, but still relatively accessible for observational and experimental research. DNSE habitats rely upon an allochthonous energy subsidy (Polis et al. 1997) from primary producers growing in the nearshore shallow photic environments (SPE; Britton-Simmons et al. 2009, 2012). Photosynthetic organisms produce the vast majority of energy for food webs via the de novo synthesis of carbohydrates, lipids and proteins. The availability and importance of basal energy sources (including phytoplankton, terrestrial carbon, and macrophytes) to benthic invertebrate secondary production in subtidal nearshore ecosystems may vary considerably with distance from the source of production (Krumhansl and Scheibling 2012*a*). The relative contribution of these disparate sources has been the subject of many studies, yet remains unresolved (Duggins et al. 1989; Nadon and Himmelman 2006; Miller and Page 2012).

Much of what we know about trophic relationships between marine consumers and their prey is based upon inferences made from 'biomarker' (e.g., fatty acid [FA] and multiple stable isotope [MSI]) signature analysis (Kelly and Scheibling 2012; Peterson 1999). There are two general methods for estimating the trophic pathways and importance of different energy sources to subtidal communities: a non source-specific approach: calculate estimates of net primary productivity (NPP) and flux rates of various sources, assuming that sources with the highest NPP are most important (Cebrian 1999); or a potentially source-specific approach: examine differences in biomarker signatures of producers and track these biomarkers into consumer

tissues (Dunton and Schell 1987; Duggins et al. 1989). Many small benthic invertebrates, including suspension feeders, consume prey that cannot be reliably identified from traditional stomach content analysis (Cranford and Grant 1990). For such cases, a FA and MSI biomarker approach is essential for investigating trophic relationships.

A diverse assemblage of FA is found in whole lipid extractions of tissues from organisms. As such, FA 'signatures' for different sources can be quite informative as trophic markers (Budge et al. 2006; Kelly and Scheibling 2012). FA can be used for qualitative assessment of trophic relationships (e.g., tracing presence of certain source-specific biomarkers), or for quantitative modeling of predator diet when FA signatures of all likely prey sources are known and fractionation of FA from diet to tissues of the consumer is accounted for (Iverson 2009). It is becoming increasingly evident that FA signatures are tied to species' phylogenetic relationships, e.g., in marine macrophytes (Galloway et al. 2012), phytoplankton (Lang et al. 2011), invertebrates and fishes (Budge et al. 2002).

Multiple stable isotope (MSI; $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) analysis is an additional valuable biomarker tool for ecologists (Fry 2006). Carbon isotope ratios are changed very little (~+1‰) between trophic levels, and remain close to the ratio at which the carbon was photosynthetically 'fixed' by an autotroph. The type of photosynthesis used to fix the carbon is thus imprinted into its signature (Fry 2006). Nitrogen isotope ratios are enriched by an average value of ~+3.4 ‰ between trophic levels, and are therefore used to calculate the number of trophic levels between a consumer and its diet (Cabana and Rasmussen 1996; Peterson 1999). The sulfur isotope ratio is not known to change substantively between trophic levels, and can indicate whether the sulfur came from oceanic or terrestrial sources, making it useful as an indicator of organic material origin (Peterson and Fry 1987; Fry 2006).

Here, we used FA and MSI as trophic biomarkers to compare tissues from conspecifics of a suite of primary and secondary consumers between photic and subphotic habitats (15 and 100 m depth) at three sites in the San Juan Archipelago, Washington. This approach allows us to evaluate the consequences of an energy subsidy on the trophic ecology of conspecifics in both habitats; if biomarker signatures differ across these depths it may indicate either that animals eat different diets or that resources are changing during the aging process as they transit to deeper habitats. Specifically, we asked: 1) Do FA and MSI signatures differ among invertebrate species? 2) Do FA and MSI signatures of subsidy-dependent organisms in deep water differ from conspecifics in shallow-water habitats? And 3) If differences exist, do summaries of FA categories or MSI differ consistently across depths? To test a hypothesis on a potential mechanism for the differences observed in consumer tissues, we also investigated the FA response of two common types of macroalgae to aging and colonization by microbial biofilms from a concurrent lab experiment designed to simulate the processes affecting detritus during transport to deep habitats. Here, we ask: Does algal aging and microbial biofilm colonization change FA signatures of algae? Are the results of this experiment consistent with patterns observed in the consumer biomarker data?

Methods

Study system

Three study sites in the San Juan Archipelago (SJA; Fig. 3.1) were selected to maximize an a priori hypothesized natural gradient of influence of freshwater (terrestrial origin) inputs (at the northernmost site Skipjack; SKP) to the influence of higher salinity oceanic water (at the westernmost site, Pillar; PIL) with one site intermediate (Point Caution; PTC). These anticipated

patterns have been confirmed with in-situ subtidal temperature and salinity loggers (D. O. Duggins unpublished data). The bathymetry of the SJA is complex, with channel depths up to 300 m and strong tidally driven hydrodynamic forces that can result in currents in excess of 100 cm s^{-1} (Eckman et al. 2003). Britton-Simmons et al. (2012) used a remotely operated vehicle to show that detached macrophytes (drift) are abundant at all depths surveyed below the photic zone (30-170 m) in the San Juan Channel and are associated with large aggregations of consumers, including shrimp. Furthermore, previous research in our system showed that MSI signatures of macrophytes can differ among sites (Dethier et al. 2013; among-site FA signatures not evaluated). Because site-level differences in MSI and FA signatures were found in invertebrate consumer tissues in other systems (e.g., Tasmania: Guest et al. 2010), we explicitly designed our study to capture potential variation at multiple sites.

Invertebrate biomarker signatures

We used a benthic trawl to collect organisms in the DNSE from a depth of 90-100 m at each site. We identified species in the field and froze (-20°C) 3-10 whole individuals of each within 6 hours of collection. From a list of the species (n=12) common to all sites, we chose a range of trophic positions and feeding modes, and then collected 'shallow' (10-15 m) conspecific individuals in the SPE at each site using SCUBA. All shallow and deep specimens were collected in August-September 2011. We were left with a total of 7 taxa from all sites and depths (Table 3.1), and most (36 of 42) analyses had 5 site and depth replicates.

Due to logistical constraints, we extracted FA from only 5 of the 7 taxa studied with MSI, representing each of the dominant feeding modes in our list (see Table 3.1). Within 6 months, all animals were thawed, dissected, and muscle tissue was removed, lyophilized for 48 h and ground in acetone pre-washed stainless steel mortar and pestles. Each FA and MSI analysis was

conducted on separate 10 mg aliquots of the same dried, ground muscle tissue from each specimen, so that all biomarker analyses are fundamentally linked to shared tissue. MSI analyses of invertebrate muscle tissue followed Howe and Simenstad (2007). Lyophilized MSI samples were weighed using a microbalance and enclosed in tin capsules for analysis at Washington State University's Stable Isotope Core lab. FA extractions were performed in-house (described below).

Algal aging experiment

An algal aging study was conducted in July and August 2011 on specimens of two common NE Pacific macroalgae, *Saccharina subsimplex* and *Agarum fimbriatum* (both Laminariales). These kelps are the top two contributors to benthic drift macroalgae (and therefore a potentially important energy source for consumers) within the SJA (37% and 11% of the total drift, respectively; Britton-Simmons et al. 2009). They further provide a contrast in concentrations of phlorotannin (i.e., phenolic) chemical defenses, with *Agarum* having substantially higher content than *Saccharina* (Van Alstyne 1999).

Results of other analyses performed in the algal aging experiment (e.g., MSI values, C:N ratios, microbial counts, phlorotannin concentrations) are presented in Sosik (2012) and Sosik and Simenstad (in revision). The experiment ran concurrently to the consumer data collection, and was not designed a priori to evaluate the patterns found in the observational dataset. Here, we evaluate a subset of the samples from Sosik (2012) specifically for the FA response to algal aging and microbe colonization. Four replicate blades from each species were collected from the field, cleaned of any visible epibionts by gentle scrubbing, and aged for five weeks in the lab in separate, darkened tanks in flow-through seawater. Samples (5 cm^2) were taken from aging blades at 1 and 5 weeks from approximately the same location on each blade (avoiding meristem

and sori), and frozen (-20°C) for later analysis (<6 months). We extracted FA from the four replicate samples from each species and age (1 or 5 weeks).

Fatty acid extraction, methylation, and quantification

FA extractions were performed using a modified Folch method (Taipale et al. 2011). Ten mg of lyophilized tissue sample were weighed into a culture tube. Two mL of chloroform were added, followed by 1 mL of methanol, 1 mL of 2:1 chloroform: methanol mix, and 0.5 mL of deionized water. This mixture was sonicated for 10 minutes, vortexed for 30 seconds, and finally centrifuged for 3 min at 3000 revolutions $minute^{-1}$ (rpm) to separate the phases. The lower (organic) phase was removed to a separate culture tube. An additional 2.7 mL of chloroform was added to the original sample to replenish the volume removed, and the process of sonication, vortexing, centrifugation, and removal was repeated two more times, for a total of three lower phase extractions. The extracted organic layers were pooled and evaporated under nitrogen. Once dry, 1 mL of toluene and 2 mL of a 1% solution of sulfuric acid in methanol were added to re-suspend the lipids. This mixture was vortexed to ensure uniformity, and then placed into a 50°C water bath for 16 h to allow for methylation of the FA. Once cooled, 2 mL of 2% $KHCO_3$ solution and 5 mL of 1:1 hexane: diethyl ether were added, followed by vortexing and centrifugation for 2 minutes at 1500 rpm to separate the phases. The organic layer was again removed (this time the upper phase). To ensure total removal of FA methyl esters (FAME), we added an additional 5 mL of hexane: diethyl ether, repeated the previous step and pooled the organic layers from the extractions. These organics were evaporated under nitrogen, and the FAME subsequently dissolved into 1.5 mL hexane for gas chromatography (GC) analysis.

Chromatograms were generated from a GC equipped with a flame ionization detector (GC-FID; HP 6958), with an Agilent DB-23 column, and an 85-minute method (following

Taipale et al. 2011) designed to separate 16 and 18 monounsaturated FA (MUFA) and polyunsaturated FA (PUFA). Peak identification was achieved by analyzing a subset of samples using GC-mass spectrometry (GC-MS) with the same column and under an identical temperature program. Mass spectral information from each species improved identification beyond comparison of known retention times, and was able to distinguish FAME which had co-eluted or which had shifted elution times. To quantify the sample FA weights (mg FAME g^{-1} dry tissue), dilutions of a 569B standard purchased from Nu-chek Prep were run through the GC-FID from which our data was generated. The concentrations of FAME (2, 1, 0.5, 0.25, 0.1, 0.05, and 0.0025 mg mL^{-1}) were correlated with areas from the chromatogram. After accounting for hexane volume and dry weight of the sample, an estimate of the proportion of FAME per unit mass of dry weight was calculated.

Data analysis

We used permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) (Euclidean distance) to evaluate differences in biomarker signatures of invertebrates. We identified 54 different FA using a combination of GC-FID and GC-MS. Before breaking the analysis into within-species evaluations across depths (see below), we first evaluated the significance of the factor 'species' with the entire FA dataset, and found FA signatures to be very different among species (see Results). For our depth comparisons of conspecifics, the mean proportion of each FA across all samples for each species was calculated, and all FA that constituted <0.1% of the total for that species were removed (Kelly et al. 2008; see '#FA' in Table 3.1). All subsequent FA analyses, on both consumers and algae, are therefore species-specific, as different suites of FA passed this abundance test for each species (Table 3.1). We transformed each species' FA dataset using the log-ratio (LN(*x*/18:0)) transformation, which

has been recommended particularly for proportional FA data (Iverson et al. 2002) without zeros in the dataset. Non-metric multidimensional scaling (NMDS) plots are used to visualize multivariate FA patterns and box-plots are presented for each SI result.

Because site level variation is potentially significant for biomarker signatures (Guest et al. 2010; Dethier et al. 2013), to analyze our fundamental question about differences between depths we first pooled sites in 1-way analyses on the factor depth. We also performed two-way PERMANOVAs (for both FA and MSI) to investigate site x depth interactions, which were present in most tests (see Results). However, because site differences were not of primary interest in this study, we also conducted 1-way analyses (test of factor depth) for each species at each site to determine if the interaction of site and depth affected our conclusions. MSI PERMANOVAs were run on untransformed MSI data that were first normalized to a common scale (Anderson et al. 2008). All PERMANOVAs used Type III sums of squares, treating depth and site as fixed and random factors, respectively.

We evaluated FA response in the algal aging experiment with a 1-way PERMANOVA for each species. For both algae, all rare FA were removed, and remaining FA were log-ratio transformed (same approach defined above for consumers), and normalized prior to analysis. The factor time (1 and 5 weeks) was treated as fixed. For all PERMANOVA analyses, significance of tests was determined using unrestricted permutation of the raw data (9999 permutations). When the number of unique permutations for a test <200, we used the Monte-Carlo generated *p*-values (Anderson et al. 2008).

We used similarity percentages (SIMPER) analyses to identify the FA that are important to differences found in the PERMANOVA tests of depth. Datasets used for SIMPER analyses were not transformed because the power of their interpretation is based upon comparisons of the

mean proportional FA abundance within each species. All multivariate analyses were performed using Plymouth Routines In Multivariate Ecological Research (PRIMER; version 6.0 with PERMANOVA+ add on). Univariate analysis of variance (ANOVA) and associated 2-sample *t*-tests were used to compare log-transformed FAME weight (mg FAME g^{-1} dry tissue mass) summary categories, including: total FA, saturated FA (SAFA), MUFA, PUFA, ω3 FA, ω6 FA, highly unsaturated FA (HUFA; e.g., FA with ≥20 C and ≥3 double bonds, Bell and Tocher 2009), and bacterial biomarker FA (*i*-15:0, 15:0, *i*-17:0, *ai*-17:0, 16:1-branched, 17:0, 18:1ω7; Kelly and Scheibling 2012) across depths (for consumers) or across treatments (for algal aging experiment). Univariate tests were performed with JMP.

Results

Species specific FA and MSI signatures

An initial PERMANOVA analysis of the factor species (using the full 54 FA dataset for 5 species) and associated NMDS plot (Fig. 3.2) clearly showed that FA signatures differed among species regardless of site or depth (PERMANOVA, Pseudo-F_4=408.1, P=0.0001). This is due to the same FA not always being present above trace levels in all the species. The subsequent separation of each species into its own analytical unit and removal of the extremely rare or absent FA resulted in 5 separate FA datasets (see Appendix B) with only the most important FA for a given species (see *n* FA for each species in Table 3.1). Across-species MSI signatures were also different (PERMANOVA, Pseudo-F_6=53.68, P=0.0001; not illustrated with NMDS). MSI means (±SD) for each species x site x depth combination are presented in Appendix C.

Within consumer FA signatures across depths

When replicates from all sites are pooled, FA composition differed across depths for all five species (PERMANOVA, $P<0.005$; Table 3.2). NMDS plots present visual comparisons of the multivariate FA signatures for each species across depths (Fig. 3.3a-e). Collection sites are indicated as a factor in the Fig. 3.3 plots (see below for result on site differences). SIMPER analyses revealed there was little consistency among species in the FA primarily responsible for driving differences across depths (Table 3.3). The PUFA 20:5ω3 (EPA) was an exception to this; it was the only FA that in all species was ranked in the top 5 FA for discriminating across depths. All deep individuals were depleted in EPA content relative to shallow conspecifics except for the predatory snail *Fusitriton oregonensis* (Gastropoda). The SIMPER results further show that the number of FA that substantively contribute to differences between depths varies among taxa (10 FA in the green urchin *Strongylocentrotus droebachiensis* [Echinoidea], versus only 3 in the scallop *Chlamys hastata* [Bivalvia]: Table 3.3), and appears to be unrelated to feeding mode of the organism (3 FA in *Chlamys* and 9 in the sea cucumber *Psolus chitonoides* [Holothuroidea], both suspension feeders).

Two-way PERMANOVAs (site and depth as factors) found significant ($P<0.05$) site x depth interactions for all species except the urchin *Strongylocentrotus*. We therefore conducted individual PERMANOVAs testing depth only for each site and taxon combination ($n=15$ tests; Table 3.4). Urchins were included in this second series of tests (even though they did not initially return a significant overall interaction) to be consistent in our analytical approach; i.e., to do the same tests to all species. Twelve of these 15 within-site comparisons were significant for depth (PERMANOVAs, $P<0.025$); the exceptions were: *Fusitriton* at PIL, *Strongylocentrotus* at PTC, and the crab *Cancer oregonensis* [Decapoda] at PTC (Table 3.4). Thus, FA differentiate taxa

across depths in multivariate space despite the site interactions (*see* groupings of deep and shallow samples in Fig. 3.3a-e). The direction of the interaction across sites is generally consistent (Fig. 3.3a-e).

Univariate analyses on FA summary categories (where replicates are pooled across sites) show significant declines in both total mg FAME and PUFA g^{-1} of tissue for the deep suspension feeders *Chlamys* (FAME: *t*-test=3.45, df=26, *P*=0.0019, PUFA: *t*-test=2.39, df=26, *P*=0.0246) and *Psolus* (FAME: *t*-test=2.38, df=27, *P*=0.0246, PUFA: *t*-test=2.09, df=27, *P*=0.0462), but not other organisms (Table 3.5). Total ω3 FA were significantly lower in deep *Chlamys* (*t*-test=2.28, df=26, *P*=0.0309), *Psolus* (*t*-test=2.36, df=27, *P*=0.0256), and *Strongylocentrotus* (*t*-test=2.58, df=26, *P*=0.0160), but did not differ among depths in the predators *Cancer* and *Fusitriton*. The proportion of bacterial marker FA (*n*=7 FA included in this analysis) to total FA was variable among species. The shallow suspension feeders had higher total bacterial marker FA in their tissues compared with deep conspecifics (*Chlamys*: *t*-test=3.17, df=26, *P*=0.0038; *Psolus*: *t*-test=2.95, df=27, *P*=0.0066), whereas the deep predators had more total bacterial marker FA than shallow (*Cancer*: *t*-test=-2.29, df=27, *P*=0.0299; *Fusitriton*: *t*-test=-2.48, df=25, *P*=0.0204; Table 3.5).

Within consumer MSI signatures across depths

MSI signatures differed across depths for six of the seven taxa tested (PERMANOVAs, *P*<0.005; Table 3.2), with the exception of *Fusitriton* (PERMANOVA, Pseudo-F_1=2.55, *P*=0.0766). Univariate comparisons of individual isotopes for each species across depths show that $\delta^{13}C$ is consistently significantly enriched in deep animal tissues relative to shallow for all taxa (Fig. 3.4a). $\delta^{15}N$ results showed two general patterns; the predators *Cancer* and *Fusitriton* did not differ across depths (*P*>0.05) and both were enriched (~3‰) relative to the other species;

furthermore, deep 'non-predators' (n=5 species) were all significantly enriched relative to shallow conspecifics (Fig. 3.4b). The average (±SD) enrichment for $\delta^{13}C$ and $\delta^{15}N$ from shallow to deep was greater for primary consumers ($\delta^{13}C$: -1.00±0.17‰; $\delta^{15}N$: -1.22±0.46‰; n=5) than for predators ($\delta^{13}C$: -0.70±0.01‰; $\delta^{15}N$: -0.33±0.08‰; n=2; Fig. 3.4a, b). $\delta^{34}S$ differed across depths for only *Cancer* (t-test=3.01, df=27, P=0.0057; Fig. 3.4c). $\delta^{34}S$ values were highly variable in shallow *Psolus* and deep *Strongylocentrotus* (Fig. 3.4c).

Algal aging experiment: FA

FA signatures changed in both algae after aging 5 weeks (PERMANOVAs: *Saccharina*: Pseudo-F_1=7.35, P=0.0043; *Agarum*: Pseudo-F_1=3.70, P=0.0327). Sosik (2012) evaluated subsamples of these same aged algal blades and found that microbe abundance had no clear trend through time in the chemically defended *Agarum* but did significantly increase in the non-defended *Saccharina*. In *Saccharina*, total mg ω3 FA and FAME g^{-1} of tissue declined significantly with time (ω3 FA; t-test=-3.24, df=6, P=0.0178; Fig. 5; FAME: t-test=-3.55, df=6, P=0.0121) but differences were not statistically significant for the increase in bacterial FA (t-test=2.32, df=6, P=0.0594; Fig. 3.5). Total ω3 FA and bacterial FA did not change in *Agarum* (Fig. 3.5), but total FAME did decline through time (t-test=-3.55, df=6, P=0.0121).

Discussion

Both FA and MSI differed between shallow and deep habitats for a diverse assemblage of organisms (Mollusca, Arthropoda, Echinodermata, Brachiopoda) that use a range of feeding modes (suspension feeders, omnivores, herbivores, predators). The pattern of enrichment in $\delta^{13}C$ and $\delta^{15}N$ from shallow to deep animals is clearly discernable despite generally significant site-level differences (with exception of the predator $\delta^{15}N$ signatures). These results show that

FA signatures and MSI values of organisms are generally a function of their environment. Site differences in biomarker signatures are not unexpected (Guest et al. 2010; Dethier et al. 2013). Nevertheless, to our knowledge, the finding of consistent changes in FA and MSI values between conspecifics across a depth gradient is novel. The pattern of enrichment in $\delta^{15}N$ of deep consumers is consistent with the observations of Mintenbeck et al. (2007); our FA data offer additional information regarding potential mechanisms causing the observed patterns (see below). The relationship between consumer tissue $\delta^{13}C$ signatures with depth may depend upon local dynamics of primary production and the composition of the seston; for all 7 invertebrates studied here, $\delta^{13}C$ ratios were more enriched with depth, whereas in offshore habitats, others have reported depletion in consumer $\delta^{13}C$ ratios with increased depth (Fry 1988; Nerot et al. 2012). Importantly, future researchers should anticipate depth as a significant factor that is likely to contribute variability to invertebrate biomarker signatures.

Our results concur with other studies showing that FA signatures of different groups of organisms are related to phylogeny (Budge et al. 2002; Galloway et al. 2012). Invertebrate taxa studied here clearly differed from each other regardless of depth and site variation (FA and MSI: P=0.0001; Fig. 3.2 shows FA patterns). This result suggests that differences between taxa exist regardless of diet. Before FA can be used for modeling consumer diets in a quantitative mixing model approach (quantitative FA signature analysis, QFASA; Iverson 2004), signatures of the potential prey and the associated variation of those signatures with environmental context must be quantified. Furthermore, the basal resolution of a QFASA (or similar) model output can only be as source-specific as the level of resolution of FA signatures of the potential prey items. Our work provides biochemical signatures and estimates of variation with site and depth for several taxa not heretofore evaluated. Additional research is needed to catalogue FA signatures of other

subtidal benthic invertebrates, which are often overlooked compared to pelagic fish and invertebrates.

Despite significant phylogenetic biomarker signals, FA signatures differed and $\delta^{13}C$ and $\delta^{15}N$ were consistently enriched from shallow to deep animals, particularly for primary consumers. What is consistently different among depths for all of these animals? Several non-exclusive hypotheses exist: 1) different food sources are available to deep and shallow benthic ecosystems, consisting of unique or novel prey items that are available at one depth but not the other (trophic explanation); 2) different abiotic environmental characteristics (e.g., temperature, pressure, light levels) between habitats cause changes in consumer behavior or metabolism that result in differing biomarker signatures (environmental explanation); or 3) the same food sources are available to deep and shallow benthic habitats via the energy subsidy from the shallow photic zone (not hypothesis 1) but the foods themselves have undergone some biochemical change as they age during the transit from shallow to deep water (diagenesis explanation; Mintenbeck et al. 2007). While we cannot refute any of these hypotheses, below we discuss each within the context of our study system and the results of the algal aging experiment.

The food sources available to a consumer likely vary between habitats due to changes in their surroundings. For example, Britton-Simmons et al. (2009) found differences between percent cover and composition of shallow (10 m: dominated by macroalgae) and deeper (30 m: dominated by invertebrates) communities. The strength and nature of subsidies will also drive differences in food availability (Polis et al. 1997). Therefore, changes in biomarkers may be caused by access to different trophic sources (hypothesis 1). The trophic processes are likely to be different among the three feeding modes of the consumers studied. Predators (*Fusitriton* and *Cancer*) forage directly on the benthic invertebrate community, so might reasonably reflect

differences in local (i.e., within-depth) prey biomarker signatures. Omnivores (the snail *Amphissa* [Gastropoda], *Strongylocentrotus*) are known to forage directly on macrodetritus, drift, and benthic invertebrates; as such they are logically tied to biomarkers of both the in-situ benthic community and variable allochthonous detrital sources. Finally, suspension feeders (e.g., *Psolus*, the lamp-shell *Terebratalia transversa* [Brachiopoda], *Chlamys*) capture particulate organic matter (POM) in suspension; POM is a notoriously complex blend of material from different sources that may vary between depths due to a lack of primary productivity in deep water. POM is difficult to characterize isotopically as an end-member because values depend upon composition and growing conditions (Miller and Page 2012).

We found a consistent enrichment in $\delta^{13}C$ and $\delta^{15}N$ in all deep primary consumers relative to shallow consumers. Traditional isotope approaches often attribute enrichment in $\delta^{13}C$ in nearshore marine organisms to a shift from phytoplankton, which is generally more deplete (e.g., mean value of -20.4‰; Miller at al. 2013), to macrophyte sources. The deep areas sampled in the SJA have considerable drift algal biomass (Britton-Simmons et al. 2012). The main species contributing to the drift are relatively enriched kelps (Laminariales) that have average (±SD) $\delta^{13}C$ signatures of -15.9±3.7‰ (*n*=75 replicates across 4 kelp taxa, multiple seasons, and the same 3 sites) or seagrasses (e.g., *Zostera* [Anthophyta]) which have $\delta^{13}C$ signatures of -10.9±1.9‰ (*n*=20 replicates in 2 seasons at the same 3 sites; data from Dethier et al. 2013). The degradation and resuspension of this material could be contributing disproportionately to deep benthic food webs. Evidence for the increased use of macroalgal detritus by deep consumers may lie in the increased proportion of 20:4ω6, a commonly reported brown-algal biomarker (Kelly and Scheibling 2012), by the suspension feeding *Psolus*. However, the enrichment in $\delta^{13}C$ in deep suspension feeders is of the same magnitude as the enrichment in *Strongylocentrotus*, a

direct herbivore (Fig. 3.4c.). A key limitation of the strictly trophic explanation for the differences in biomarkers between depths is the observed directional consistency (e.g., $\delta^{13}C$ enrichment with depth) for such a diverse group of consumers. The consistency of this broad pattern logically implies the importance of a common mechanism that would affect all animals in a similar manner.

Environmental characteristics that could potentially differ between the depths studied include temperature, light, and pressure. Temperature can affect FA of bivalves (Pernet et al. 2007) and MSI signatures of euphausiids (Frazer et al. 1997), likely as a function of growth and metabolism. However, our data suggest: temperatures do not differ greatly between these depths (D. O. Duggins unpublished data) because the subtidal water column is so well mixed due to tidal currents and complex bathymetry; and if temperatures do differ, the scale of the difference is very small compared to interannual (seasonal) and diel (i.e., from tidal mixing of Fraser River freshwater lens) temperature range recorded at the same 3 study sites (at 5 m depth) in our system (winter average of 7.7±0.4°C, summer average of 11.4±1.0°C, D. O. Duggins unpublished data). Light and pressure could contribute to differences in metabolism. Depths of 100 m are well beyond the euphotic zone (Harrison et al. 1999) in this system. The difference between the dark, deep environment and the seasonally variable light levels in the shallow environment could lead to significant changes in seasonal metabolism. It is theoretically conceivable that differences in pressure of these depths could affect cellular respiration (Sebert et al. 2004), however, very little information is available about these processes and the response of FA and MSI. In this context, it is unlikely that the depth differences in biomarker signatures (related to environmental factors) would be greater than the site differences.

The observed isotope enrichment and concurrent decrease in ω3 FA in deep animals is consistent with the changes observed in aging algae. $\delta^{15}N$ enrichment has also been observed in suspension feeding bivalves with increasing depth (Mintenbeck et al. 2007), suggesting a change in the biochemical composition of all food sources available to suspension feeders and herbivores during the transfer to deep areas. It is therefore feasible that differences in FA signatures of consumers between depths, as well as general enrichment in $\delta^{13}C$ and $\delta^{15}N$ with depth, are due to deeper consumers eating foods (e.g., POM, detritus, prey) that have been altered and enriched by increased microbe abundance on older detrital substrates (McArthur et al. 1992; Chen et al. 2008; diagenesis explanation). The algal aging experiment showed that FA signatures for both algae change after 5 weeks. The relatively undefended *Saccharina* showed declines in total ω3 FA, and a trend of increasing bacterial marker FA with age (Fig. 3.5). No decline in ω3 FA was found in the chemically defended *Agarum*. Sosik (2012) also found that $\delta^{15}N$ from the same experimental blades were enriched in aged *Saccharina* but not in *Agarum*, which corresponded to changes in microbial abundance on the respective kelps.

The specific declines in total ω3 FA found in primary consumers with increasing depth are congruent with the results from the algal aging experiment. However, total bacterial FA were not also higher in deep animals. We were not able to evaluate whether there is a causal relationship between ω3 FA declines in algae and concurrent increases in microbe abundance. However, recent research by Thiansilakul et al. (2013) has shown that adding low levels of phenolics to seafood during processing and storage reduces both bacterial growth and oxidation (i.e., loss) of ω3 FA over time. If microbes are responsible for the ω3 declines in the aged, non-defended alga in our experiment (e.g., through selective consumption and catabolization of ω3 FA from their substrates) this could have important food web implications, as this category of

FA is critical for heterotrophs (Müller-Navarra 2008) and has been hypothesized as a limiting resource in food webs (Litzow et al. 2006). Additional research is needed to further understand the roles that microbes, which can quickly colonize and abandon suspended POM (Kiørboe et al. 2002), may play in diagenesis of POM and macrodetrital biomarker signatures. The lack of significant differences in *Agarum* total ω3 FA and bacterial marker FA, when paired with the fact that microbe abundance did not increase on *Agarum* through time (Sosik 2012), implies a relationship between microbes and ω3 FA (Thiansilakul et al. 2013) that warrants further research.

Some biomarker patterns support a priori expectations, e.g., the enrichment of *Strongylocentrotus* in $\delta^{13}C$ relative to other consumers, regardless of depth, to a range of values that we would expect for a direct kelp consumer. Other patterns in our biomarker data identify new questions about the ecology of these poorly studied subtidal organisms. One such pattern is the lack of difference between depths in $\delta^{15}N$ found in either predator, *Cancer* and *Fusitriton*, implying that small differences in biomarkers at the base of the food chain are mediated by primary consumers and not transferred to higher predators (Hall et al. 2006). *Fusitriton* in particular only differed in one FA summary category across depths (bacterial FA). Why are predator total bacterial marker FA higher in deep animals compared with conspecifics? This is currently unexplained and deserves further research. Interestingly, *Psolus* total bacterial marker FA levels were generally very high relative to other organisms studied (Table 3.5) and SIMPER identified the bacterial marker FA iso-15:0 as a particularly relevant contributor to the depth differences. The FA 23:1 is rarely reported in marine systems, but was also identified in the SIMPER analysis as being particularly abundant and important for driving depth differences in *Psolus*. While the exact source of this relatively novel FA is unknown, it has also been identified

in abyssal holothurians (Drazen et al. 2008) and may be a bacterial biomarker FA (odd-carbon numbered; Budge et al. 2001).

A recent review of the fate of detrital material (Krumhansl and Scheibling 2012*a*) estimated average kelp forest (Laminarian macroalgae) productivity to be 864 g C m^{-2} yr^{-1}, with the vast majority (>80%) of this production entering detrital pathways. Wilmers et al. (2012) estimated that 3-450 g C m^{-1} yr^{-1} of the production from kelp forests is not consumed in the SPE but is transferred out of the photic zone to the deep subtidal. The order of magnitude in the uncertainty of this flux estimate assumes that 1-50% of annual in situ kelp NPP is transported to DNSE (also see Buesseler et al. 2007). These approaches (e.g., estimates of export-based NPP) do not account for dissolved organic carbon (DOC), which may account for up to 34% of the total annual energy production by kelps (Newell et al. 1980). During transit from the SPE to DNSE and deeper, what changes does detritus undergo and what are the implications of these changes to deep consumers? Changes in algal FA signatures as a result of aging and/or decomposing are generally unexplored (but see Krumhansl and Scheibling 2012b), which is surprising given how closely coupled many marine food webs are to decomposing material. The combination of FA and MSI in this study ultimately suggested diagenesis as a mechanism for the consumer patterns, an explanation that may have been missed with MSI alone. The results also highlight the importance of considering primary producer aging, and subsequent biochemical changes that affect conclusions drawn of trophic relationships using mixing models (Sosik 2012, Dethier et al. 2013), an area of potential variation which is often overlooked.

Literature Cited

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology **26**:32-46.

Anderson, M. J., R. N. Gorley, and K. R. Clarke. 2008. PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E Ltd.

Bell, M. V., and D. R. Tocher. 2009. Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: general pathways and new directions. Pages 211-236. *In* M. T. Arts, M. T. Brett and M. Kainz, editors. Lipids in aquatic ecosystems. Springer, New York.

Britton-Simmons, K. H., G. Foley, and D. Okamoto. 2009. Spatial subsidy in the subtidal zone: Utilization of drift algae by a deep subtidal sea urchin. Aquatic Biology **5**:233-243.

Britton-Simmons, K. H., A. L. Rhoades, R. E. Pacunski, A. W. E. Galloway, A. T. Lowe, E. A. Sosik, M. N. Dethier, and D. O. Duggins. 2012. Habitat and bathymetry influence the landscape-scale distribution and abundance of drift macrophytes and associated invertebrates. Limnology and Oceanography **57**:176-184.

Budge, S. M., C. C. Parrish, and C. H. McKenzie. 2001. Fatty acid composition of phytoplankton, settling particulate matter and sediments at a sheltered bivalve aquaculture site. Marine Chemistry **76**:285-303.

Budge, S. M., S. J. Iverson, W. D. Bowen, and R. G. Ackman. 2002. Among- and within-species variability in fatty acid signatures of marine fish and invertebrates on the Scotian Shelf, Georges Bank, and southern Gulf of St. Lawrence. Canadian Journal of Fisheries and Aquatic Sciences **59**:886-898.

Budge, S. M., S. J. Iverson, and H. N. Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Marine Mammal Science **22**:759-801.

Buesseler, K. O., C. H. Lamborg, P. W. Boyd, P. J. Lam, T. W. Trull, R. R. Bidigare, J. K. B. Bishop, K. L. Casciotti, F. Dehairs, M. Elskens, M. Honda, D. M. Karl, D. A. Siegel, M.

W. Silver, D. K. Steinberg, J. Valdes, B. Van Mooy, and S. Wilson. 2007. Revisiting carbon flux through the ocean's twilight zone. Science **316**:567-570.

Cabana, G., and J. B. Rasmussen. 1996. Comparison of aquatic food chains use in nitrogen isotopes. Procedings of the National Academy of Sciences of the United States of America **93**:10844-10847.

Cebrian, J. 1999. Patterns in the fate of production in plant communities. American Naturalist **154**:449-468.

Chen, F., L. Zhang, Y. Yang, and D. Zhang. 2008. Chemical and isotopic alteration of organic matter during early diagenesis: evidence from the coastal area off-shore the Pearl River estuary, south China. Journal of Marine Systems **74**:372-380.

Cranford, P. J., and J. Grant. 1990. Particle clearance and absorption of phytoplankton and detritus by the sea scallop *Placopecten magellanicus* (Gmelin). Journal of Experimental Marine Biology and Ecology **137**: 105-121.

Dethier, M. N., E. A. Sosik, A. W. E. Galloway, D. O. Duggins, and C. A. Simenstad. 2013. Addressing assumptions: variation in stable isotopes and fatty acids in marine macrophytes can confound conclusions of food web studies. Marine Ecology Progress Series **478**:1-14.

Drazen, J. C., C. F. Phleger, M. A. Guest, and P. D. Nichols. 2008. Lipid, sterols and fatty acid composition of abyssal holothurians and ophiuroids from the North-East Pacific Ocean: food web implications. Comparative Biochemistry and Physiology, Part B **151**:79-87.

Duggins, D. O., C. A. Simenstad, and J. A. Estes. 1989. Magnification of secondary production by kelp detritus in coastal marine ecosystems. Science **245**:170-173.

Dunton, K. H., and D. M. Schell. 1987. Dependence of consumers on macroalgal (*Laminaria solidungula*) carbon in an arctic kelp community: $\delta^{13}C$ evidence. Marine Biology **93**:615-625.

Eckman, J. E., D. O. Duggins, and C. E. Siddon. 2003. Current and wave dynamics in the shallow subtidal: implications to the ecology of understory and surface-canopy kelps. Marine Ecology Progress Series **265**:45-56.

Frazer, T. K., R. M. Ross, L. B. Quetin, and J. P. Montoya. 1997. Turnover of carbon and nitrogen during growth of larval krill, *Euphausia superba* Dana: a stable isotope approach. Journal of Experimental Marine Biology and Ecology **212**:250-275.

Fry, B. 1988. Food web structure on Georges Bank from stable C, N, and S isotopic compositions. Limnology and Oceanography **33**:1182-1190.

Fry, B. 2006. Stable isotope ecology. Springer.

Galloway, A. W. E., K. H. Britton-Simmons, D. O. Duggins, P. W. Gabrielson, and M. T. Brett. 2012. Fatty acid signatures differentiate marine macrophytes at ordinal and family ranks. Journal of Phycology **48**:956-965.

Guest, M. A., A. J. Hirst, P. D. Nichols, and S. D. Frusher. 2010. Multi-scale spatial variation in stable isotope and fatty acid profiles amongst temperate reef species: implications for design and interpretation of trophic studies. Marine Ecology Progress Series **410**:25-41.

Harrison, P. J., P. W. Boyd, D. E. Varela, and S. Takeda. 1999. Comparison of factors controlling phytoplankton productivity in the NE and NW subarctic Pacific gyres. Progress in Oceanography **43**:205-234.

Hall, D., S. Y. Lee, and T. Meziane. 2006. Fatty acids as trophic tracers in an experimental estuarine food chain: tracer transfer. Journal of Experimental Marine Biology and Ecology **336**:42-53.

Howe, E. R., and C. A. Simenstad. 2007. Restoration trajectories and food web linkages in San Francisco Bay's estuarine marshes: a manipulative translocation experiment. Marine Ecology Progress Series **351**:65-76.

Iverson, S. J., K. J. Frost, and S. L. C. Lang. 2002. Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound, Alaska: factors contributing to among and within species variability. Marine Ecology Progress Series **241**:161-181.

Iverson, S. J., C. Field, W. D. Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. Ecological Monographs **74**:211-235.

Iverson, S. J. 2009. Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. Pages 281-307. *In* M. T. Arts, M. T. Brett and M. Kainz, editors. Lipids in aquatic ecosystems. Springer, New York.

Kelly, J. R., and R. E. Scheibling. 2012. Fatty acids as dietary tracers in benthic food webs. Marine Ecology Progress Series **446**:1-22.

Kelly, J. R., R. E. Scheibling, S. J. Iverson, and P. Gagnon. 2008. Fatty acid profiles in the gonads of the sea urchin *Strongylocentrotus droebachiensis* on natural algal diets. Marine Ecology Progress Series **373**:1-9.

Kiørboe, T., H. P. Grossart, H. Ploug, and K. Tang. 2002. Mechanisms and rates of bacterial colonization of sinking aggregates. Applied and Environmental Microbiology **68**:3996.

Krumhansl, K. A., and R. E. Scheibling. 2012a. Production and fate of kelp detritus. Marine Ecology Progress Series **467**:281-302.

Krumhansl, K. A., and R. E. Scheibling. 2012b. Detrital subsidy from subtidal kelp beds is altered by the invasive green alga *Codium fragile* ssp. *fragile*. Marine Ecology Progress Series **456**:73-85.

Lang, I. K., L. Hodac, T. Friedl, and I. Feussner. 2011. Fatty acid profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection. BMC Plant Biology **11**:124, doi:10.1186/1471-2229-11-124

Litzow, M. A., K. M. Bailey, F. G. Prahl, and R. Heintz. 2006. Climate regime shifts and reorganization of fish communities: the essential fatty acid limitation hypothesis. Marine Ecology Progress Series **315**: 1-11.

McArthur, J. M., R. V. Tyson, J. Thomson, and D. Mattey. 1992. Early diagenesis of marine organic matter: alteration of the carbon isotopic composition. Marine Geology **105**:51-61.

Miller, R. J., and H. M. Page. 2012. Kelp as a trophic resource for marine suspension feeders: A review of isotope-based evidence. Marine Biology **159**:1391-1402.

Miller, R. J., H. M. Page, and M. A. Brzezinski. 2013. $\delta^{13}C$ and $\delta^{15}N$ of particulate organic matter in the Santa Barbara Channel: drivers and implications for trophic inference. Marine Ecology Progress Series **474**:53-66.

Mintenbeck, K., U. Jacob, R. Knust, W. E. Arntz, and T. Brey. 2007. Depth-dependence in stable isotope ratio of $\delta^{15}N$ of benthic POM consumers: the role of particle dynamics and organism trophic guild. Deep Sea Research, Part I **54**:1015-1023.

Muller-Navarra, D. C. 2008. Food web paradigms: the biochemical view on trophic interactions. International Review of Hydrobiology **93**:489-505.

Nadon, M. O., and J. H. Himmelman. 2006. Stable isotopes in subtidal food webs: have enriched carbon ratios in benthic consumers been misinterpreted. Limnology and Oceanography **51**:2828-2836.

Nerot, C., A. Lorrain, J. Grall, D. P. Gillikin, J. Munaron, H. Le Bris, and Y. Paulet. 2012. Stable isotope variations in benthic filter feeders across a large depth gradient on the continental shelf. Estuarine, Coastal and Shelf Science **96**:228-235.

Newell, R. C., M. I. Lucas, B. Velimirov, and L. J. Seiderer. 1980. Quantitative significance of dissolved organic losses following fragmentation of kelp (*Ecklonia maxima* and *Laminaria pallida*). Marine Ecology Progress Series **2**:45-59.

Pernet, F., R. Tremblay, L. Comeau, and H. Guderley. 2007. Temperature adaptation in two bivalve species from different thermal habitats: energetics and remodelling of membrane lipids. Journal of Experimental Biology **210**:2999-3014.

Peterson, B. J. 1999. Stable isotopes as tracers of organic matter input and transfer in benthic food webs: a review. Acta Oecologica-International Journal of Ecology **20**:479-487.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics **18**:293-320.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Towards an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annual Review of Ecology and Systematics **28**:289-316.

Ramirez-Llodra, E., A. Brandt, R. Danovaro, B. De Mol, E. Escobar, C. R. German, L. A. Levin, P. M. Arbizu, L. Menot, P. Buhl-Mortensen, B. E. Narayanaswamy, C. R. Smith, D. P. Tittensor, P. A. Tyler, A. Vanreusel, and M. Vecchione. 2010. Deep, diverse and

definitely different: unique attributes of the world's largest ecosystem. Biogeosciences **7**:2851-2899.

Sebert, P., M. Theron, and A. Vettier. 2004. Pressure and temperature interactions on cellular respiration: a review. Cellular and Molecular Biology **50**:491-500.

Sosik, E. A. 2012. Isotopic evidence of microbial pathways in macroalgal-detritus based coastal food webs. Master's thesis. University of Washington.

Sosik, E. A., and C. A. Simenstad. Microbial trophic levels: isotopic evidence and consequences in macroalgal detritus-based coastal food webs. *in revision*, March 2013.

Taipale, S. J., M. J. Kainz, and M. T. Brett. 2011. Diet-switching experiments show rapid accumulation and preferential retention of highly unsaturated fatty acids in *Daphnia*. Oikos **120**:1674-1682.

Thiansilakul, Y., S. Benjakul, and M. P. Richards. 2013. Effect of phenolic compounds in combination with modified atmospheric packaging on inhibition of quality losses of refrigerated Eastern little tuna slices. LWT-Food Science and Technology **50**:146-152.

Van Alstyne, K. L. V., J. J. M. Iii, C. L. Hustead, and L. J. Kearns. 1999. Phlorotannin allocation among tissues of Northeastern Pacific kelps. Journal of Phycology **35**:483-492.

Wilmers, C. C., J. A. Estes, M. Edwards, K. L. Laidre, and B. Konar. 2012. Do trophic cascades affect the storage and flux of atmospheric carbon? An analysis of sea otters and kelp forests. Frontiers in Ecology and the Environment **10**:409-415.

Table 3.1. Consumers sampled, commonly known higher order group classification, primary trophic role, feeding mechanism, analyses performed, and number of FA variables averaging >0.1% of the total FA for each consumer.

Genus species	Group name	Role	Feeding mechanism	Analyses	FA
Chlamys hastata	Bivalvia	suspension	mucus (ctenidia)	FA+MSI	35
Psolus chitonoides	Holothuroidea	suspension	mucus (tentacles)	FA+MSI	43
Terebratalia transversa	Brachiopoda	suspension	cilia (tentacles)	MSI	--
Amphissa columbiana	Gastropoda	omnivore	scraping (radula)	MSI	--
Strongylocentrotus droebachiensis	Echinoidea	omnivore	biting (Aristotle's lantern)	FA+MSI	33
Cancer oregonensis	Decapoda	predator	crushing (claws, mandibles)	FA+MSI	33
Fusitriton oregonensis	Gastropoda	predator	scraping, boring (radula)	FA+MSI	30

Table 3.2. One-way PERMANOVA results for FA and MSI ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) biomarkers. These analyses pool all replicates across sites to test the factor depth for each species. All analyses run type III Sums of Squares, treat the factor depth as fixed, use unrestricted permutation of the raw data with 9999 perms. MSI PERMANOVAs are run on untransformed, normalized MSI data.

Species:	Biomarker category			FA				MSI
Source	df	MS	Pseudo-*F*	*p*(perm)	df	MS	Pseudo-*F*	*p*(perm)
Chlamys								
depth	1	44.35	20.00	0.0001	1	11.58	24.57	0.0001
residual	26	2.22			26	0.47		
total	27				27			
Psolus								
depth	1	36.83	4.25	0.0024	1	15.11	13.47	0.0002
residual	27	8.67			27	1.12		
total	28				28			
Strongylocentrotus								
depth	1	32.08	5.33	0.0003	1	12.60	5.88	0.0027
residual	26	6.02			28	2.14		
total	27				29			
Cancer								
depth	1	30.86	5.30	0.0001	1	4.56	10.96	0.0002
residual	27	5.82			27	0.41		
total	29				28			
Fusitriton								
depth	1	36.21	9.77	0.0001	1	2.80	2.55	0.0766
residual	25	3.71			25	1.10		
total	26				26			
Terebratalia								
depth	--	--	--	--	1	6.28	11.09	0.0002
residual	--	--			27	0.57		
total	--				28			
Amphissa								
depth	--	--	--	--	1	6.47	15.26	0.0001
residual	--	--			28	0.42		
total	--				29			

Table 3.3. SIMPER results showing FA that are primarily responsible for driving differences in species tissues with depth. The total number of FA that account for >90% of the total variation in this comparison are listed (#FAΣ>90%), followed by the mean FA proportion of up to the top 5 contributing FA in the raw samples. The final columns show the % contribution of a particular FA in describing the differences between shallow and deep samples and the direction of the difference for each FA from shallow to deep.

			Mean FA% (Raw)		Comparison %	
Species	#FAΣ>90%	Top 5 FA	shallow	deep	contribution	direction
Chlamys	3	22:6ω3	25.2	31.1	67.7	+
		20:5ω3	22.1	20.2	18.0	-
		16:0	17.8	16.2	4.9	-
Psolus	9	20:4ω6	5.7	9.6	29.9	+
		23:1	5.6	8.3	14.6	+
		i-15:0	5.4	2.8	14.4	-
		20:5ω3	19.7	17.8	14.1	-
		20:1ω9	8.8	7.1	5.6	-
Strongylocentrotus	10	20:4ω6	19.8	23.4	54.4	+
		20:5ω3	15.3	13.4	14.9	-
		20:0	9.9	10.2	5.1	~
		22:6ω3	1.25	0.3	4.5	-
		16:0	9.6	8.7	3.9	-
Cancer	6	20:5ω3	34.1	32.7	38.9	-
		22:6ω3	12.1	13.3	22.0	+
		18:1ω9	8.7	9.2	10.6	+
		16:1ω7	3.3	3.8	7.1	+
		16:0	14.9	15.5	6.9	-
Fusitriton	9	20:5ω3	14.4	15.9	48.2	+
		22:4ω6	4.3	4.2	11.4	~
		22:5ω3	12.8	13.2	6.5	+
		18:0	14.8	14.0	5.0	-
		22:2	8.3	7.5	4.7	-

Table 3.4. PERMANOVA (FA) summary table of results for each test of the factor depth for each species at each site. Significance for tests was determined with Monte Carlo simulations for these comparisons because several of the comparisons had less than 100 unique permutations (Anderson et al. 2008).

Site:	PIL	SKP	PTC
Species	*p*(MC)	*p*(MC)	*p*(MC)
Chlamys	0.001	0.004	0.005
Psolus	0.007	0.001	0.009
Strongylocentrotus	0.027	0.009	0.357
Cancer	0.003	0.021	0.186
Fusitriton	0.175	0.006	0.003

Table 3.5. Summary of univariate FA depth comparisons, with replicates (*n*~15 per category) pooled across 3 sites. Full species names are in Table 1, data are avg (±SD) sums (mg FAME g^{-1} of dry tissue). See Methods for category abbreviations. *P*-values are results of 2-sample *F*-tests.

		Chlamys			*Psolus*			*Strongylocentrotus*			*Cancer*			*Fusitriton*		
mg g^{-1} tissue	Depth	avg	SD	*p*	avg	SD	*p*	avg	SD	*p*	avg	SD	*p*	avg	SD	*p*
Total FAME	shallow	20.2	1.5	0.002	22.3	10.2	0.025	16.2	5.3	0.262	13.4	0.9	0.306	10.0	1.0	0.712
	deep	18.2	1.5		13.4	6.7		14.0	1.8		13.8	1.3		10.4	2.1	
SAFA	shallow	5.6	0.4	<0.001	4.5	2.4	0.073	4.8	1.7	0.247	3.3	0.2	0.782	3.1	0.3	0.344
	deep	4.9	0.5		2.7	1.4		4.0	0.6		3.3	0.3		3.0	0.6	
MUFA	shallow	1.8	0.2	<0.001	8.5	3.9	0.019	2.1	0.6	0.223	2.4	0.3	0.028	1.4	0.2	0.185
	deep	1.3	0.2		5.0	2.2		1.9	0.2		2.7	0.4		1.3	0.3	
PUFA	shallow	12.4	1.0	0.025	7.6	3.5	0.046	9.3	2.9	0.290	7.3	0.5	0.800	4.9	0.6	0.284
	deep	11.6	0.8		4.9	2.7		8.1	1.0		7.4	0.8		5.4	1.2	
Omega-3s	shallow	11.4	0.9	0.031	5.3	2.6	0.026	3.4	1.2	0.016	6.9	0.5	0.552	3.1	0.7	0.260
	deep	10.6	0.8		3.0	2.0		2.5	0.4		7.1	0.7		3.5	1.0	
Omega-6s	shallow	1.0	0.1	0.053	1.2	0.3	0.427	3.7	1.0	0.799	0.3	0.1	0.009	0.8	0.3	0.752
	deep	0.9	0.1		1.3	0.4		3.7	0.6		0.2	0.1		0.7	0.2	
HUFA	shallow	11.1	0.9	0.560	6.1	2.7	0.047	6.7	2.1	0.296	6.9	0.4	0.408	3.5	0.6	0.237
	deep	10.9	0.7		4.1	2.2		5.9	0.8		7.1	0.7		3.9	0.9	
Bacterial FA	shallow	1.0	0.1	0.004	2.2	1.3	0.007	0.4	0.1	0.084	0.9	0.1	0.030	0.6	0.1	0.020
	deep	0.9	0.1		1.0	0.8		0.3	0.0		1.0	0.1		0.7	0.1	

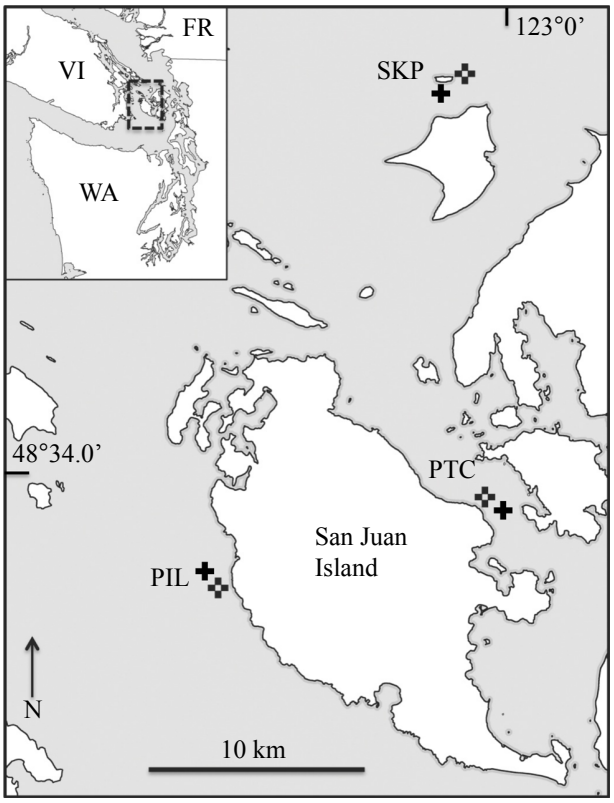

Fig. 3.1. Area map showing the western San Juan Archipelago in Washington (WA) and the three core study sites where samples were collected for fatty acid and stable isotope comparisons. Deep and shallow collection areas at each site are indicated by filled in and open crosshairs, respectively. The study sites cover a gradient of exposure to terrestrial freshwater inputs from the Fraser River (FR) to the North (Skipjack Island; SKP) and the higher salinity open ocean to the far west (Pillar; PIL) with the intermediate Point Caution (PTC).

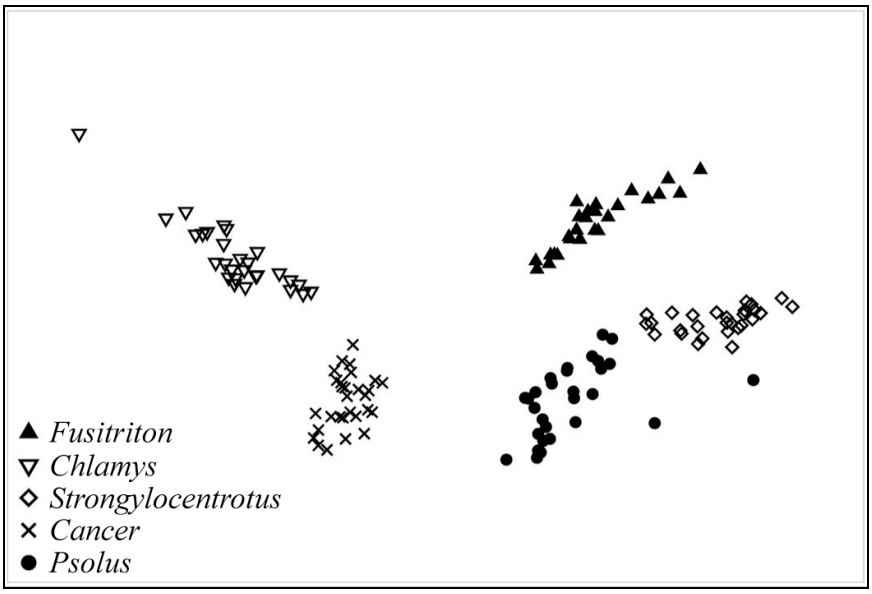

Fig. 3.2. NMDS plot of FA signatures of 5 species studied (54 FA untransformed) showing taxonomic distinctness of different invertebrates regardless of site or depth. The plot was generated using Euclidean distance, 2D stress=0.06.

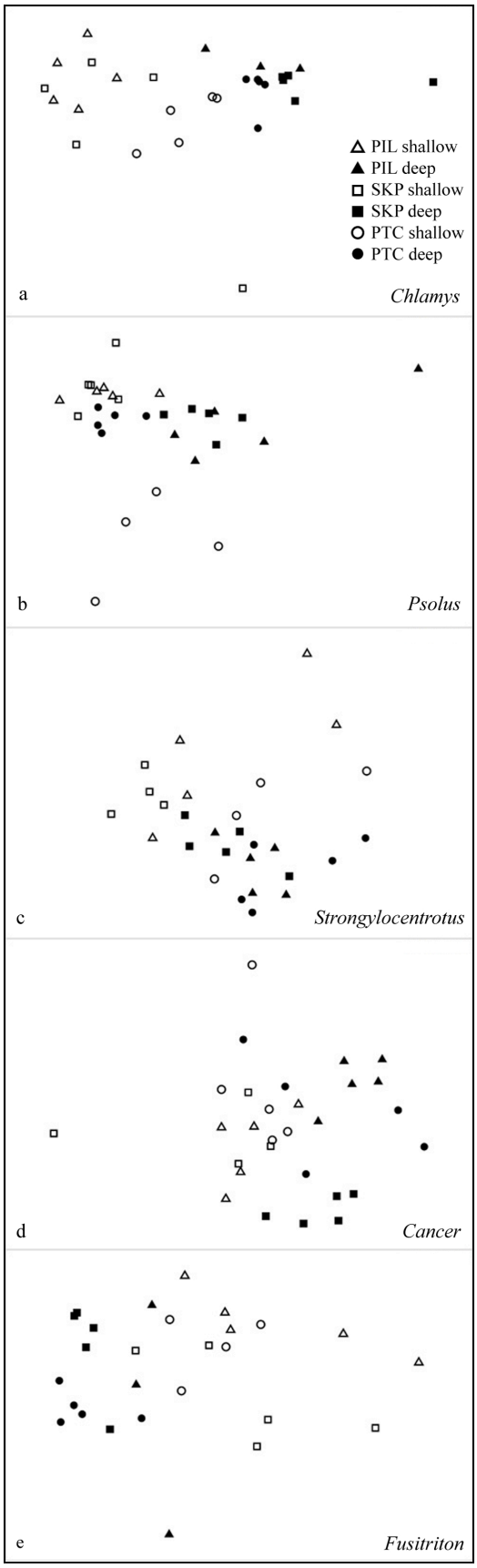

Fig. 3.3. NMDS plots of the 5 species studied, coded to show factors of site and depth. All plots are generated from Euclidean resemblance matrices on log-ratio transformed FA datasets reduced to only the FA that contribute >0.1% of total FA across samples within that species (see Methods). The species (and 2D stress) in each NMDS analysis is: (a) *Chlamys hastata* (0.06), (b) *Psolus chitonoides* (0.09), (c) *Strongylocentrotus droebachiensis* (0.13), (d) *Cancer oregonensis* (0.17), and (e) *Fusitriton oregonensis* (0.11).

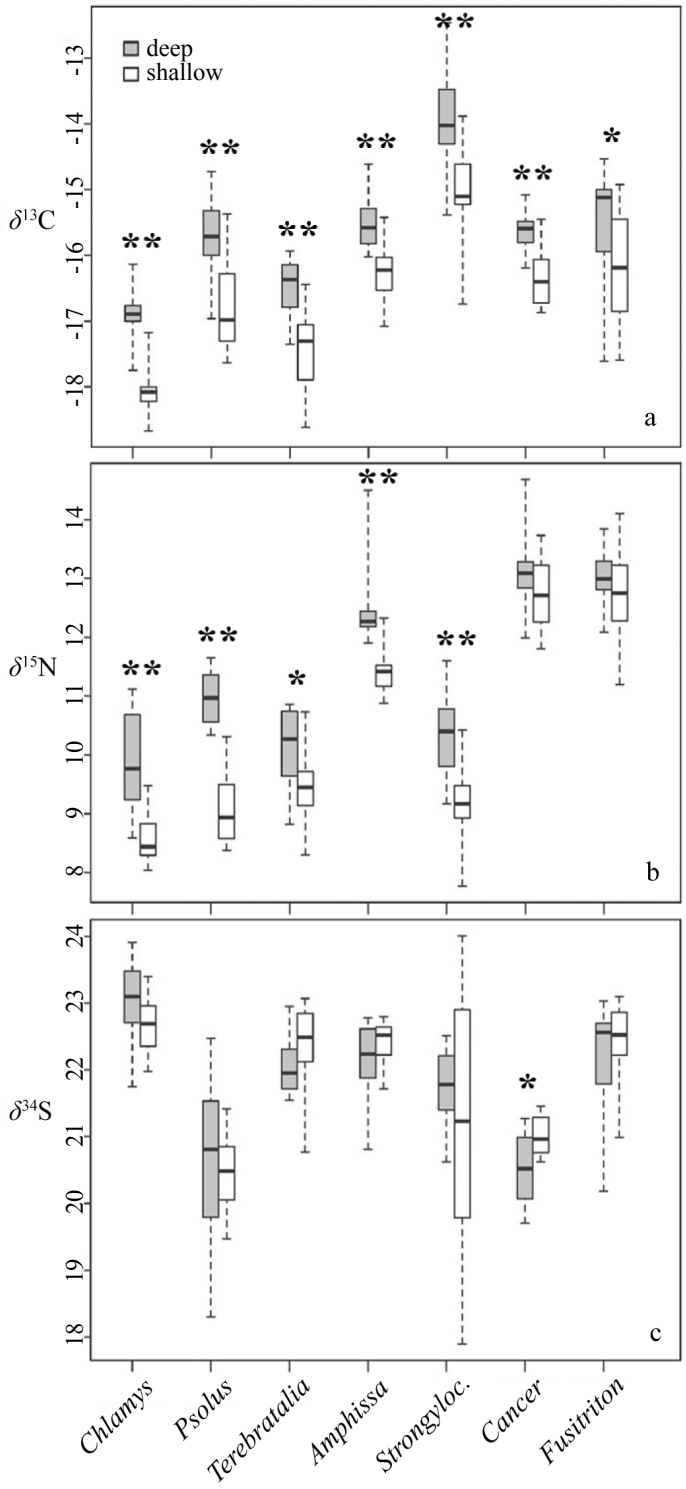

Fig. 3.4. Box and whisker plots of MSI ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) data for each of the 7 species included in MSI analyses (see Table 3.1), pooled across all 3 study sites. Grey-shaded boxes are animals collected deep (100 m) and white boxes are shallow (15 m). Heavy line in each box is the mean, box edges are the quartiles, and whiskers extend to minimum and maximum values measured. Significance levels of 2-sample *t*-tests are indicated (*:$P<0.05$, **; $P<0.001$).

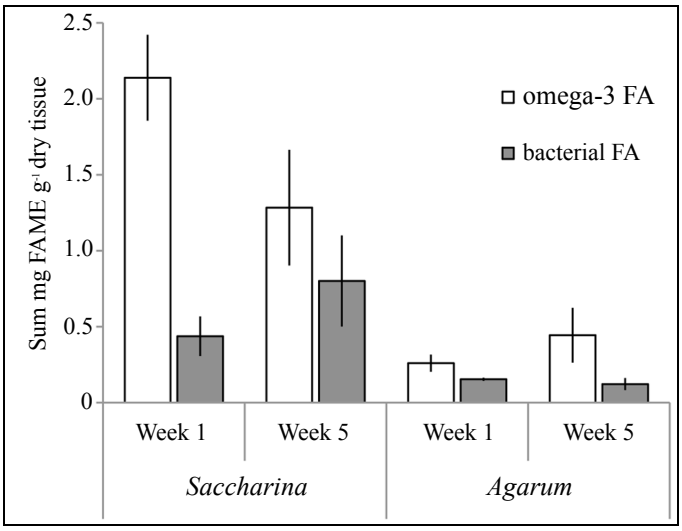

Fig. 3.5. Total average (±SD) FA weight (mg FAME g^{-1} algal tissue dry weight) of two summary categories (ω3 FA, open bars; and bacterial FA, grey-shaded bars) in 5-week algal aging experiment. Algae are *Saccharina subsimplex* and *Agarum fimbriatum*. In *Saccharina*, ω3 FA (*t*-test=-3.24, df=6, *P*=0.0178) declined through time but was not differences were not statistically significant for bacterial FA (*t*-test=2.32, df=6, *P*=0.0594). These summary FA categories did not differ through time in *Agarum*.

CHAPTER 4 – DIET-SPECIFIC FRACTIONATION OF FATTY ACIDS AND $\delta^{15}N$ IN AN HERBIVOROUS MARINE ISOPOD

Abstract

Biochemical analyses using fatty acids (FA) and multiple stable isotopes (MSI) have become essential tools for investigating trophic interactions, particularly for tracking sources of basal production through food webs. Experimental feeding trials that are designed to verify the key assumptions of these methods are critically needed. Here we asked whether FA and isotope-specific signatures of algal diets are assimilated consistently and predictably into consumer tissues, and if biomarker fractionation is diet-specific. Using fast growing juveniles of an herbivorous isopod (*Idotea wosnesenskii*) as a model organism, we show in a ten-week feeding trial that animals eating diets from three phyla doubled in size, and that FA signatures were closely related between the isopods and their diets. We identify several classes of polyunsaturated FA (PUFA) that were highly correlated between diet and consumer (e.g., ΣC_{18} $\omega 6 + C_{18}$ $\omega 3$, $r^2=0.93$). $\delta^{13}C$ fractionation from diet to consumer was highly variable, and the mean (±SD) $\delta^{15}N$ fractionation across all diets (1.21±1.14‰) was lower than generally indicated by the literature. Most importantly, we show that the relationships between dietary and consumer PUFA and $\delta^{15}N$ are diet dependent, highlighting the need for empirically derived fractionation values when applying mixing models to back-calculate contributions of basal resources to consumers.

Introduction

Marine food webs are supported by energy from photosynthetic organisms, including phytoplankton, seagrasses, and benthic micro- and macro-algae. Macroalgae and seagrasses (collectively macrophytes) account for a large proportion of nearshore primary production (e.g., Mann 1973, Duggins 1980, Duarte and Cebrian 1996). Some of this production is directly consumed by herbivores, but most (>80%) enters nearshore food webs as detritus (Krumhansl and Scheibling 2012). Effective ecosystem management requires a greater understanding of the basal energy sources that ultimately support upper trophic level consumers. However, tracking energy flow through food webs is very challenging, especially when it is not possible to quantify gut contents (e.g., in animals that consume small food particles).

Fatty acids (FA) and multiple stable isotopes (MSI; FAMSI) are commonly used as biochemical markers for tracing sources of basal production into consumer diets (reviewed by Dalsgaard et al. 2003, Fry 2006, Kelly and Scheibling 2012). The particular strength of the FAMSI approach, relative to direct observation or gut content approaches, is that it has the potential to provide information about both what consumers are eating over long time scales and which dietary sources are most valuable (Duggins et al. 1989). Wild animal FAMSI signatures are typically evaluated using either a qualitative 'biomarker' approach, or a quantitative mixing model approach (reviewed in Budge et al. 2006, Fry 2013). The qualitative approach involves comparing experimental or wild animal signatures with potential source diets in multidimensional plots such as PCA or NMDS (FA) or bivariate plots of two stable isotopes (SI). Quantitative modeling approaches are common for MSI, through the use of Bayesian mixing models (e.g., Parnell et al. 2010), which are now being adapted for additional FA variables (Dethier et al. 2013). A FA specific approach, quantitative FA signature analysis (QFASA;

Iverson et al. 2004) was developed for marine mammals and has recently been applied to fish (Budge 2012), but has not yet been applied to invertebrates because of the paucity of information about diet-to-consumer FA "fractionation".

Very few studies have measured FA fractionation (but see Taipale et al. 2011). MSI fractionation is known to vary among consumer taxa and tissues (e.g., Caut et al. 2009), and the question of diet-specific MSI fractionation has only recently been evaluated experimentally (Prado et al. 2012). This gap is problematic for quantitative mixing model analyses that solve for dietary contributions of different resources to consumers. For MSI, researchers generally apply SI-specific fractionation coefficients from the literature without knowing whether such values are relevant for the species of study, or measuring them using controlled feeding trials (Gannes et al. 1997, del Rio et al. 2009). For FA, fractionation is important because invertebrates can selectively catabolize, modify, or synthesize new FA molecules via desaturase and elongase enzymes (Kelly and Scheibling 2012). Animals cannot synthesize long-chain (e.g., $\geq C_{20}$) ω3 or ω6 PUFA *de novo* (Bell and Tocher 2009), but some can bioconvert them through modification of related short carbon chain precursor FAs such as 18:3ω3 (ALA) and 18:2ω6 (LIN; Dalsgaard et al. 2003).

We conducted a controlled experiment to test the hypothesis that the FA signatures and MSI ($\delta^{13}C$ and $\delta^{15}N$) ratios of basal resources are assimilated consistently and predictably into consumer tissues. We measured molecule and isotope-specific FAMSI fractionation from diet to whole-body tissues in juvenile *Idotea wosnesenskii* fed macroalgal diets with distinct biochemical signatures. It is critical that diet-to-consumer biomarker fractionation is measured in organisms that have accrued the large majority of their tissues while consuming the treatment diets. Juvenile *Idotea* are an ideal model organism because very small animals can be removed

from brooding mothers and grown quickly on diverse algal diets in the lab. For each suite of biomarkers (FA and MSI) we specifically asked: 1) Do animals raised on different macroalgal resources differ in their total biomarker signatures? 2) Is biomarker fractionation diet specific? 3) Which biomarkers in consumers are the best indicators of their diets?

Methods

Collection and feeding trial procedures

We collected brooding adult female *Idotea* from the intertidal zone at Eagle Cove, San Juan Island, WA. Adults were transferred to air stone-plumbed 2 L aquaria in 0.3 μm filtered seawater in a climate-controlled room with a natural diel light cycle. Juvenile animals (3.1±0.1 mm, hereafter mean ± 1 SD) removed from brooding females, distributed randomly (n=105 per replicate) into 12 aquaria (3 replicates per diet), and starved 2 d. We used four macroalgal diets from 3 phyla (*Nereocystis luetkeana*, *Fucus distichus* (Ochrophyta), *Mazzaella splendens* (Rhodophyta) and *Ulva* sp. (Chlorophyta), known to have very distinctive biochemical signatures (Galloway et al. 2012). Algal diets were collected in the vicinity of Friday Harbor Laboratories from the subtidal (*Nereocystis*) or intertidal (other taxa). We changed the water and provided new food at 48-72 hr intervals, carefully removing unconsumed algae, and adding sufficient ~4 cm^2 pieces of fresh algae to provide *ad libitum* feeding. Algal tissue samples were frozen for biochemical analysis at roughly the beginning, middle, and end of the feeding trial. The mean daily experimental temperature throughout the 10-week trial (17-June through 28-August 2012) was 12.3±0.5°C. Trial duration was based upon pilot studies showing that juvenile *Idotea* would double in size during this time frame. At the end of the trial, animals were measured (tip of the head to the end of the uropod) under a dissecting scope, and frozen.

Biochemical extraction

Samples were stored at -20°C for <2 months, lyophilized for 48 h and ground with acetone pre-washed stainless steel mortar and pestles prior to extraction. Each FA and SI analysis was from aliquots of the same pooled, whole-body tissues from each tank replicate. SI analyses followed Howe and Simenstad (2007). SI tissue samples were not lipid-extracted because both the lipids and the non-lipid tissues in the isopods are of critical interest to our question; i.e., the degree to which these animals synthesize lipids or obtain them directly from dietary sources is unknown (see Matthews and Mazumder 2005). SI samples were weighed (2 mg) into tin capsules and sent for analysis at Washington State University's SI Core lab. FA methyl esters (FAME) were extracted in house on 5 mg of tissue using a modified Folch method (e.g., Galloway et al. 2012). We analyzed FAME with GC-FID (HP 6958, Agilent DB-23 column), and an 85-minute temperature program (Taipale et al. 2011) designed to separate C_{16} and C_{18} monounsaturated FA (MUFA) and polyunsaturated FA (PUFA). Peak identification was achieved primarily using GC-FID and a 40 FA standard (Nu-chek Prep standard 569B). We determined the identity of putative and unknown peaks with GC-MS.

Analyses

We calculated the mean proportion of each FA across all samples and excluded rare FA that comprised <0.1% of the total FA (Kelly et al. 2008). A second, post-hoc visualization analysis was done on the FA dataset comprised solely of PUFA (see below). The reduced datasets (28 and 13 FA) were renormalized and arcsine square root transformed ($x' = \sin^{-1}\sqrt{x}$) prior to analysis. The FA signatures of the algal diets and *Idotea* fed those diets (28 FA) were compared separately using PERMANOVA followed by pairwise tests (with fixed factors and Type III SS). Significance was determined using unrestricted permutation of the raw data (9999

permutations), using Monte-Carlo generated *P*-values when the number of unique permutations <200. We used PERMDISP to compare the changes in multivariate dispersions among *Idotea*-algal diet pairs when groups of FA that did not correlate with diet were removed (below). We used non-metric multidimensional scaling (NMDS) to visualize PUFA signatures (13 FA) and both bivariate and box-plots to present growth, raw SI, and SI fractionation results. All multivariate analyses were performed using PRIMER v. 6.0 and PERMANOVA+ add on (PRIMER-E Ltd., Plymouth UK).

We evaluated the individual SI of diets, *Idotea* fed those diets, fractionation (i.e., the difference) between diet and *Idotea* for each isotope, and total *Idotea* growth (in mm) across treatments using univariate ANOVA with Tukey's LSD post-hoc tests. Eight FA categories with 3 replicates for each algal diet and the *Idotea* fed those diets were compared with a series of correlations (FA categories and abbreviations in Table 4.1). All univariate ANOVAs, coefficients of determination, and associated Pearson *p*-values were calculated with SPSS v. 19.0 for Mac and multiple test questions (correlations) were Bonferroni corrected.

Results

Growth

Across all treatments, *Idotea* increased by a mean of 3.2±1.1 mm, an average increase of 103.1±35.9% (Fig. 4.1a). *Idotea* growth across treatments differed overall (ANOVA, F_3=151.92, P<0.0001) and post-hoc tests found that all treatments differed (Tukey's LSD, P<0.0001). Treatment specific % size increases were 112.7±31.1% for *Ulva* sp., 129.5±28.8% for *Nereocystis*, 83.8±24.6% for *Fucus*, and 67.5±17.6% for *Mazzaella*. Differences in coloration of *Idotea* across diets were clearly visible (Fig 4.1b-d).

Fatty acids

Algal FA signatures differed overall (PERMANOVA, Pseudo-F_3=56.53, P=0.0001) and between all species (P<0.002) except for the two brown algae (P=0.152). *Idotea* FA signatures differed overall (PERMANOVA, Pseudo-F_3=186.97, P=0.0001), and between all diets in post-hoc pairwise tests (P<0.0005). The FA similarities (13 FA) among both diets and consumers are visualized in Fig. 4.2a (see Appendix D for FA summary data). The summary categories SAFA and MUFA (Fig. 4.3a-b) were not correlated between diet and consumer (Table 4.1 shows all 8 calculated bivariate correlations). Some, but not all, FA were highly correlated between the algal diets and the *Idotea* fed those diets (Table 4.1; Fig. 4.3b-c). A post-hoc removal of SAFA and MUFA resulted in higher within-group, *Idotea*-algal diet similarities (i.e., lower dispersions) for all diet-consumer pairs except for the animals fed *Ulva* sp. (Fig. 4.4).

Stable Isotopes

Algal diets differed in both $\delta^{13}C$ and $\delta^{15}N$ (F_3=15.15, P=0.001; F_3=11.29, P=0.003, respectively), but for both isotopes, post-hoc tests showed that these differences were due entirely to *Mazzaella* (P<0.005; Fig. 4.5). *Idotea* fed different diets differed in both $\delta^{13}C$ and $\delta^{15}N$ (F_3=298.32, P<0.0001; F_3=37.65, P<0.0001, respectively). For $\delta^{13}C$ all *Idotea* treatments differed (P<0.0001) except for *Nereocystis* and *Ulva* sp. For $\delta^{15}N$ all *Idotea* treatments differed (P<0.02) except for *Nereocystis* and *Mazzaella* (Fig. 4.5). The SI bivariate plots are shown in Fig. 4.2b and diet specific fractionations for each isotope are shown in Fig. 4.6a-b, with mean fractionation across all diet treatments of 0.59±1.84‰ for $\delta^{13}C$ and 1.21±1.14‰ for $\delta^{15}N$. Fractionation for $\delta^{13}C$ was highly variable and did not differ across treatments (F_3=1.95, P=0.200). Conversely, $\delta^{15}N$ fractionation did differ overall (F_3=8.55, P=0.007); *Ulva* sp. differed

from *Fucus* (P=0.013) and *Mazzaella* (P=0.001), and *Nereocystis* differed from *Mazzaella* (P=0.016). Appendix D shows SI data for all algal diets and *Idotea*.

Discussion

We found striking associations between the FA signatures in *Idotea* and their algal diets. *Idotea* grew readily in the lab on controlled mono-specific diets, offering rare insights into the trophic transfer of individual FA from diet to a consumer. Analysis of bulk FA signatures on the two datasets (28 and 13 FA) show two critically important results: as animals, *Idotea* have FA that are clearly distinct from their algal diets; yet, the consumers are nonetheless separated from each other and grouped strongly in multivariate space with their diet (Fig 4.2a). The associations between diet and consumer are not visually evident in the SI bivariate plot (Fig. 4.2b). SAFA and MUFA in *Idotea* were generally insensitive to the differences in the algal diets (Fig. 4.3a-b, Table 4.1), and the post-hoc removal of these FA reduced the multivariate dispersion between the diets and *Idotea* for all diets except *Ulva* sp. (Fig. 4.4). The relatively high levels of the PUFA 16:4ω3 in *Ulva* sp. (~18% of total FA vs. 0% in other diets) likely accounted for this; animals fed *Ulva* sp. also retained this green algal marker (16:4ω3=3.4±0.4% compared with 0% in *Idotea* fed other diets). When using a qualitative biomarker approach with wild consumers, researchers should consider removing the FA categories that have been shown to be unresponsive to diet (if feeding trials have been done) and renormalizing datasets prior to analysis.

Our data show that the fractionation of FA from dietary sources to consumer tissues depends quantitatively on the food source. The proportions of ΣARA+EPA were strongly correlated between algal diet and *Idotea* tissue (Fig. 4.3c-d, Table 4.1), making these FA useful biomarkers for this macroalgal consumer. These same FA were also identified as excellent

dietary biomarkers in the freshwater zooplankter *Daphnia pulex* (Brett et al. 2006). The correlation between diet and consumer of ΣALA+LIN is interesting because these two precursor PUFA can be used by animals to bioconvert to metabolically active ω3 and ω6 PUFA (see Dalsgaard 2003). C_{18} PUFA were enriched in the diets (Fig. 4.3c), while C_{20} PUFA were enriched in the consumer relative to the diet (Fig 4.3d). The difference in the slope of the results line and the 1:1 relationship (Fig. 4.3c-d) shows how different diets affect FA fractionation. For example, a diet with a high percentage of ALA and LIN (*Ulva* sp.) transfers relatively less of these FA to the consumer than does the brown algal *Nereocystis* diet. *Idotea* maintain minimum levels of ARA (~2.5%) and EPA (~10.5%), even on the *Ulva* sp. diet, which had very low levels of these FA (~1.5%). Galloway et al. (2012) found that macroalgal taxonomic orders could be discriminated with the sum of all C_{18} and C_{20} ω3 and ω6 FA alone; this study clarifies that the best tracers for interpreting animal diets are physiologically similar FA types such as C_{18} or C_{20} ω3 and ω6 PUFA.

Trophic SI fractionation of $\delta^{15}N$ from algal diets to whole-body *Idotea* tissues (1.21±1.14‰) differed among algal diets (Fig. 4.6b), and was lower than the enrichment range most commonly applied (~3-4‰; Peterson and Fry 1987). This result is of critical importance to interpretation of $\delta^{15}N$ ratios in trophic studies, and is corroborated by research by Prado et al. (2012) and Bunn et al. (in press). For example, a predator of *Idotea* might be enriched in its $\delta^{15}N$ ratio by only 3-4‰ above the algal baseline, and thus could mistakenly be interpreted as an herbivore. Fractionation estimates of 3-4‰ per trophic level may actually indicate multiple trophic levels, omnivory, or consumption of microbes (Galloway et al. in revision), thus conflating actual trophic enrichment. We found that the diets leading to the highest consumer growth also exhibited the lowest $\delta^{15}N$ fractionation (*Ulva* sp.: -0.10±0.41‰ and *Nereocystis*:

0.88±0.86‰). These are locally preferred diets for adult *Idotea* (M. N. Dethier, unpublished data), so it is likely that wild animals consuming these algae would have depleted $\delta^{15}N$ ratios. We observed some incidences of isopod cannibalism in the treatments with *Mazzaella* and *Ulva* sp.; however we did not find biochemical evidence of carnivory (enriched $\delta^{15}N$) in the these animals; $\delta^{15}N$ enrichment in the *Mazzaella* treatment is still <20% of what would be expected for a 2nd order consumer (6-8‰). Our results raise potential concerns about whether the general trophic fractionation coefficients used are valid across taxa, and furthermore whether such values even approximate the true relationship within a given species under different diets.

The diet-specific fractionation coefficients measured here for $\delta^{13}C$ were highly variable (0.59±1.84‰), but fall within the range of commonly used values (e.g., ~0-1‰ per trophic level; Peterson and Fry 1987). The fact that we did not lipid or acid-extract our samples prior to SI analyses may be relevant for comparing our results with other studies, as lipid extraction on whole body marine invertebrates can cause a mean $\delta^{13}C$ enrichment of 1.3±0.44‰, with negligible effects for $\delta^{15}N$ (-0.1±0.12‰; Logan et al. 2008) because FA do not contain N. Acid-treating whole crustaceans significantly changes $\delta^{13}C$ ratios but not $\delta^{15}N$ ratios in *Neotrypaea* (Yokoyama et al. 2005). We argue that despite its widespread use, lipid extraction is potentially counterproductive given that lipids may be the primary component of an animal's biomass that is actually relevant for tracking diet (e.g., Matthews and Mazumder 2005).

There are several critical uncertainties to the biomarker approach where important progress is occurring: 1) FA and SI signatures are distinct among primary producer groups (see Galloway et al. 2012, Kelly and Scheibling 2012, Dethier et al. 2013); 2) these source biomarker signatures are preserved in the consumer's tissues in a predictable way (e.g., Dalsgaard et al. 2003; Hall et al. 2006, Kelly et al. 2008, this study); and 3) 'fresh' producer biomarker signatures

(typically what is measured) do not change during aging and break-down into detritus (i.e., diagenesis; Galloway et al. in revision, Sosik and Simenstad in revision). Few studies have measured SI and FA fractionation from the diet to the resulting consumer tissues (but see Yokoyama et al. 2005, Taipale et al. 2011). Both biomarker approaches need additional research on diet-specific fractionation and the importance to biomarker signatures of consumer metabolism (tissue turnover due to growth rate) (Brett et al. 2006, Budge et al. 2012), particularly under mixed-diet, experimental conditions. Our findings show that diet-specific variation in fractionation can be significant for both FA and $\delta^{15}N$, a fact that is very important for future attempts at meaningful quantitative mixing model analyses for both suites of biomarkers. Finally, these results demonstrate the potential for using FA signature analyses to achieve phylum-level resolution of algal diet in an invertebrate herbivore.

Literature Cited

Bell, M. V. and D. R. Tocher. 2009. Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: general pathways and new directions. Pages 211-236 *in* M. T. Arts, M. T. Brett, and M. Kainz, editors. Lipids in aquatic ecosystems. Springer, New York.

Brett, M. T., D. C. Muller-Navarra, A. P. Ballantyne, J. L. Ravet, and C. R. Goldman. 2006. *Daphnia* fatty acid composition reflects that of their diet. Limnology and Oceanography **51**:2428-2437.

Budge, S. M., S. J. Iverson, and H. N. Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Marine Mammal Science **22**:759-801.

Budge, S. M., S. N. Penney, and S. P. Lall. 2012. Estimating diets of Atlantic salmon (*Salmo salar*) using fatty acid signature analyses; validation with controlled feeding studies.

Canadian Journal of Fisheries and Aquatic Sciences **69**:1033-1046.

Bunn, S. E., C. Leigh, T. Jardine. in press. Diet-tissue fractionation of $\delta^{15}N$ by consumers from streams and rivers. Limnology and Oceanography.

Caut, S., E. Angulo, and F. Courchamp. 2009. Variation in discrimination factors ($\delta^{15}N$ and $\delta^{13}C$): the effect of diet isotopic values and applications for diet reconstruction. Journal of Applied Ecology **46**:443-453.

Dalsgaard, J., M. St. John, G. Kattner, D. C. Muller-Navarra, and W. Hagen. 2003. Fatty acid trophic markers in the pelagic marine food environment. Advances in Marine Biology **46**:225-340.

del Rio, C. M., N. Wolf, S. A. Carleton, and L. Z. Gannes. 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biological Reviews **84**:91-111.

Dethier, M. N., E. A. Sosik, A. W. E. Galloway, D. O. Duggins, and C. A. Simenstad. 2013. Addressing assumptions: variation in stable isotopes and fatty acids in marine macrophytes can confound conclusions of food web studies. Marine Ecology Progress Series **478**:1-14.

Duarte, C. M. and J. Cebrian. 1996. The fate of marine autotrophic production. Limnology and Oceanography **41**:1758-1766.

Duggins, D. O. 1980. Kelp beds and sea otters: an experimental approach. Ecology **61:** 447-453.

Duggins, D. O., C. A. Simenstad, and J. A. Estes. 1989. Magnification of secondary production by kelp detritus in coastal marine ecosystems. Science **245**:170-173.

Fry, B. 2006. Stable isotope ecology. Springer, New York.

Fry, B. 2012. Alternative approaches for solving undetermined isotope mixing problems. Marine Ecology Progress Series **472**:1-13.

Galloway, A. W. E., K. H. Britton-Simmons, D. O. Duggins, P. W. Gabrielson, and M. T. Brett. 2012. Fatty acid signatures differentiate marine macrophytes at ordinal and family ranks. Journal of Phycology **48**:956-965.

Galloway, A. W. E., A. T. Lowe, E. A. Sosik, J. S. Yeung, and D. O. Duggins. *in press.* Fatty acid and stable isotope biomarkers suggest microbe-induced differences in benthic food webs between depths. Limnology and Oceanography.

Gannes, L. Z., D. M. Obrien, and C. M. del Rio. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology **78**:1271-1276.

Hall, D., S. Y. Lee, and T. Meziane. 2006. Fatty acids as trophic tracers in an experimental estuarine food chain: tracer transfer. Journal of Experimental Marine Biology and Ecology **336**:42-53.

Howe, E. R. and C. A. Simenstad. 2007. Restoration trajectories and food web linkages in San Francisco Bay's estuarine marshes: a manipulative translocation experiment. Marine Ecology Progress Series **351**:65-76.

Iverson, S. J., C. Field, W. D. Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. Ecological Monographs **74**:211-235.

Kelly, J. R., R. E. Scheibling, S. J. Iverson, and P. Gagnon. 2008. Fatty acid profiles in the gonads of the sea urchin *Strongylocentrotus droebachiensis* on natural algal diets. Marine Ecology Progress Series **373**:1-9.

Kelly, J. R. and R. E. Scheibling. 2012. Fatty acids as dietary tracers in benthic food webs. Marine Ecology Progress Series **446**:1-22.

Krumhansl, K. A. and R. E. Scheibling. 2012. Production and fate of kelp detritus. Marine Ecology Progress Series **467**:281-302.

Logan, J. M., T. D. Jardine, T. J. Miller, S. E. Bunn, R. A. Cunjak, and M. E. Lutcavage. 2008. Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. Journal of Animal Ecology **77**:838-846.

Mann, K. H. 1973. Seaweeds: their productivity and strategy for growth. Science **182**:975-981.

Matthews, B. and A. Mazumder. 2005. Temporal variation in body composition (C:N) helps explain seasonal patterns of zooplankton $\delta^{13}C$. Freshwater Biology **50**:502-515.

Parnell, A. C., R. Inger, S. Bearhop, and A. L. Jackson. 2010. Source partitioning using stable isotopes: coping with too much variation. Plos One **5(3)**:e9672.doi:9610.1371.

Peterson, B. J. and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics **18**:293-320.

Prado, P., R. H. Carmichael, S. A. Watts, J. Cebrian, and K. L. Heck Jr. 2012. Diet-dependent $\delta^{13}C$ and $\delta^{15}N$ fractionation among sea urchin *Lytechinus variegatus* tissues: implications for food web models. Marine Ecology Progress Series **462**:175-190.

Sosik, E. A., and C. A. Simenstad. Microbial trophic levels: isotopic evidence and consequences in macroalgal detritus-based coastal food webs. *in revision*, March 2013.

Taipale, S. J., M. J. Kainz, and M. T. Brett. 2011. Diet-switching experiments show rapid accumulation and preferential retention of highly unsaturated fatty acids in *Daphnia*. Oikos **120:** 1674-1682.

Yokoyama, H., A. Tamaki, K. Harada, K. Shimoda, K. Koyama, and Y. Ishihi. 2005. Variability of diet-tissue isotopic fractionation in estuarine macrobenthos. Marine Ecology Progress Series **296**:115-128.

Table 4.1. Definitions of FA categories and abbreviations used and results of bivariate correlations of individual FA or sums of FA within categories (see Methods) between the diets and the *Idotea* fed those diets. Each comparison reports the coefficients of determination (r^2) and associated *P*-values. The Bonferroni-corrected significance for the 8 tests is α=0.006.

FA category (definition)	Abbreviation	Diet-*Idotea* correlation r^2	*P* –value
Saturated	SAFA	-0.019	0.670
Monounsaturated	MUFA	-0.007	0.796
18:2ω6 (LIN) + 18:3ω6 (GLA)	$\Sigma C_{18}\omega 6$	0.846	<0.001
18:3ω3 (ALA) + 18:4ω3 (SDA)	$\Sigma C_{18}\omega 3$	0.890	<0.001
LIN + GLA + ALA + SDA	$\Sigma C_{18}\omega 6+C_{18}\omega 3$	0.926	<0.001
20:4ω6 (ARA)	ARA	0.824	<0.001
20:5ω3 (EPA)	EPA	0.943	<0.001
ARA + EPA	ΣARA+EPA	0.907	<0.001

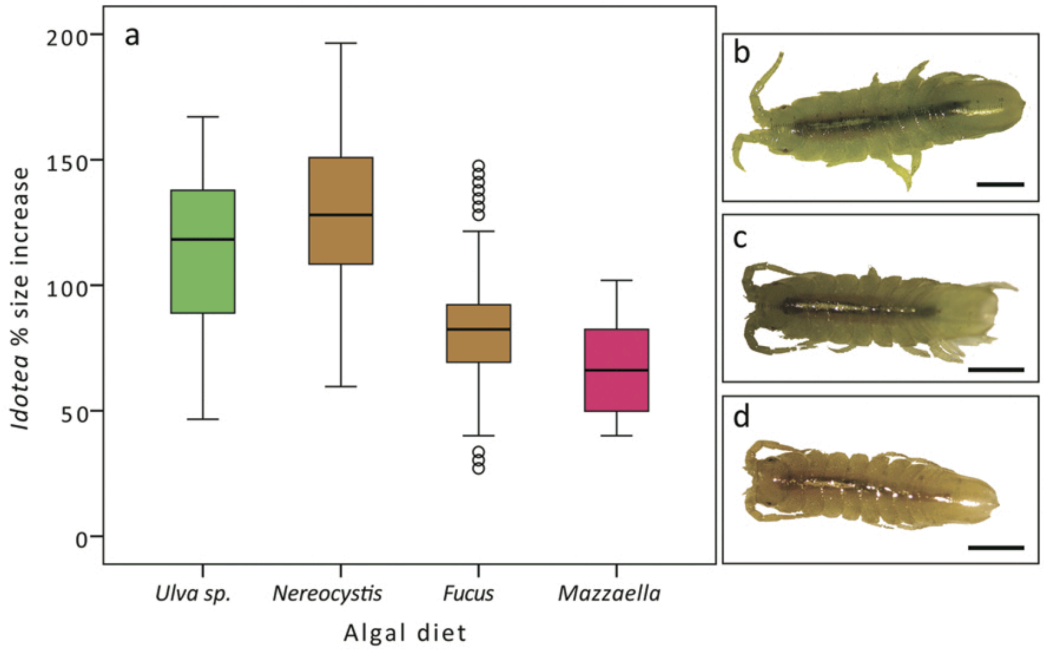

Figure 4.1. (a) Boxplot (median, quartile range and 95% CI) of percent size (mm) increase of juvenile *Idotea* during 10-week feeding trial, color coded by algal division of the 4 macroalgal diets (Chlorophyta, green; Ochrophyta, brown, Rhodophyta, red). Representative photos (with 1 mm scale bar) of *Idotea* fed (b) *Ulva* sp. (green), (c) *Nereocystis* (brown), and (d) *Mazzaella* (red).

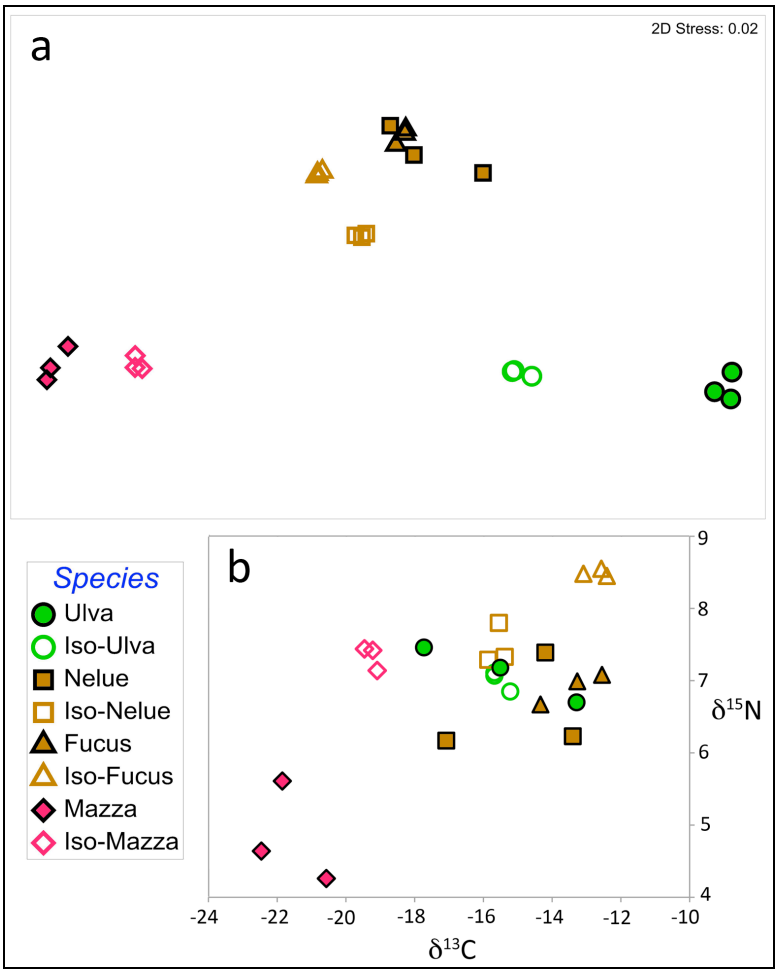

Figure 4.2. (a) NMDS plot of the reduced, transformed, and normalized (see Methods) 13 FA dataset (just PUFA) in *Idotea* (open symbols) and their algal diets (filled symbols). (b) Bivariate SI plot showing raw $\delta^{13}C$ (x-axis) and $\delta^{15}N$ (y-axis) ratios for *Idotea* and diets. Legend abbreviations for both plots are: Algal diets (Ulva [*Ulva* sp.], Nelue [*Nereocystis*], Fucus [*Fucus*], Mazza [*Mazzaella*]); Idotea fed the algal diets (Iso-Ulva [fed *Ulva* sp.], Iso-Nelue [fed *Nereocystis*], Iso-Fucus [fed *Fucus*], Iso-Mazza [fed *Mazzaella*]).

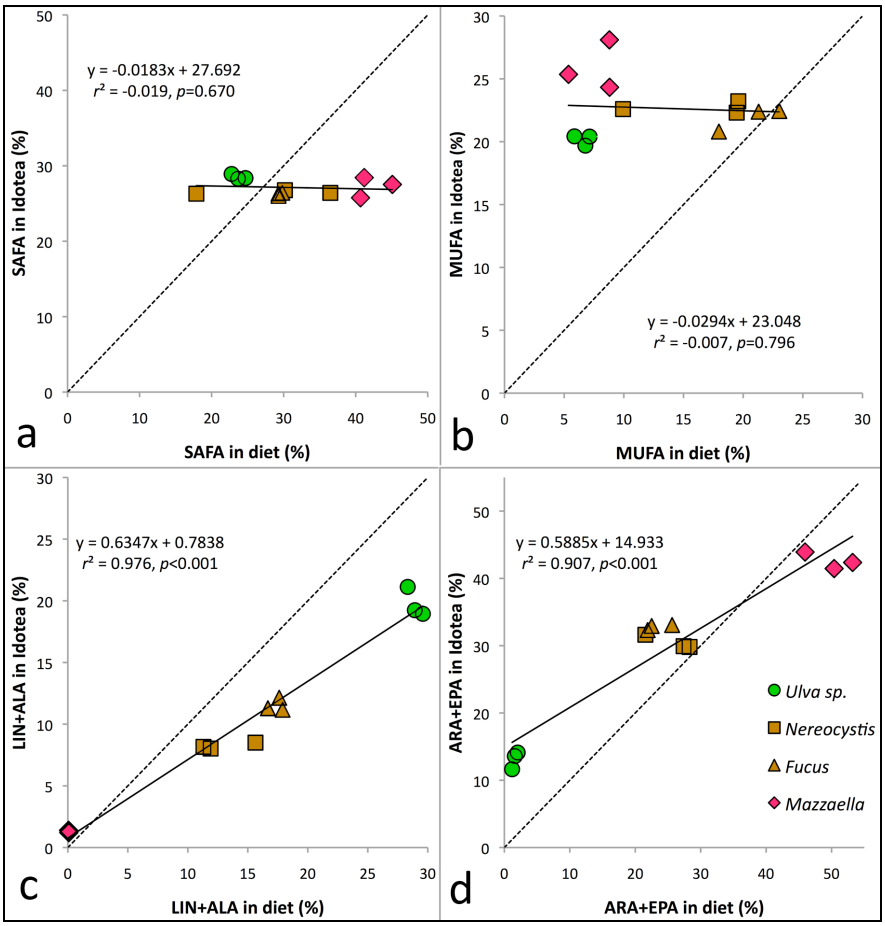

Figure 4.3. Bivariate plots of correlations and linear fit equations between FA groups in the diet and the *Idotea* fed those diets. All plots also show the 1:1 relationship as dotted reference lines. See Table 4.1 for categories and abbreviations. (a) SAFA, (b) MUFA, (c) ΣLIN+ALA, (d) ΣARA+EPA. Diets, symbols, and colors as in Figures 4.1 and 4.2.

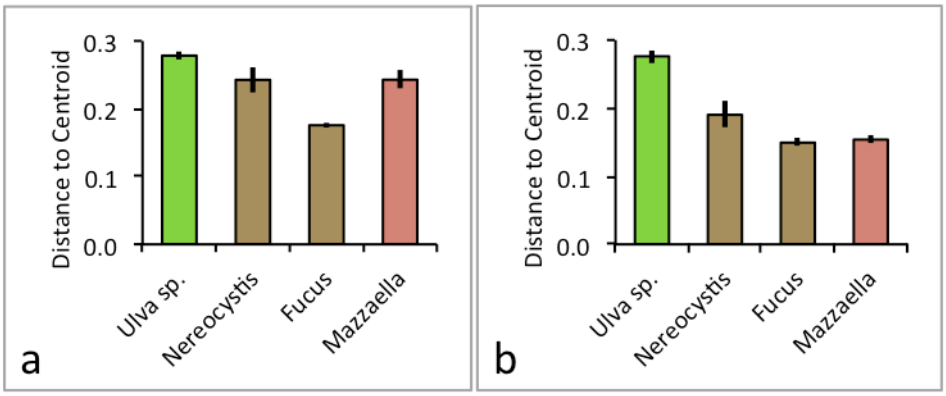

Fig. 4.4. Results of tests of multivariate FA dispersions comparing total distance from centroid of pooled values of *Idotea* and their specific algal diets in (a) the 28 FA dataset and (b) the 13 FA dataset. PERMDISP tests are significant overall for both analyses (P<0.001); the plots show how removing the FA that are not correlated between algal diet and consumer (e.g., MUFA and SAFA) reduces the dispersion (i.e., distance) between the diets and consumer for all treatments except animals fed *Ulva* sp., which was not affected by the reduction.

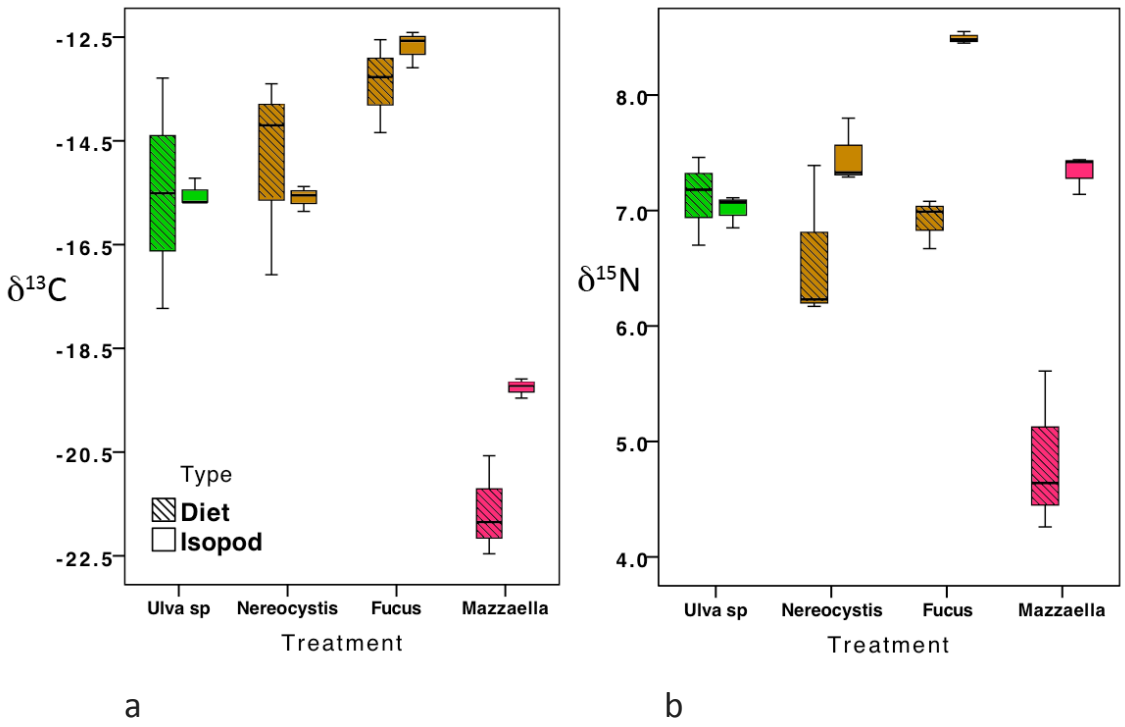

Fig. 4.5. Boxplots (median, quartile range and 95% CI) of SI ratios of the four algal diets (see Methods) and *Idotea* fed those diets. (a) $\delta^{13}C$, (b) $\delta^{15}N$.

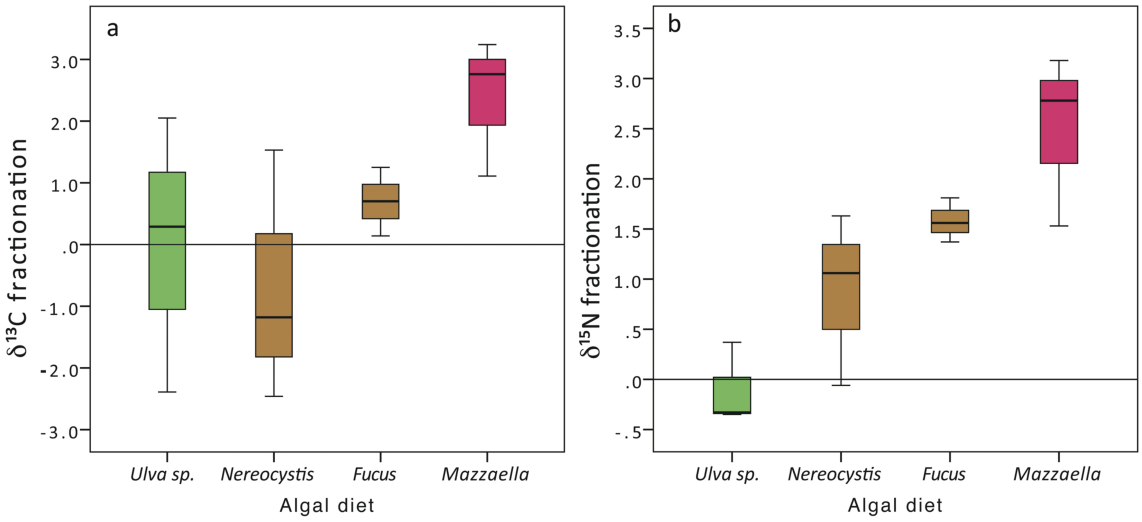

Fig. 4.6. Boxplots (median, quartile range and 95% CI) of trophic fractionation (i.e., enrichment) between algal diet SI ratios and *Idotea* fed those diets. (a) $\delta^{13}C$, (b) $\delta^{15}N$.

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Chapter 2

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APPENDIX A – CHAPTER 2 FA DATA

Appendix A. Chapter 2 - 40 macrophyte taxa studied in the San Juan Archipelago. Data are mean FA percentages of total FA (44 identified FA, see Methods) ± 1 SD, where *n*=3 replicates except for *Syringoderma*, where *n*=1.

Phylum	Anthophyta				Chlorophyta					Ochrophyta							
Order	Alismatales		Bryopsidales	Cladophorales	Prasiolales	Ulvales		Desmarestiales	Dictyotales	Ectocarpales			Fucales				
FA	PHSCO	ZOMAR	COFRA	CLCOL	PRMER	ULINT	ULLAC	DEMUN	DIBIN	SOULV	SCLOM	FUDIS	SAMUT				
14:0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.3	7.5 ± 5.1	3.9 ± 0.1	0.9 ± 0.3	0.4 ± 0.1	4.1 ± 0.5	7.8 ± 0.6	5.9 ± 0.4	6.3 ± 0.2	9.6 ± 0.9	2.6 ± 0.1				
14:1ω5	0.5 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.3 ± 0.1	0.5 ± 0.3	0.1 ± 0.0	0.2 ± 0.0	0.7 ± 0.1	0.3 ± 0.1	0.3 ± 0.0				
15:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.0				
16:0	23.1 ± 2.1	18.2 ± 1.8	27.6 ± 1.0	24.2 ± 3.4	15.1 ± 0.2	19.7 ± 7.3	16.8 ± 0.9	10.8 ± 0.5	10.8 ± 0.4	18.6 ± 1.5	21.1 ± 1.9	16.3 ± 2.0	28.0 ± 0.9				
16:1ω11	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.2	0.6 ± 0.2	0.7 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.1	0.2 ± 0.2				
16:1ω7	0.6 ± 0.3	0.0 ± 0.0	1.3 ± 0.4	6.7 ± 3.8	0.9 ± 0.3	0.7 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	0.7 ± 0.1	0.5 ± 0.0	1.4 ± 0.2	2.6 ± 0.5				
16:1ω*7**	7.5 ± 4.1	7.2 ± 1.0	8.0 ± 0.7	8.2 ± 2.9	5.2 ± 0.4	5.5 ± 0.8	5.6 ± 2.2	4.3 ± 0.4	5.9 ± 0.4	5.0 ± 0.3	5.4 ± 0.8	2.3 ± 0.8	5.7 ± 0.1				
16:1ω5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	6.6 ± 0.6	7.8 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	0.0 ± 0.1	0.1 ± 0.1				
i-17:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0				
16:2ω4	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.7 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.1	0.0 ± 0.0	0.9 ± 0.1	0.3 ± 0.1	0.9 ± 0.2	0.5 ± 0.0				
17:0	0.8 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.0				
16:3ω4/5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0				
16:3ω3	2.8 ± 0.8	8.0 ± 1.6	11.5 ± 0.4	0.9 ± 1.5	12.0 ± 3.0	2.4 ± 0.3	2.1 ± 0.2	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0				
17:1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0				
16:4ω3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4.9 ± 4.2	0.0 ± 0.0	14.0 ± 1.6	18.6 ± 3.3	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0				
i-18:0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.4 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0				
16:4ω1	0.0 ± 0.0	0.2 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2				
18:0	2.4 ± 0.9	2.1 ± 0.3	1.3 ± 0.1	1.4 ± 0.3	1.7 ± 0.2	1.3 ± 0.5	0.6 ± 0.3	0.8 ± 0.2	0.6 ± 0.0	2.1 ± 0.1	1.5 ± 0.2	0.6 ± 0.1	1.0 ± 0.0				
18:1ω9	1.0 ± 0.3	1.0 ± 0.2	8.5 ± 0.3	6.4 ± 4.8	23.6 ± 2.4	0.1 ± 0.2	0.1 ± 0.2	7.4 ± 0.6	8.5 ± 0.2	11.4 ± 0.7	13.2 ± 1.4	16.5 ± 5.1	7.4 ± 0.9				
18:1ω7	1.3 ± 0.5	0.1 ± 0.1	0.8 ± 0.1	3.9 ± 0.8	1.4 ± 0.1	7.8 ± 3.2	5.1 ± 0.8	0.0 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.0 ± 0.1	0.0 ± 0.0				
18:1ω5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0				
18:2ω6	9.0 ± 2.5	6.7 ± 1.9	2.9 ± 0.3	3.5 ± 1.6	9.7 ± 1.9	6.1 ± 2.1	4.1 ± 0.9	3.6 ± 0.2	2.2 ± 0.2	5.0 ± 0.2	5.0 ± 0.1	11.3 ± 0.5	4.1 ± 0.1				
18:3ω6	0.0 ± 0.0	0.0 ± 0.0	1.5 ± 0.1	0.2 ± 0.1	0.0 ± 0.0	0.6 ± 0.2	0.8 ± 0.4	0.7 ± 0.1	0.4 ± 0.0	1.4 ± 0.1	0.7 ± 0.0	0.8 ± 0.1	0.8 ± 0.1				
18:3ω3	42.4 ± 6.9	50.7 ± 2.6	17.6 ± 1.2	11.6 ± 5.8	14.6 ± 0.8	19.8 ± 1.8	22.4 ± 2.3	11.8 ± 0.4	7.8 ± 0.2	7.9 ± 0.6	5.6 ± 0.7	5.8 ± 0.8	8.0 ± 0.3				
18:4ω3	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 0.1	4.8 ± 7.3	0.0 ± 0.0	10.9 ± 2.5	15.6 ± 1.3	20.9 ± 0.2	22.4 ± 1.5	15.8 ± 1.3	11.2 ± 1.4	6.9 ± 2.2	8.8 ± 0.6				
20:0	0.9 ± 0.2	1.6 ± 0.2	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.1	0.9 ± 0.0	1.5 ± 0.2	0.1 ± 0.1	0.2 ± 0.1				
20:1ω11	0.0 ± 0.0	0.0 ± 0.0	2.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.1	1.6 ± 0.1				
20:1ω9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0				
20:1ω7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.5 ± 0.1				
20:2ω6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0				
20:3ω6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.1	0.7 ± 0.2	0.4 ± 0.0	0.5 ± 0.1	0.9 ± 0.1	0.9 ± 0.1				
20:4ω6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.3	2.9 ± 1.8	0.0 ± 0.0	0.1 ± 0.2	12.8 ± 0.9	9.5 ± 0.4	8.2 ± 0.6	9.2 ± 0.5	14.4 ± 0.9	12.2 ± 0.4				
20:4ω3	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.0 ± 0.0	0.4 ± 0.7	0.3 ± 0.3	0.9 ± 0.1	1.0 ± 0.1	0.5 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.0				
20:5ω3	0.0 ± 0.0	0.0 ± 0.0	3.8 ± 0.1	4.2 ± 1.4	0.0 ± 0.0	1.1 ± 0.4	0.8 ± 0.1	12.2 ± 1.0	6.7 ± 0.3	10.6 ± 0.5	15.0 ± 1.8	8.9 ± 0.9	10.1 ± 1.4				
22:0	6.1 ± 1.7	2.7 ± 0.3	3.8 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.5	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.0				
22:1ω11	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	1.5 ± 0.1				
22:1ω9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0				
22:2ω6	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0				
22:4ω6	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.1	0.0 ± 0.0	0.7 ± 0.7	0.4 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	3.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0				
22:3ω3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0				
22:5ω3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.1	0.0 ± 0.0	2.6 ± 0.4	2.1 ± 0.5	0.0 ± 0.0	0.8 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0				
22:6ω3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 2.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0				
24:0	1.0 ± 0.2	1.1 ± 0.2	1.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0				
24:1ω9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0				

NOTE: The exact identity of 16:1ω*7* is currently unresolved. This compound elutes right after 16:1ω7 in our program and likely co-elutes with 16:1ω7 in shorter GC programs.

Phylum	Ochrophyta										Rhodophyta				
Order	Laminariales							Ralfsiales	Syringodermatales		Bonemaisoniales	Ceramiales			
FA	ALMAR	AGFIM	COCOS	NELUE	SALAT	SASES	SASUB	EGMEN	ANJAP	SYABY	BOCAL	RHPLU	POLAT		
14:0	3.7 ± 0.8	4.9 ± 1.2	7.8 ± 0.4	11.2 ± 1.0	10.5 ± 0.9	8.1 ± 0.4	9.0 ± 1.1	6.9 ± 0.8	6.4 ± 0.5	7.4 ± na	5.3 ± 0.7	3.1 ± 0.6	5.0 ± 0.6		
14:1ω5	0.2 ± 0.1	0.4 ± 0.1	0.6 ± 0.2	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.7 ± na	0.7 ± 0.1	0.3 ± 0.1	0.7 ± 0.1		
15:0	0.2 ± 0.3	0.1 ± 0.1	0.4 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± na	0.1 ± 0.3	0.3 ± 0.0	0.0 ± 0.0		
16:0	14.3 ± 2.0	15.3 ± 5.2	14.7 ± 1.3	18.3 ± 3.4	21.3 ± 2.6	15.1 ± 0.5	21.6 ± 3.7	21.6 ± 2.7	23.9 ± 1.1	22.7 ± na	49.2 ± 2.3	37.1 ± 5.4	40.4 ± 4.6		
16:1ω11	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
16:1ω7	0.7 ± 0.2	8.1 ± 0.7	2.0 ± 1.5	0.6 ± 0.1	4.5 ± 1.0	1.2 ± 0.3	3.3 ± 0.6	1.6 ± 0.4	0.4 ± 0.0	5.8 ± na	0.0 ± 0.0	1.1 ± 0.1	0.5 ± 0.4		
16:1ω*?*	4.6 ± 1.5	6.5 ± 2.3	5.9 ± 1.1	3.0 ± 0.6	5.1 ± 0.2	4.8 ± 0.6	4.0 ± 0.2	7.3 ± 0.3	5.6 ± 0.3	10.5 ± na	4.8 ± 0.1	5.7 ± 0.6	6.9 ± 1.3		
16:1ω5	0.1 ± 0.1	0.8 ± 0.1	1.8 ± 0.2	0.6 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
i-17:0	0.0 ± 0.0	0.2 ± 0.2	0.1 ± 0.1	0.0 ± 0.0	1.1 ± 0.3	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	5.3 ± 0.8	0.0 ± 0.0	0.0 ± 0.0		
16:2ω4	1.7 ± 0.4	4.4 ± 0.7	0.7 ± 0.2	0.5 ± 0.2	0.5 ± 0.4	0.4 ± 0.0	0.3 ± 0.3	1.1 ± 0.1	0.0 ± 0.0	0.7 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
17:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
16:3ω4/5	0.0 ± 0.0	4.1 ± 1.9	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.1 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
16:3ω3	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0		
17:1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 0.2		
16:4ω3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
i-18:0	0.0 ± 0.0	1.5 ± 0.3	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.2 ± 0.3	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
16:4ω1	0.0 ± 0.0	0.3 ± 0.3	0.4 ± 0.1	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0		
18:0	1.0 ± 0.2	1.7 ± 0.3	1.0 ± 0.0	1.6 ± 0.2	1.8 ± 0.7	1.4 ± 0.1	1.9 ± 0.2	1.5 ± 0.2	1.1 ± 0.1	5.8 ± na	3.3 ± 0.7	1.7 ± 0.5	2.9 ± 0.6		
18:1ω9	8.0 ± 2.0	3.2 ± 1.5	9.4 ± 1.6	13.1 ± 1.9	9.5 ± 2.7	11.7 ± 1.0	12.8 ± 1.2	9.8 ± 0.6	12.8 ± 0.4	9.0 ± na	4.0 ± 1.1	7.0 ± 0.2	2.9 ± 0.6		
18:1ω7	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± na	0.0 ± 0.0	2.1 ± 0.4	1.8 ± 0.2		
18:1ω5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
18:2ω6	3.8 ± 0.8	9.7 ± 0.4	2.6 ± 0.9	6.0 ± 1.0	5.5 ± 0.9	5.5 ± 0.3	6.9 ± 0.7	5.7 ± 0.4	5.6 ± 0.2	6.5 ± na	0.2 ± 0.3	0.6 ± 0.0	0.7 ± 0.0		
18:3ω6	0.9 ± 0.3	2.4 ± 0.8	0.3 ± 0.1	0.8 ± 0.3	2.3 ± 0.7	0.9 ± 0.1	1.6 ± 0.4	0.1 ± 0.2	0.5 ± 0.1	0.0 ± na	0.0 ± 0.0	0.5 ± 0.0	0.0 ± 0.0		
18:3ω3	10.1 ± 0.7	1.6 ± 0.1	9.0 ± 2.1	7.1 ± 1.3	3.8 ± 1.8	6.9 ± 0.6	4.8 ± 0.9	9.2 ± 0.6	5.8 ± 0.2	7.9 ± na	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.6		
18:4ω3	20.9 ± 4.2	1.5 ± 0.2	15.2 ± 4.3	12.4 ± 4.4	8.8 ± 4.7	13.5 ± 0.9	7.3 ± 2.5	10.7 ± 2.6	11.2 ± 0.7	3.4 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
20:0	0.3 ± 0.3	0.4 ± 0.1	0.1 ± 0.1	0.1 ± 0.2	0.0 ± 0.0	0.3 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.1 ± 0.2	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
20:1ω11	0.0 ± 0.0	0.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
20:1ω9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.5		
20:1ω7	0.0 ± 0.0	1.4 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	3.4 ± 1.5	0.0 ± 0.0	0.0 ± 0.0		
20:2ω6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
20:3ω6	0.5 ± 0.5	0.8 ± 0.1	0.5 ± 0.4	0.9 ± 0.1	0.6 ± 0.1	0.0 ± 0.0	0.4 ± 0.3	0.2 ± 0.2	0.8 ± 0.2	0.0 ± na	0.0 ± 0.0	1.8 ± 0.1	0.0 ± 0.0		
20:4ω6	10.4 ± 1.8	13.2 ± 0.6	7.0 ± 3.5	11.3 ± 1.2	13.0 ± 2.9	21.7 ± 1.4	15.0 ± 2.2	13.8 ± 0.5	12.6 ± 0.8	8.7 ± na	8.8 ± 0.8	8.9 ± 1.0	28.8 ± 11.4		
20:4ω3	0.9 ± 0.2	0.1 ± 0.2	0.5 ± 0.1	0.7 ± 0.2	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.2	1.2 ± 0.2	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
20:5ω3	17.6 ± 1.6	13.8 ± 1.5	18.5 ± 1.9	8.3 ± 0.5	8.2 ± 3.1	7.7 ± 0.2	6.7 ± 1.4	8.5 ± 1.0	10.9 ± 0.3	6.7 ± na	9.2 ± 1.7	27.0 ± 8.1	1.7 ± 0.8		
22:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
22:1ω11	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
22:1ω9	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
22:2ω6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	4.7 ± 5.1	0.0 ± 0.0	0.0 ± 0.0		
22:4ω6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
22:3ω3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
22:5ω3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
22:6ω3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	4.8 ± 5.5		
24:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
24:1ω9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± na	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		

Phylum	Rhodophyta																							
Order	Ceramiales				Corallinales		Erythropeltidales		Gigartinales										Halymeniales		Palmariales		Plocamiales		Rhodymeniales	
FA	NELAR		OSSPE		CATUB		SMNAI		COSUB		ENMUR		OPCAL		CHEXA		MASPL		CAFLA		PRSTE		HAGLA		PLPAC		SPPER	
14:0	3.9	0.7	8.4	0.7	0.0	0.0	0.2	0.0	2.8	0.2	0.0	0.0	1.8	0.4	4.4	0.5	4.5	2.2	4.8	1.1	4.6	0.3	21.1	2.2	7.1	0.9	2.3	0.2
14:1ω5	0.9	0.2	0.6	0.0	1.4	2.5	0.2	0.0	1.3	0.2	13.7	0.9	0.8	0.5	2.4	0.7	1.7	0.5	1.1	0.1	1.9	0.3	2.4	0.0	0.7	0.2	0.3	0.0
15:0	0.0	0.0	0.6	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.6	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1
16:0	23.7	2.1	46.6	3.6	24.4	2.7	21.6	1.1	28.1	0.6	69.1	0.8	36.3	4.8	53.5	4.9	43.6	4.8	61.0	6.5	40.6	2.6	46.3	3.5	29.0	3.2	31.2	2.0
16:1ω11	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.2	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.0	0.0
16:1ω7	9.0	3.2	3.2	0.5	0.0	0.0	2.5	0.1	2.0	0.4	0.0	0.0	0.5	0.9	1.6	2.8	0.0	0.0	1.9	1.0	2.9	1.2	2.0	1.8	1.6	0.6	2.8	0.3
16:1ω*?*	7.4	1.0	5.1	0.5	1.6	2.7	6.4	0.5	3.4	0.3	0.0	0.0	4.3	0.4	4.9	0.4	4.5	0.8	6.1	0.7	4.2	0.9	7.9	0.6	5.7	0.7	4.6	0.8
16:1ω5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
i-17:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16:2ω4	3.7	0.5	0.3	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.6	1.0	0.0	0.0	0.6	0.6	0.0	0.0
17:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	17.0	7.1	0.0	0.0
16:3ω4/5	3.8	0.6	0.8	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.9	0.0	0.0	0.0	0.0	0.0	0.0
16:3ω3	0.2	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17:1	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.5	0.4	0.0
16:4ω3	0.2	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
i-18:0	1.9	0.5	1.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16:4ω1	0.0	0.0	0.5	0.5	0.0	0.0	0.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.7	0.0	0.0	0.0	0.0	1.5	0.1	0.0	0.0
18:0	2.4	0.4	3.0	0.5	0.0	0.0	0.6	0.0	3.5	0.4	17.2	0.7	3.6	0.3	4.8	0.9	4.1	1.0	3.7	0.5	4.3	0.9	5.6	1.7	4.3	0.8	1.5	0.2
18:1ω9	2.1	0.4	6.2	0.5	0.0	0.0	0.2	0.0	2.0	0.2	0.0	0.0	5.6	0.4	6.1	1.1	3.9	0.7	4.0	0.6	5.2	1.1	0.0	0.0	0.5	0.5	2.8	0.1
18:1ω7	3.7	0.4	0.9	0.2	0.0	0.0	7.7	0.1	0.0	0.0	0.0	0.0	0.2	0.4	0.0	0.0	0.0	0.0	0.0	0.0	3.2	0.4	0.0	0.0	0.0	0.0	0.4	0.1
18:1ω5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:2ω6	1.6	0.2	0.5	0.4	0.0	0.0	1.2	0.2	0.0	0.0	0.0	0.0	0.6	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.6	0.0	0.0	0.0	0.0	0.6	0.1
18:3ω6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.1
18:3ω3	4.2	1.0	0.3	0.5	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	1.1	0.0	0.0	0.0	0.0	0.0	0.0
18:4ω3	4.5	1.8	0.3	0.5	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
20:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:1ω11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:1ω9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:1ω7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.0	8.2	0.0	0.0
20:2ω6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:3ω6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	0.9	0.2	0.3
20:4ω6	4.2	1.3	7.4	1.7	30.4	8.3	0.5	0.0	18.2	0.5	0.0	0.0	29.1	3.0	2.3	2.1	4.7	1.6	11.2	7.0	10.8	1.6	0.0	0.0	3.5	1.1	18.6	1.5
20:4ω3	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:5ω3	21.1	1.6	9.8	2.9	42.3	10.2	55.5	1.3	19.3	2.0	0.0	0.0	6.6	1.3	6.7	1.5	27.1	6.5	0.4	0.7	15.0	1.4	14.6	6.2	14.2	4.5	21.8	4.9
22:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.4	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.3	2.4	1.0
22:1ω11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.3
22:1ω9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:2ω6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.5	2.0
22:4ω6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:3ω3	1.3	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:5ω3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:6ω3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24:1ω9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

APPENDIX B – CHAPTER 3 FA DATA

Chlamys

Appendix B. Chapter 3 - FA summary table for 5 taxa studied at 3 sites and two depths (see Methods for codes/depths).

Species	*Chlamys*		*Chlamys*		*Chlamys*		*Chlamys*		*Chlamys*		*Chlamys*	
Site	PIL		PIL		SKP		SKP		PTC		PTC	
Depth	Shallow		Deep		Shallow		Deep		Shallow		Deep	
n	5		3		5		5		5		5	
FA	Ave	SD	Ave	SD	Ave	SD	Ave	SD	Ave	SD	Ave	SD
c14:0	1.02	0.16	0.85	0.10	1.04	0.19	0.78	0.07	0.92	0.09	0.83	0.05
c15:0	0.27	0.02	0.40	0.01	0.37	0.05	0.48	0.02	0.33	0.06	0.40	0.02
i-16:0	0.69	0.11	0.79	0.05	0.69	0.08	0.89	0.06	0.74	0.04	0.87	0.07
c16:0	17.96	0.52	17.40	0.33	18.15	0.68	15.57	0.63	17.40	0.35	16.10	0.29
c16:1	0.46	0.16	0.34	0.07	0.48	0.09	0.30	0.02	0.28	0.06	0.29	0.03
16:1n-7	0.39	0.04	0.39	0.03	0.35	0.08	0.46	0.04	0.39	0.01	0.45	0.04
16:1n-5	0.52	0.13	0.37	0.08	0.48	0.12	0.33	0.03	0.32	0.04	0.32	0.01
i-17:0	0.55	0.03	0.96	0.10	0.59	0.05	0.89	0.06	0.62	0.05	0.81	0.04
a-17:0	0.24	0.05	0.32	0.01	0.26	0.01	0.32	0.04	0.28	0.03	0.34	0.04
c17:0	0.75	0.04	1.04	0.09	0.85	0.05	1.14	0.06	0.85	0.07	1.02	0.06
i-18:0	0.18	0.01	0.25	0.03	0.17	0.01	0.27	0.02	0.19	0.02	0.23	0.02
a-18:0	0.08	0.04	0.22	0.04	0.06	0.01	0.21	0.02	0.09	0.06	0.17	0.06
c18:0	6.74	0.37	7.15	0.68	6.96	0.71	7.65	0.56	6.81	0.44	7.27	0.28
18:1n-9	1.52	0.12	0.99	0.07	1.66	0.17	0.83	0.05	1.12	0.13	0.87	0.11
18:1n-7	3.65	0.07	2.59	0.26	3.24	0.33	2.45	0.13	3.32	0.25	2.55	0.20
18:1n-5	0.26	0.05	0.21	0.06	0.20	0.02	0.13	0.05	0.15	0.01	0.16	0.06
18:2n-6	0.76	0.10	0.37	0.06	0.72	0.09	0.28	0.03	0.50	0.06	0.33	0.03
18:3n-6	0.82	0.16	0.41	0.13	0.61	0.20	0.27	0.12	0.62	0.06	0.45	0.06
c19:0	0.31	0.05	0.26	0.04	0.28	0.07	0.25	0.01	0.25	0.04	0.27	0.02
18:3n-3	0.87	0.10	0.41	0.08	0.74	0.16	0.36	0.06	0.66	0.06	0.43	0.04
18:4n-3	4.08	0.52	2.06	0.50	3.34	0.75	1.90	0.29	3.32	0.28	2.24	0.30
20:1n-11	0.80	0.06	0.79	0.02	0.75	0.06	0.94	0.08	0.75	0.06	0.78	0.06
20:1n-9	1.23	0.09	1.05	0.10	1.27	0.23	1.06	0.12	1.00	0.07	0.94	0.09
20:1n-7	1.29	0.08	1.06	0.11	1.17	0.32	0.86	0.12	1.06	0.14	0.99	0.06
20:2n-6	0.76	0.10	0.49	0.13	0.65	0.14	0.36	0.07	0.54	0.04	0.39	0.04
20:3n-6	0.23	0.02	0.06	0.01	0.17	0.10	0.06	0.00	0.05	0.00	0.06	0.00
20:4n-6	1.70	0.26	2.43	0.34	2.10	0.44	2.95	0.42	1.94	0.24	2.73	0.23
20:3n-3	2.06	0.22	2.03	0.16	2.00	0.21	2.23	0.13	1.97	0.18	2.18	0.16
20:4n-3	0.51	0.11	0.28	0.11	0.40	0.16	0.20	0.07	0.38	0.07	0.27	0.03
20:5n-3	23.05	0.87	20.48	1.60	20.76	1.46	17.79	3.22	22.35	1.02	22.51	1.14
21:5n-3	1.33	0.06	1.06	0.08	1.22	0.11	1.08	0.10	1.28	0.11	1.25	0.03
22:4n-6	0.13	0.06	0.28	0.02	0.12	0.08	0.29	0.08	0.17	0.07	0.28	0.01
22:5n-6	0.51	0.13	0.64	0.05	0.63	0.08	0.73	0.15	0.44	0.05	0.57	0.03
22:5n-3	1.31	0.11	1.30	0.04	1.40	0.13	1.40	0.07	1.39	0.19	1.39	0.19
22:6n-3	22.63	1.77	29.95	1.76	25.78	2.40	33.93	3.81	27.21	0.49	28.95	1.02

Psolus

Appendix B.

Species	*Psolus*		*Psolus*		*Psolus*		*Psolus*		*Psolus*		*Psolus*	
Site	PIL		PIL		SKP		SKP		PTC		PTC	
Depth	Shallow		Deep		Shallow		Deep		Shallow		Deep	
n	5		5		5		5		4		5	
FA	Ave	SD	Ave	SD	Ave	SD	Ave	SD	Ave	SD	Ave	SD
c14:0	3.73	0.60	2.16	0.84	3.86	0.75	2.60	0.45	1.42	0.62	2.71	0.53
i-15:0	4.07	1.08	1.93	1.06	5.72	3.05	1.91	0.64	6.65	4.34	4.48	2.05
c15:0	0.85	0.32	0.56	0.32	1.14	0.63	0.53	0.14	1.65	0.74	1.35	0.76
i-16:0	1.09	0.17	3.16	0.96	1.26	0.22	2.05	0.23	4.54	2.33	1.88	0.17
c16:0	3.81	0.16	3.31	0.60	4.13	0.54	3.32	0.61	2.72	0.57	3.59	0.51
16:1n-7	6.98	0.81	4.42	1.51	7.67	0.38	4.69	1.06	4.64	0.77	7.10	1.03
16:1n-5	0.17	0.05	0.13	0.05	0.21	0.05	0.12	0.03	0.39	0.39	0.22	0.09
a-17:0	1.02	0.31	0.79	0.18	0.97	0.22	0.74	0.14	0.99	0.31	1.20	0.11
16:2n-4	1.03	0.16	0.69	0.28	1.07	0.11	0.75	0.14	0.55	0.21	1.01	0.16
c17:0	0.27	0.03	0.38	0.10	0.32	0.05	0.38	0.05	0.34	0.06	0.39	0.04
16:3n-4	0.83	0.18	0.35	0.10	0.94	0.13	0.40	0.14	0.38	0.10	0.67	0.09
16:4n-1	1.50	0.33	0.74	0.33	1.52	0.16	1.00	0.26	0.65	0.28	1.38	0.17
c18:0	6.11	0.59	6.50	1.57	6.79	0.65	7.06	0.72	4.79	0.51	6.54	0.49
18:1n-9	4.09	0.89	3.45	1.03	4.17	0.46	3.25	0.59	1.68	0.35	2.73	0.58
18:1n-7	1.91	0.20	1.82	0.44	2.28	0.27	2.08	0.13	1.70	0.35	2.41	0.29
18:2n-6	0.43	0.04	0.40	0.18	0.38	0.03	0.47	0.10	0.34	0.11	0.40	0.03
c18:2	0.46	0.05	0.28	0.11	0.42	0.05	0.31	0.08	0.22	0.06	0.46	0.06
c19:0	0.73	0.08	0.67	0.04	0.75	0.05	0.72	0.07	0.58	0.23	0.72	0.08
18:4n-3	0.72	0.15	0.30	0.12	0.85	0.08	0.39	0.14	0.35	0.07	0.65	0.05
18:4n-1	0.53	0.10	0.28	0.11	0.49	0.04	0.33	0.18	0.11	0.10	0.47	0.06
c20:0	1.57	0.16	1.84	0.45	1.53	0.23	1.77	0.30	1.30	0.21	1.62	0.14
20:1n-11	3.60	0.82	5.08	1.06	3.13	0.40	4.81	0.24	5.19	0.97	3.13	0.38
20:1n-9	8.96	1.11	7.26	1.95	10.23	0.75	7.12	0.37	6.95	1.53	6.82	0.71
20:1n-7	3.30	0.74	3.78	0.79	3.62	0.43	4.01	0.84	5.44	0.98	2.68	0.41
20:2n-6	0.55	0.04	0.97	0.12	0.47	0.07	0.83	0.04	0.56	0.24	0.73	0.11
20:4n-6	5.52	1.74	12.35	5.17	2.84	0.56	9.76	1.09	9.46	2.64	6.77	0.82
c21:0	0.57	0.13	1.14	0.35	0.48	0.06	1.04	0.20	1.19	0.27	0.73	0.15
21:1/20:3n-4	0.26	0.11	0.13	0.05	0.29	0.06	0.12	0.03	0.13	0.08	0.34	0.05
20:4n-3	0.53	0.10	0.13	0.05	0.56	0.09	0.19	0.15	0.21	0.14	0.45	0.08
20:5n-3	21.01	2.33	14.86	2.77	19.07	1.20	17.65	3.01	18.93	1.59	21.01	0.60
c22:0	1.28	0.14	2.20	0.39	1.23	0.18	1.96	0.39	1.41	0.20	1.43	0.18
c22:1	1.07	0.07	1.43	0.13	0.83	0.17	1.32	0.17	1.09	0.59	1.26	0.06
c22:1	0.05	0.02	0.13	0.05	0.13	0.20	0.12	0.03	0.13	0.08	0.06	0.01
22:1n-9	1.30	0.14	1.42	0.16	1.50	0.13	1.73	0.53	1.23	0.22	1.32	0.19
c22:1	0.70	0.07	0.99	0.18	0.78	0.13	1.00	0.16	0.72	0.37	0.87	0.18
21:5n-3	0.86	0.10	0.58	0.21	0.74	0.10	0.67	0.10	0.42	0.12	0.90	0.10
c22:2	0.17	0.06	0.13	0.05	0.18	0.05	0.12	0.03	0.13	0.08	0.16	0.08
c23:0	0.17	0.05	0.44	0.34	0.17	0.04	0.31	0.19	0.20	0.12	0.25	0.06
c23:1	5.01	0.82	9.91	2.66	3.83	0.49	9.40	1.75	8.37	1.65	5.57	0.84
c22:4n-1?	0.30	0.14	0.13	0.05	0.32	0.04	0.12	0.03	0.13	0.08	0.32	0.10
22:5n-3	0.52	0.07	0.13	0.05	0.43	0.12	0.17	0.10	0.19	0.10	0.42	0.04
22:6n-3	1.27	0.28	1.16	0.53	1.49	0.16	1.41	0.16	0.89	0.45	1.80	0.23
24:1n-9	1.10	0.05	1.45	0.18	1.23	0.18	1.25	0.15	1.03	0.55	0.98	0.11

Strongylocentrotus

Appendix B.

Species	*Strongy.*		*Strongy.*		*Strongy.*		*Strongy.*		*Strongy.*		*Strongy.*	
Site	PIL		PIL		SKP		SKP		PTC		PTC	
Depth	Shallow		Deep		Shallow		Deep		Shallow		Deep	
n	5		5		4		5		4		5	
FA	Ave	SD	Ave	SD	Ave	SD	Ave	SD	Ave	SD	Ave	SD
c14:0	3.18	0.27	3.39	0.27	3.73	0.33	3.52	0.40	3.13	0.78	3.09	0.75
c14:1	0.24	0.07	0.32	0.04	0.25	0.02	0.33	0.07	0.30	0.11	0.26	0.03
c15:0	0.30	0.09	0.25	0.06	0.24	0.02	0.33	0.07	0.27	0.11	0.31	0.08
c16:0	9.62	0.66	8.67	0.66	10.07	0.11	8.60	0.52	8.98	0.58	8.85	0.89
c16:1	0.32	0.20	0.20	0.02	0.26	0.06	0.22	0.03	0.23	0.08	0.21	0.11
16:1n-7	1.35	0.09	1.44	0.12	1.47	0.19	1.49	0.02	2.11	0.59	2.37	0.23
16:1n-5	1.15	0.14	1.54	0.26	1.13	0.15	1.69	0.33	1.41	0.30	1.51	0.07
c18:0	5.75	0.79	6.04	0.51	5.59	0.27	5.58	0.44	5.82	0.28	5.86	0.54
18:1n-9	0.41	0.23	0.65	0.16	1.17	0.28	0.78	0.09	0.52	0.28	0.52	0.25
18:1n-7	2.24	0.24	1.78	0.19	1.95	0.04	1.99	0.10	2.11	0.22	1.86	0.18
c18:2	0.37	0.16	0.36	0.18	0.31	0.17	0.51	0.09	0.53	0.22	0.50	0.05
18:2n-6	0.30	0.13	0.40	0.08	0.67	0.10	0.37	0.09	0.26	0.18	0.28	0.15
c19:0	0.28	0.06	0.17	0.08	0.20	0.05	0.24	0.02	0.21	0.13	0.09	0.02
18:3n-3	0.20	0.11	0.45	0.10	0.47	0.16	0.32	0.15	0.27	0.20	0.29	0.17
18:4n-3	0.35	0.23	0.55	0.07	1.08	0.60	0.55	0.17	0.42	0.25	0.46	0.26
c20:0	10.71	1.22	9.33	0.28	9.18	1.12	10.44	0.31	9.61	0.68	10.94	0.59
20:1n-11	0.66	0.11	0.67	0.08	0.84	0.11	0.66	0.08	0.85	0.30	0.74	0.16
20:1n-9	2.54	0.26	2.78	0.24	3.70	0.18	2.90	0.22	2.70	0.37	2.81	0.22
20:2/20:1n-7	6.82	0.41	7.04	0.30	7.71	0.93	7.25	0.25	7.22	1.15	7.33	0.40
20:2/20:1n-5	1.33	0.40	1.34	0.33	1.04	0.28	1.39	0.09	1.24	0.30	1.54	0.33
20:3n-9	0.75	0.28	0.62	0.17	0.84	0.38	0.85	0.21	0.72	0.28	0.63	0.08
20:2n-6	2.07	0.42	1.74	0.23	2.05	0.32	1.53	0.14	1.88	0.27	1.46	0.08
20:3n-6	0.20	0.10	0.08	0.01	0.33	0.06	0.20	0.11	0.16	0.11	0.13	0.08
20:4n-6	20.15	2.20	24.21	1.87	17.90	1.94	23.79	1.60	21.38	2.62	22.15	1.24
20:3n-3	2.36	0.66	3.34	0.36	2.53	0.41	2.49	0.33	3.21	0.35	2.81	0.38
c21:1	2.50	0.61	2.73	0.29	1.84	0.30	2.31	0.18	3.06	0.55	2.96	0.56
20:4n-3	0.70	0.13	0.57	0.08	0.83	0.20	0.48	0.09	0.46	0.24	0.45	0.19
20:5n-3	15.75	0.44	13.98	1.63	15.39	0.56	12.75	1.19	14.80	0.33	13.47	1.18
22:1n-9	1.30	0.11	0.99	0.14	1.63	0.28	1.31	0.16	1.25	0.34	1.07	0.07
c22:2	0.45	0.10	0.19	0.10	0.37	0.05	0.33	0.05	0.27	0.11	0.18	0.10
c22:2	3.84	0.36	3.65	0.19	2.94	0.30	4.03	0.30	3.71	0.53	4.43	0.48
22:5n-3	0.31	0.15	0.08	0.01	0.36	0.05	0.11	0.08	0.16	0.09	0.09	0.02
22:6n-3	1.38	1.24	0.27	0.19	1.78	0.40	0.44	0.26	0.57	0.41	0.15	0.14

Cancer

Appendix B.

Species	*Cancer*		*Cancer*		*Cancer*		*Cancer*		*Cancer*		*Cancer*	
Site	PIL		PIL		SKP		SKP		PTC		PTC	
Depth	Shallow		Deep		Shallow		Deep		Shallow		Deep	
n	5		5		4		5		5		5	
FA	Ave	SD	Ave	SD	Ave	SD	Ave	SD	Ave	SD	Ave	SD
c14:0	0.51	0.09	0.32	0.02	0.30	0.06	0.32	0.08	0.32	0.10	0.32	0.06
c15:0	0.42	0.04	0.50	0.08	0.33	0.05	0.44	0.11	0.44	0.06	0.47	0.10
i-16:0	1.28	0.07	1.03	0.09	1.37	0.04	0.75	0.09	1.35	0.20	1.22	0.24
c16:0	14.59	0.91	15.01	0.62	14.57	0.50	16.02	0.70	15.48	0.30	15.42	1.45
16:1n-7	3.26	0.46	3.41	0.53	3.43	0.34	4.20	1.09	3.29	0.57	3.93	1.84
i-17:0	0.44	0.05	0.92	0.18	0.44	0.09	0.55	0.14	0.53	0.07	0.72	0.24
a-17:0	0.18	0.06	0.45	0.13	0.16	0.11	0.21	0.08	0.23	0.02	0.38	0.17
c17:0	0.95	0.16	0.92	0.15	0.80	0.07	0.66	0.12	0.98	0.10	0.88	0.21
c17:1	0.57	0.20	0.60	0.12	0.43	0.10	0.42	0.05	0.58	0.07	0.61	0.09
i-18:0	0.52	0.17	0.77	0.16	0.35	0.11	0.38	0.06	0.49	0.23	0.75	0.30
a-18:0	0.16	0.10	0.25	0.11	0.08	0.00	0.11	0.07	0.09	0.01	0.12	0.07
c18:0	6.79	0.67	5.62	0.52	6.57	0.15	5.67	0.69	6.37	0.41	5.84	0.71
18:1n-9t	0.08	0.01	0.08	0.00	0.08	0.00	0.17	0.12	0.09	0.01	0.13	0.09
18:1n-9	9.11	0.73	8.70	1.30	8.01	0.40	9.13	1.63	8.95	1.61	9.65	0.64
18:1n-7	5.09	0.33	5.26	0.40	5.26	0.53	4.94	0.51	4.75	0.27	5.37	0.41
18:1n-5	0.25	0.02	0.52	0.14	0.28	0.03	0.32	0.15	0.29	0.10	0.34	0.20
18:2n-6	0.90	0.10	0.63	0.09	0.82	0.16	0.47	0.03	0.85	0.29	0.56	0.17
c18:2	0.38	0.09	0.28	0.12	0.37	0.16	0.37	0.04	0.29	0.13	0.34	0.06
c19:0	0.30	0.04	0.25	0.09	0.36	0.15	0.23	0.09	0.17	0.12	0.22	0.08
18:3n-3	0.48	0.06	0.37	0.03	0.58	0.25	0.11	0.07	0.41	0.19	0.19	0.14
18:4n-3	0.34	0.20	0.08	0.00	0.21	0.16	0.11	0.09	0.21	0.17	0.08	0.00
c20:0	0.30	0.05	0.11	0.07	0.20	0.08	0.11	0.05	0.12	0.08	0.15	0.10
20:1n-11	0.44	0.12	0.47	0.04	0.43	0.15	0.56	0.16	0.46	0.13	0.33	0.33
20:1n-9	0.39	0.09	0.57	0.06	0.41	0.11	0.51	0.16	0.31	0.13	0.51	0.15
20:1n-7	0.63	0.13	0.60	0.12	0.56	0.14	0.88	0.20	0.51	0.25	0.49	0.30
20:2n-6	0.85	0.18	0.78	0.19	1.11	0.46	0.56	0.10	0.93	0.17	0.69	0.20
20:3n-3	2.83	0.43	2.69	0.11	3.09	0.30	2.65	0.32	2.88	0.24	2.79	0.19
20:5n-3	33.44	1.88	31.61	2.09	34.66	1.41	34.89	1.84	34.25	1.25	31.63	2.48
21:5n-3	0.45	0.08	0.42	0.09	0.36	0.09	0.84	0.17	0.34	0.16	0.46	0.13
22:4n-6	0.27	0.18	0.41	0.05	0.54	0.44	0.08	0.01	0.38	0.08	0.33	0.04
22:5n-6	0.12	0.09	0.29	0.12	0.08	0.00	0.08	0.01	0.09	0.01	0.13	0.10
22:5n-3	1.25	0.40	1.42	0.22	1.49	0.63	1.05	0.15	1.16	0.23	1.08	0.16
22:6n-3	12.14	1.64	14.40	1.23	12.01	0.83	11.98	1.30	12.15	1.80	13.63	1.33

Fusitriton

Appendix B.

Species	*Fusitriton*		*Fusitriton*		*Fusitriton*		*Fusitriton*		*Fusitriton*		*Fusitriton*	
Site	PIL		PIL		SKP		SKP		PTC		PTC	
Depth	Shallow		Deep		Shallow		Deep		Shallow		Deep	
n	5		3		5		5		4		5	
FA	Ave	SD	Ave	SD	Ave	SD	Ave	SD	Ave	SD	Ave	SD
c14:0	1.78	0.19	1.71	0.39	1.67	0.22	1.56	0.23	1.89	0.52	1.57	0.21
c15:0	0.61	0.09	0.76	0.13	0.68	0.14	0.77	0.20	0.77	0.12	0.92	0.10
i-16:0	2.54	0.37	2.22	0.29	2.31	0.37	2.01	0.16	2.29	0.11	2.40	0.20
c16:0	11.05	1.17	9.12	0.67	10.29	0.68	9.24	0.31	9.87	0.25	9.41	0.79
16:1n-7	1.39	0.18	1.13	0.25	1.23	0.14	1.14	0.11	1.29	0.08	1.34	0.09
i-17:0	0.60	0.20	0.87	0.11	0.54	0.14	0.80	0.07	0.79	0.22	0.80	0.10
a-17:0	0.32	0.19	0.43	0.05	0.60	0.36	0.54	0.05	0.50	0.13	0.99	0.08
16:1-branched	1.16	0.26	1.60	0.37	1.69	0.33	1.92	0.56	1.77	0.26	2.25	0.40
c17:0	1.13	0.30	1.25	0.14	0.93	0.18	1.17	0.09	1.23	0.17	1.11	0.10
i-18:0	0.41	0.13	0.58	0.17	0.64	0.16	0.71	0.11	0.62	0.15	1.34	0.11
a-18:0	0.11	0.01	0.31	0.23	0.12	0.02	0.55	0.09	0.11	0.00	0.21	0.09
c18:0	15.23	1.68	14.05	0.62	14.47	1.41	13.64	0.24	14.63	1.23	14.21	0.45
18:1n-9	2.52	0.68	2.06	0.22	2.60	0.31	1.88	0.08	2.37	0.45	2.08	0.17
18:1n-7	1.62	0.44	1.96	1.26	1.40	0.27	1.15	0.22	1.34	0.10	0.91	0.11
18:2n-6	0.93	0.26	0.83	0.56	0.88	0.17	0.51	0.14	0.81	0.16	0.80	0.15
c19:0	0.57	0.13	0.42	0.11	0.44	0.18	0.33	0.18	0.62	0.06	0.30	0.15
c20:0	0.23	0.26	0.10	0.01	0.55	0.42	0.10	0.03	0.11	0.00	0.13	0.03
20:1n-11	4.36	0.15	4.58	0.89	5.12	0.68	4.48	0.85	4.37	0.29	3.84	0.30
20:1n-9	2.90	0.90	2.53	0.65	3.63	0.71	3.03	0.51	2.93	0.67	2.52	0.42
20:1n-7	1.90	0.58	1.97	0.41	1.85	0.14	1.79	0.12	1.90	0.27	1.62	0.08
20:2n-6	2.48	0.42	2.04	0.40	2.86	0.57	2.21	0.51	2.63	0.20	2.40	0.29
20:3n-6	0.11	0.01	0.27	0.28	0.18	0.13	0.10	0.03	0.11	0.00	0.32	0.15
20:5n-3	15.18	6.06	15.13	1.99	12.89	3.10	18.55	2.98	15.42	1.40	13.77	1.73
c22:2	7.68	1.32	7.53	0.52	9.04	1.36	7.17	0.55	8.13	0.99	7.79	0.63
c22:2	2.00	0.69	4.32	1.80	2.54	0.31	3.23	0.30	2.64	0.44	3.09	0.34
21:5n-3	0.19	0.18	0.24	0.24	0.20	0.18	0.49	0.20	0.20	0.17	0.43	0.16
c22:2	0.16	0.10	0.67	0.57	0.24	0.16	0.58	0.09	0.24	0.25	0.75	0.11
22:4n-6	4.54	3.43	4.03	0.74	4.76	1.28	3.25	1.21	3.34	0.53	5.33	1.31
22:5n-3	12.39	1.01	13.62	3.05	12.75	1.66	13.48	0.89	13.25	1.07	12.71	0.24
22:6n-3	3.92	1.73	3.67	0.56	2.87	0.95	3.60	0.46	3.81	0.63	4.67	0.52

APPENDIX C – CHAPTER 3 MSI DATA

Appendix C. Chapter 3 - MSI data for 7 taxa studied at 3 sites and two depths (see Methods for codes/depths).

			$\delta^{13}C$		$\delta^{15}N$		$\delta^{34}S$	
Species	Site	Depth	Ave	SD	Ave	SD	Ave	SD
Amphissa	PIL	Deep	-15.27	0.09	13.25	1.07	21.85	0.06
Amphissa	PTC	Deep	-15.60	0.57	12.11	0.20	22.27	0.84
Amphissa	SKP	Deep	-15.75	0.21	12.22	0.09	22.43	0.21
Amphissa	PIL	Shallow	-15.88	0.28	11.37	0.15	22.42	0.39
Amphissa	PTC	Shallow	-16.35	0.21	11.30	0.16	22.30	0.37
Amphissa	SKP	Shallow	-16.60	0.46	11.67	0.70	22.58	0.18
Cancer	PIL	Deep	-15.35	0.20	13.86	0.64	20.49	0.64
Cancer	PTC	Deep	-15.92	0.21	13.08	0.09	20.86	0.42
Cancer	SKP	Deep	-15.60	0.08	12.54	0.41	20.16	0.35
Cancer	PIL	Shallow	-16.50	0.34	12.91	0.64	21.12	0.33
Cancer	PTC	Shallow	-16.29	0.49	12.78	0.61	21.03	0.28
Cancer	SKP	Shallow	-16.16	0.53	12.61	0.70	20.79	0.14
Chlamys	PIL	Deep	-17.10	0.86	9.29	0.89	22.73	0.39
Chlamys	PTC	Deep	-16.81	0.15	9.43	0.30	22.64	0.55
Chlamys	SKP	Deep	-16.91	0.11	10.78	0.26	23.61	0.20
Chlamys	PIL	Shallow	-18.27	0.22	8.28	0.16	22.38	0.38
Chlamys	PTC	Shallow	-17.68	0.42	8.76	0.49	22.91	0.34
Chlamys	SKP	Shallow	-18.19	0.28	8.74	0.41	22.79	0.40
Fusitriton	PIL	Deep	-15.86	1.52	12.92	0.09	21.79	1.40
Fusitriton	PTC	Deep	-14.92	0.26	13.13	0.25	22.04	0.58
Fusitriton	SKP	Deep	-15.88	0.56	12.84	0.72	22.60	0.34
Fusitriton	PIL	Shallow	-16.49	1.19	12.46	1.23	22.76	0.29
Fusitriton	PTC	Shallow	-16.51	0.39	12.59	0.39	21.59	0.55
Fusitriton	SKP	Shallow	-15.65	0.54	13.02	0.26	22.70	0.23
Psolus	PIL	Deep	-15.27	0.49	11.00	0.30	21.80	0.49
Psolus	PTC	Deep	-16.34	0.48	10.58	0.26	20.00	1.13
Psolus	SKP	Deep	-15.50	0.43	11.29	0.41	20.29	0.92
Psolus	PIL	Shallow	-16.71	0.86	9.04	0.74	20.55	0.72
Psolus	PTC	Shallow	-16.12	0.57	9.57	0.30	20.09	0.45
Psolus	SKP	Shallow	-17.26	0.20	8.68	0.26	20.71	0.23
Strongylocentrotus	PIL	Deep	-14.49	0.52	10.66	0.70	22.10	0.40
Strongylocentrotus	PTC	Deep	-13.13	0.42	9.49	0.33	21.23	0.55
Strongylocentrotus	SKP	Deep	-14.15	0.25	10.70	0.33	21.93	0.35
Strongylocentrotus	PIL	Shallow	-15.30	1.08	8.64	0.74	23.25	0.46
Strongylocentrotus	PTC	Shallow	-14.55	0.39	9.85	0.55	19.96	1.25
Strongylocentrotus	SKP	Shallow	-15.37	0.59	8.93	0.52	20.40	1.52
Terebratalia	PIL	Deep	-16.54	0.50	10.53	0.29	21.97	0.34
Terebratalia	PTC	Deep	-16.50	0.53	9.36	0.36	22.07	0.50
Terebratalia	SKP	Deep	-16.41	0.28	10.55	0.39	22.11	0.40
Terebratalia	PIL	Shallow	-17.52	0.66	9.23	0.32	22.63	0.23
Terebratalia	PTC	Shallow	-16.91	0.38	9.21	0.54	21.82	0.61
Terebratalia	SKP	Shallow	-17.76	0.60	10.00	0.60	22.76	0.36

APPENDIX D – CHAPTER 4 FA AND MSI DATA

Appendix D. Chapter 4 - Mean (±1SD) % fatty acid (FA) and stable isotope (SI) ratios for algal diets and *Idotea* fed those diets in 10 week trial (see Methods)

	Algal diets								*Idotea*							
	Ulva sp.		*Nereocystis*		*Fucus*		*Mazzaella*		Iso-*Ulva*		Iso-*Nelue*		Iso-*Fucus*		Iso-*Mazza*	
FA/SI	Ave	SD	Ave	SD	Ave	SD	Ave	SD	Ave	SD	Ave	SD	Ave	SD	Ave	SD
14:0	0.54	0.16	9.09	2.81	9.94	0.25	3.37	0.85	0.49	0.01	1.54	0.09	2.17	0.10	0.37	0.18
15:0	0.04	0.04	0.13	0.04	0.27	0.04	0.04	0.01	0.40	0.01	0.14	0.00	0.25	0.01	0.46	0.03
iso–16:0	0.03	0.06	0.03	0.05	0.00	0.00	0.00	0.00	0.89	0.08	0.68	0.04	0.54	0.04	0.91	0.13
16:0	18.47	0.16	17.65	6.04	18.11	0.20	35.65	1.85	17.49	0.44	15.72	0.34	17.23	0.16	16.22	1.97
16:1	0.22	0.05	0.03	0.05	0.14	0.01	0.00	0.00	0.12	0.03	0.17	0.01	0.13	0.01	0.14	0.03
16:1ω7	0.50	0.11	0.62	0.21	1.76	0.15	0.49	0.39	1.74	0.01	3.09	0.33	2.12	0.03	2.46	0.41
16:1ω5	0.07	0.04	0.61	0.34	0.14	0.02	0.02	0.02	0.04	0.00	0.24	0.01	0.10	0.00	0.27	0.03
branched-16	3.09	0.90	0.00	0.00	0.16	0.01	0.00	0.00	1.30	0.06	0.96	0.01	0.52	0.02	0.81	0.05
17:1	0.23	0.02	0.13	0.10	0.10	0.02	0.04	0.01	0.61	0.02	0.14	0.00	0.13	0.02	0.24	0.09
16:4ω3	18.55	1.46	0.00	0.00	0.00	0.00	0.00	0.00	3.39	0.43	0.00	0.00	0.00	0.00	0.00	0.00
18:0	0.99	0.32	1.10	0.49	0.63	0.12	3.26	0.87	7.10	0.39	6.72	0.22	4.94	0.09	7.62	0.77
18:1ω9	0.47	0.01	14.91	5.25	18.45	2.57	6.39	0.99	9.08	0.47	14.97	0.31	16.26	0.68	15.09	1.14
18:1ω7	5.03	0.59	0.03	0.05	0.14	0.05	0.72	0.59	7.97	0.12	3.12	0.13	2.30	0.17	6.42	2.31
18:2ω6	3.91	0.88	6.19	1.05	11.49	0.77	0.04	0.01	4.44	0.25	3.45	0.11	8.01	0.35	0.57	0.03
18:3ω6	0.61	0.45	0.96	0.64	0.81	0.11	0.04	0.01	0.64	0.06	0.58	0.02	0.52	0.01	0.10	0.08
18:3ω3	25.03	1.42	6.76	3.31	5.90	0.48	0.04	0.01	15.33	0.95	4.78	0.23	3.52	0.18	0.71	0.08
18:4ω3	16.21	1.37	14.08	10.37	6.32	0.25	0.00	0.00	6.18	0.46	7.33	0.63	2.48	0.19	0.00	0.00
20:0	0.01	0.02	0.17	0.11	0.23	0.01	0.00	0.00	0.26	0.01	0.33	0.01	0.22	0.01	0.25	0.02
20:1ω9	0.08	0.05	0.00	0.00	0.03	0.00	0.00	0.00	0.62	0.04	0.97	0.04	0.83	0.05	1.31	0.04
20:2ω6	0.21	0.15	0.00	0.00	0.31	0.06	0.01	0.02	1.12	0.05	1.06	0.05	1.74	0.01	0.45	0.08
20:3ω6	0.00	0.00	0.89	0.44	1.11	0.21	0.04	0.01	0.60	0.02	0.49	0.03	1.40	0.08	0.38	0.04
20:4ω6	0.24	0.09	15.97	2.76	15.26	1.05	9.83	1.82	2.48	0.13	13.34	0.58	18.44	0.28	10.06	0.37
20:4ω3	0.51	0.27	0.88	0.34	0.49	0.21	0.00	0.00	2.00	0.09	1.83	0.06	0.67	0.03	0.00	0.00
20:5ω3	1.37	0.38	9.77	3.02	8.09	0.96	40.02	5.47	10.63	1.18	17.11	0.47	14.33	0.19	32.51	0.97
22:0	0.54	0.35	0.00	0.00	0.13	0.01	0.00	0.00	0.60	0.03	0.40	0.01	0.37	0.00	0.59	0.05
22:4ω6	0.10	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.38	0.04	0.19	0.01	0.20	0.01	0.16	0.01
22:5ω3	2.94	0.17	0.00	0.00	0.00	0.00	0.02	0.02	3.58	0.08	0.34	0.04	0.24	0.02	1.00	0.14
22:6ω3	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.53	0.06	0.30	0.01	0.34	0.02	0.89	0.21
$\delta^{13}C$	-15.51	2.22	-14.89	1.94	-13.39	0.90	-21.59	2.16	-15.24	0.26	-16.16	0.63	-12.69	0.36	-19.26	0.19
$\delta^{15}N$	7.11	0.38	6.60	0.69	6.91	0.22	5.17	0.60	7.70	0.29	8.17	0.79	8.49	0.05	7.33	0.17