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Fatty acid and stable isotope biomarkers suggest microbe-induced differences in benthic food webs between depths

A. W. E. Galloway,[1,2,*] A. T. Lowe,[2] E. A. Sosik,[1,2] J. S. Yeung,[3] and D. O. Duggins[2]

[1] Friday Harbor Laboratories, School of Aquatic and Fishery Sciences, University of Washington, Friday Harbor, Washington
[2] University of Washington Friday Harbor Laboratories, Friday Harbor, Washington
[3] University of Washington Civil and Environmental Engineering, Seattle, Washington

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 six of seven 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 ω-3 FA were lower in deep primary consumers, whereas predator ω-3 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 ω-3 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.

Trophic interactions in 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., multiple stable isotope [MSI] and fatty acid [FA]) signature analysis (Peterson 1999; Kelly and Scheibling 2012). 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 (Cebrian 1999), assuming that sources with the highest NPP are most important; 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).

MSI ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) analysis is an additional valuable biomarker tool for ecologists (Fry 2006). Carbon isotope ratios change 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

* Corresponding author: gway@uw.edu

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 (SJA), 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 SJA (Fig. 1) were selected to maximize an a priori hypothesized natural gradient of influence of freshwater (terrestrial origin) inputs (at the northernmost site, Skipjack Island [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 unpubl.). 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.

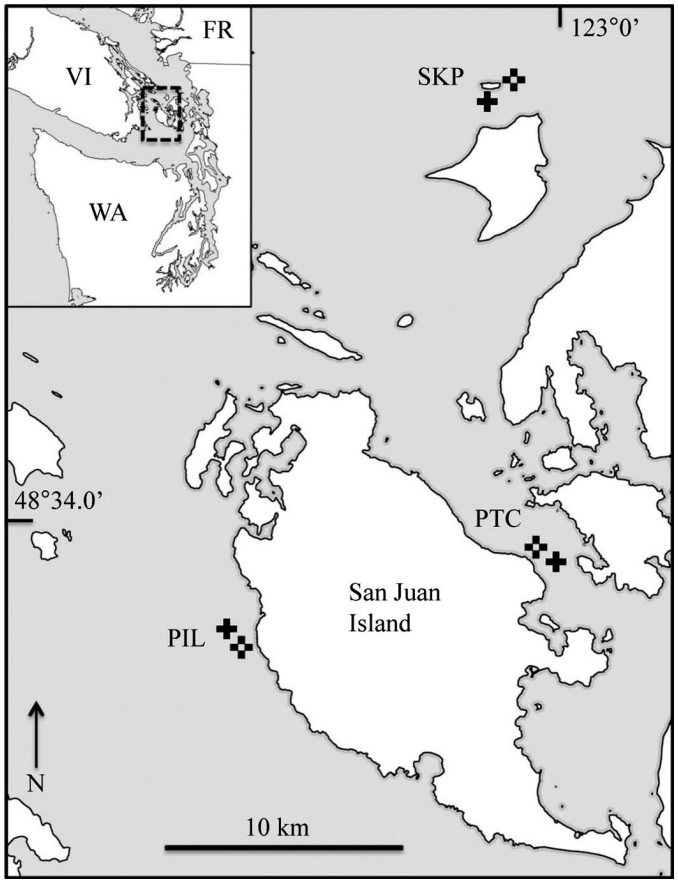

Fig. 1. Area map showing the western SJA in Washington (WA) and the three core study sites where samples were collected for FA and stable isotope comparisons. Deep and shallow collection areas at each site are indicated by filled-in and open crosshairs, respectively. The study sites cover a gradient of exposure to terrestrial freshwater inputs from the Fraser River (FR) to the north (SKP) and the higher-salinity open ocean to the far west (PIL) with the intermediate PTC.

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 3–10 whole individuals of each within 6 h 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 seven taxa from all sites and depths (Table 1), and most (36 of 42) analyses had five site and depth replicates.

Because of logistical constraints, we extracted FA from only five of the seven taxa studied with MSI, representing each of the dominant feeding modes in our list (*see* Table 1). Within 6 months, all animals were thawed and dissected, and muscle tissue was removed, lyophilized for

Table 1. Consumers sampled, commonly known higher order group classification, primary trophic role, feeding mechanism, analyses performed, and number of FA (*n*FA) variables averaging > 0.1% of the total FA for each consumer.

Genus species	Group name	Role	Feeding mechanism	Analyses	*n*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

48 h, and ground with acetone prewashed stainless steel mortars 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 northeast 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 (e.g., phenolic) chemical defenses, with *Agarum* having substantially higher content than *Saccharina* (Van Alstyne et al. 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). 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 ($-20^\circ C$) for later analysis (< 6 months). We extracted FA from the four replicate samples from each species and age (1 or 5 weeks).

FA extraction, methylation, and quantification—FA extractions were performed using a modified Folch method (Taipale et al. 2011). Ten milligrams of lyophilized tissue sample was weighed into a culture tube. Two milliliters of chloroform was 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 min, vortexed for 30 s, and finally centrifuged for 3 min at 3000 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 the layers were dry, 1 mL of toluene and 2 mL of a 1% solution of sulfuric acid in methanol were added to resuspend the lipids. This mixture was vortexed to ensure uniformity, and then placed into a $50^\circ C$ water bath for 16 h to allow for methylation of the FA. Once the mixture was 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 min 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 min 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 that had co-eluted or that 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* "*n*FA" in Table 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 1). We transformed each species's FA dataset using the log-ratio ($\ln(x/18:0)$) transformation, which has been recommended particularly for proportional FA data (Iverson et al. 2002). Nonmetric multidimensional scaling (NMDS) plots are used to visualize multivariate FA patterns and box plots are presented for each stable isotope 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 one-way analyses on the factor depth. We also performed two-way PERMANOVAs (for both FA and MSI) to investigate site $\times$ 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 one-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 one-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 was $<$ 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 and associated two-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, ω-3 FA, ω-6 FA, highly unsaturated FA (HUFA; e.g., FA with $\geq$ 20 C and $\geq$ 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.

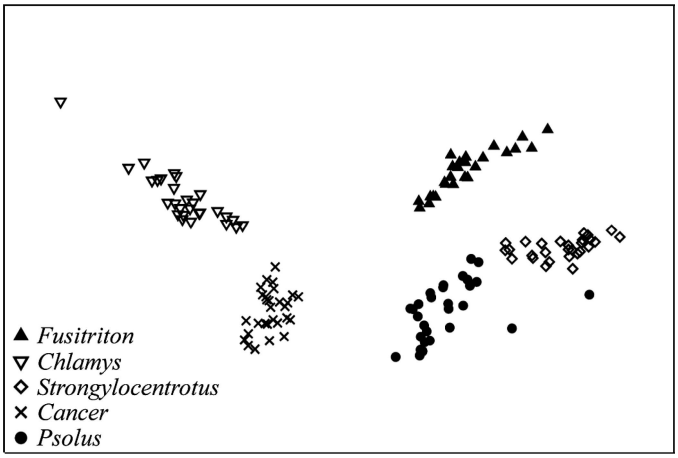

Fig. 2. NMDS plot of FA signatures of five species studied (54 FA untransformed) showing taxonomic distinctness of different invertebrates regardless of site or depth. The plot was generated using Euclidean distance, two-dimensional (2D) stress = 0.06.

Results

Species-specific FA and MSI signatures—An initial PERMANOVA analysis of the factor species (using the full 54 FA dataset for five species) and associated NMDS plot (Fig. 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 because of 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 five separate FA datasets (*see* Web Appendix, Tables A1–A5, www.aslo.org/lo/toc/vol_58/issue_4/1451a.html) with only the most important FA for a given species (*see* *n*FA for each species in Table 1). Across-species MSI signatures were also different (PERMANOVA, pseudo-$F_6 = 53.68$, $p = 0.0001$; not illustrated with NMDS).

Within-consumer FA signatures across depths—When replicates from all sites were pooled, FA composition differed across depths for all five species (PERMANOVA, $p < 0.005$; Table 2). NMDS plots present visual comparisons of the multivariate FA signatures for each species across depths (Fig. 3a–e). Collection sites are indicated as a factor in the Fig. 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). The PUFA 20:5ω3 (EPA) was an exception to this; it was the only FA that in all species was ranked in the top five FA for

Table 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, and use unrestricted permutation of the raw data with 9999 permutations (perms). MSI PERMANOVAs are run on untransformed, normalized MSI data. MS = expected mean squares.

	FA				MSI			
Species and 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				

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 (e.g., 10 FA in the green urchin *Strongylocentrotus droebachiensis* [Echinoidea], versus only 3 in the scallop *Chlamys hastata* [Bivalvia]; Table 3), and appears to be unrelated to feeding mode of the organism (e.g., three FA in *Chlamys* and nine in the sea cucumber *Psolus chitonoides* [Holothuroidea], both suspension feeders).

Two-way PERMANOVAs (site and depth as factors) found significant ($p < 0.05$) site × 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 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 4). Thus, FA differentiate taxa across depths in multivariate space despite the site interactions (*see* groupings of deep and shallow samples in Fig. 3a–e). The direction of the interaction across sites is generally consistent (Fig. 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, $t = 3.45$, df = 26, $p = 0.0019$; PUFA: *t*-test, $t = 2.39$, df = 26, $p = 0.0246$) and *Psolus* (FAME: *t*-test, $t = 2.38$, df = 27, $p = 0.0246$; PUFA: *t*-test, $t = 2.09$, df = 27, $p = 0.0462$), but not other organisms (Table 5). Total ω-3 FA were significantly lower in deep *Chlamys* (*t*-test, $t = 2.28$, df = 26, $p = 0.0309$), *Psolus* (*t*-test, $t = 2.36$, df = 27, $p = 0.0256$), and *Strongylocentrotus* (*t*-test, $t = 2.58$, 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

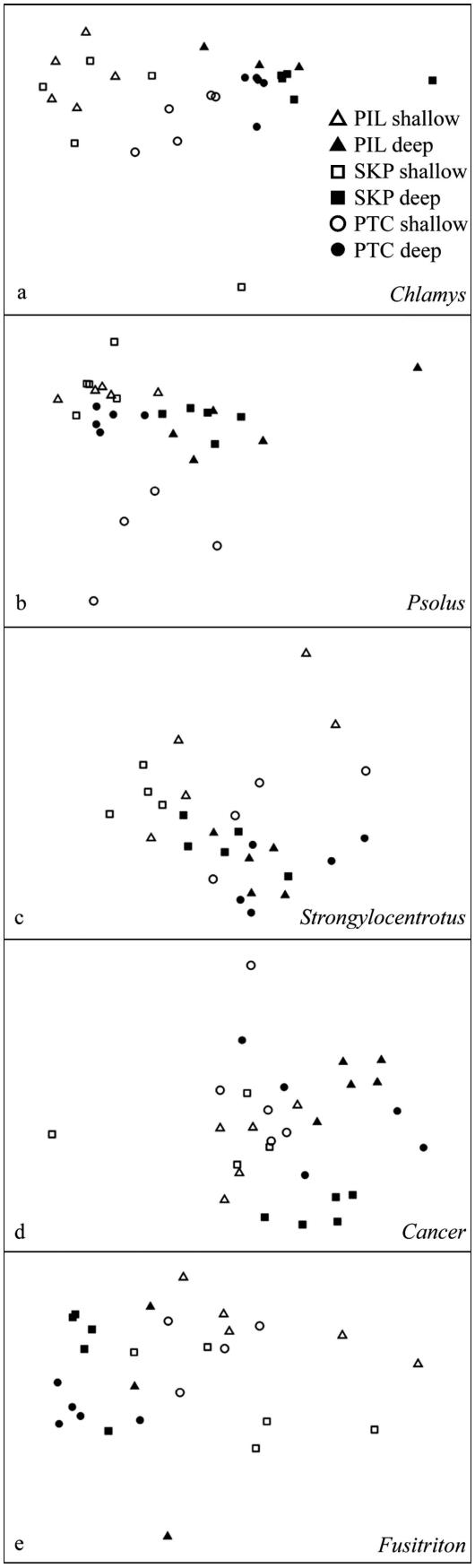

Fig. 3. NMDS plots of the five species studied, coded to show factors of site and depth. All plots are generated from Euclidean resemblance matrices on log-ratio–transformed FA datasets reduced to only the FA that contribute > 0.1% of total FA across samples within that species (*see* Methods). The species (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).

their tissues compared with deep conspecifics (*Chlamys*: *t*-test, $t = 3.17$, df = 26, $p = 0.0038$; *Psolus*: *t*-test, $t = 2.95$, df = 27, $p = 0.0066$), whereas the deep predators had more total bacterial marker FA than shallow (*Cancer*: *t*-test, $t = -2.29$, df = 27, $p = 0.0299$; *Fusitriton*: *t*-test, $t = -2.48$, df = 25, $p = 0.0204$; Table 5).

Within-consumer MSI signatures across depths—MSI signatures differed across depths for six of the seven taxa tested (PERMANOVAs, $p < 0.005$; Table 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. 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. 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. 4a,b). $\delta^{34}S$ differed across depths for only *Cancer* (*t*-test, $t = 3.01$, df = 27, $p = 0.0057$; Fig. 4c). $\delta^{34}S$ values were highly variable in shallow *Psolus* and deep *Strongylocentrotus* (Fig. 4c). A summary of the average (± SD) MSI data at each depth and site is presented in the Web Appendix, Table A6.

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 ω-3 FA and FAME g^{-1} of tissue declined significantly with time (ω-3 FA; *t*-test, $t = -3.24$, df = 6, $p = 0.0178$; Fig. 5; FAME: *t*-test, $t = -3.55$, df = 6, $p = 0.0121$) but differences were not statistically significant for the increase in bacterial FA (*t*-test, $t = 2.32$, df = 6, $p = 0.0594$; Fig. 5). Total ω-3 FA and bacterial FA did not change in *Agarum* (Fig. 5), but total FAME did decline through time (*t*-test, $t = -3.55$, df = 6, $p = 0.0121$).

Discussion

Both FA and MSI differed between shallow and deep habitats for a diverse assemblage of organisms (Mollusca,

Table 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 is listed (nFAΣ > 90%), followed by the mean FA proportion of up to the top five contributing FA in the raw samples. The final columns show the percentage 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	nFAΣ > 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	−

Arthropoda, Echinodermata, Brachiopoda) that use a range of feeding modes (suspension feeders, omnivores, herbivores, predators). The pattern of enrichment in δ^{13}C and δ^{15}N from shallow to deep animals is clearly discernible despite generally significant site-level differences (with exception of the predator δ^{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 δ^{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 δ^{13}C signatures with depth may depend upon local dynamics of primary production and the composition of the seston; for all seven invertebrates studied here, δ^{13}C ratios were more enriched with depth, whereas in offshore habitats, others have reported depletion in consumer δ^{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.

Table 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 (MC) simulations for these comparisons because several of the comparisons had less than 100 unique permutations (Anderson et al. 2008).

	Site p(MC)		
Species	PIL	SKP	PTC
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

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. 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 (e.g., 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

Table 5. Summary of univariate FA depth comparisons, with replicates ($n \sim 15$ per category) pooled across three sites. Full species names are in Table 1; data are average (Avg; ± SD) sums (mg FAME g^{-1} of dry tissue). *See* Methods for category abbreviations. *p* values are results of two-sample *F*-tests.

Category (mg g^{-1} tissue) and depth	*Chlamys*			*Psolus*			*Strongylocentrotus*			*Cancer*			*Fusitriton*		
	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	

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 nonexclusive 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 (e.g., 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). Although 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 because of changes in their surroundings. For example, Britton-Simmons et al. (2009) found differences between percentage cover and composition of shallow (e.g., 10 m: dominated by macroalgae) and deeper (e.g., 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 (e.g., 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 lampshell *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 because of 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

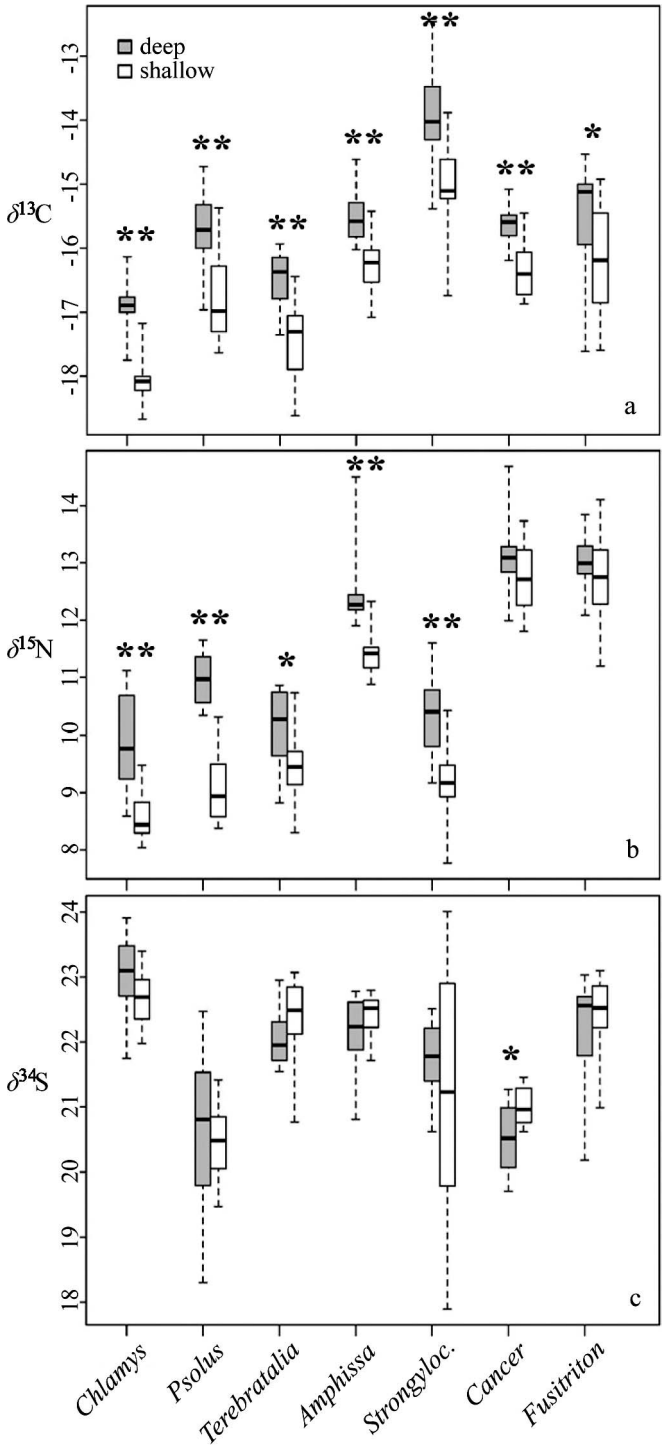

Fig. 4. Box and whisker plots of MSI (a: δ^{13}C, b: δ^{15}N, c: δ^{34}S) data for each of the seven species included in MSI analyses (*see* Table 1), pooled across all three study sites. Grey-shaded boxes are animals collected deep (100 m) and white boxes are shallow (15 m). Heavy line in each box is the mean, box edges are the quartiles, and whiskers extend to minimum and maximum values measured. Significance levels of two-sample *t*-tests are indicated (* $p < 0.05$, ** $p < 0.001$).

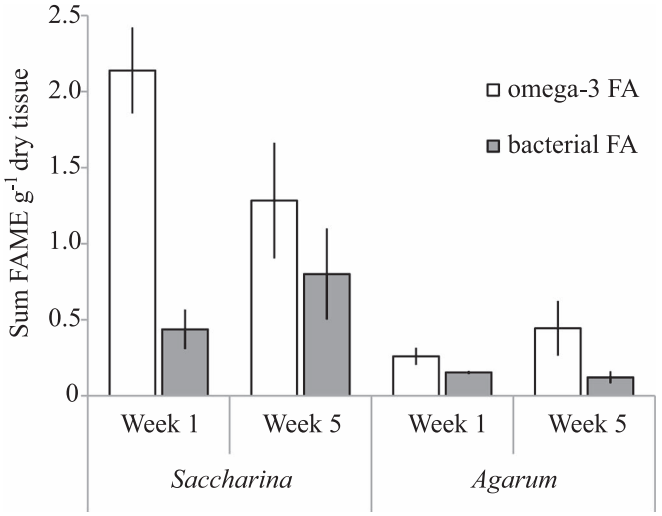

Fig. 5. Total average (± SD) FA weight (mg FAME g^{-1} algal tissue dry weight) of two summary categories (ω-3 FA, open bars; bacterial FA, grey-shaded bars) in 5-week algal aging experiment. Algae are *Saccharina subsimplex* and *Agarum fimbriatum*. In *Saccharina*, ω-3 FA (*t*-test, $t = -3.24$, df = 6, $p = 0.0178$) declined through time, but differences were not statistically significant for bacterial FA (*t*-test, $t = 2.32$, df = 6, $p = 0.0594$). These summary FA categories did not differ through time in *Agarum*.

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) δ^{13}C signatures of -15.9 ± 3.7 ($n = 75$ replicates across four kelp taxa, multiple seasons, and the same three sites) or seagrasses (e.g., *Zostera* [Anthophyta]) that have δ^{13}C signatures of -10.9 ± 1.9 ($n = 20$ replicates in two seasons at the same three 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 δ^{13}C in deep suspension feeders is of the same magnitude as the enrichment in *Strongylocentrotus*, a direct herbivore (Fig. 4c). A key limitation of the strictly trophic explanation for the differences in biomarkers between depths is the observed directional consistency (e.g., δ^{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 that temperatures do not differ greatly between these depths (D. O. Duggins unpubl.) because the subtidal water column is so well mixed

because of tidal currents and complex bathymetry; and if temperatures do differ, the scale of the difference is very small compared to interannual (seasonal) and diel (e.g., tidal mixing of Fraser River freshwater lens) temperature ranges recorded at the same three 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 unpubl.). 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 ω-3 FA in deep animals is consistent with the changes observed in aging algae. δ^{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 δ^{13}C and δ^{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 ω-3 FA, and a trend of increasing bacterial marker FA with age (Fig. 5). No decline in ω-3 FA was found in the chemically defended *Agarum*. Sosik (2012) and E. A. Sosik and C. A. Simenstad (unpubl.) also found that δ^{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 ω-3 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 ω-3 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 ω-3 FA over time. If microbes are responsible for the ω-3 declines in the aged, non-defended alga in our experiment (e.g., through selective consumption and catabolism of ω-3 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 (e.g., Budge et al. 2001). The lack of significant differences in *Agarum* total ω-3 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 ω-3 FA (Thiansilakul et al. 2013) that warrants further research.

Some biomarker patterns support a priori expectations, e.g., the enrichment of *Strongylocentrotus* in δ^{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 δ^{15}N found in either predator, *Cancer* or *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 differed in only 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 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*. Although the exact source of this relatively novel FA is unknown, it has also been identified in abyssal holothurians (Drazen et al. 2008).

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, 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 2012*b*), 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 that is often overlooked.

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References

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

———, R. N. Gorley, and K. R. Clarke. 2008. PERMANOVA+ for PRIMER: Guide to software and statistical methods. PRIMER-E.

Bell, M. V., and D. R. Tocher. 2009. Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: General pathways and new directions, p. 211–236. *In* M. T. Arts, M. T. Brett, and M. Kainz [eds.], Lipids in aquatic ecosystems. Springer.

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. Aquat. Biol. **5:** 233–243, doi:10.3354/ab00154

———, and others. 2012. Habitat and bathymetry influence the landscape-scale distribution and abundance of drift macrophytes and associated invertebrates. Limnol. Oceanogr. **57:** 176–184, doi:10.4319/lo.2012.57.1.0176

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. Mar. Chem. **76:** 285–303, doi:10.1016/S0304-4203(01)00068-8

———, 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. Can. J. Fish. Aquat. Sci. **59:** 886–898, doi:10.1139/f02-062

———, ———, and H. N. Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: A primer on analysis and interpretation. Mar. Mamm. Sci. **22:** 759–801, doi:10.1111/j.1748-7692.2006.00079.x

Buesseler, K. O., and others. 2007. Revisiting carbon flux through the ocean's twilight zone. Science **316:** 567–570, doi:10.1126/science.1137959

Cabana, G., and J. B. Rasmussen. 1996. Comparison of aquatic food chains use in nitrogen isotopes. Proc. Natl. Acad. Sci. USA **93:** 10844–10847, doi:10.1073/pnas.93.20.10844

Cebrian, J. 1999. Patterns in the fate of production in plant communities. Am. Nat. **154:** 449–468, doi:10.1086/303244

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. J. Mar. Syst. **74:** 372–380, doi:10.1016/j.jmarsys.2008.02.004

Cranford, P. J., and J. Grant. 1990. Particle clearance and absorption of phytoplankton and detritus by the sea scallop *Placopecten magellanicus* (Gmelin). J. Exp. Mar. Biol. Ecol. **137:** 105–121, doi:10.1016/0022-0981(90)90064-J

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. Mar. Ecol. Prog. Ser. **478:** 1–14, doi:10.3354/meps10310

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. Comp. Biochem. Physiol. B **151:** 79–87, doi:10.1016/j.cbpb.2008.05.013

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, doi:10.1126/science.245.4914.170

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. Mar. Biol. **93:** 615–625, doi:10.1007/BF00392799

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. Mar. Ecol. Prog. Ser. **265:** 45–56, doi:10.3354/meps265045

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. J. Exp. Mar. Biol. Ecol. **212:** 250–275, doi:10.1016/S0022-0981(96)02740-2

Fry, B. 1988. Food web structure on Georges Bank from stable C, N, and S isotopic compositions. Limnol. Oceanogr. **33:** 1182–1190, doi:10.4319/lo.1988.33.5.1182

———. 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. J. Phycol. **48:** 956–965, doi:10.1111/j.1529-8817.2012.01173.x

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. Mar. Ecol. Prog. Ser. **410:** 25–41, doi:10.3354/meps08649

Hall, D., S. Y. Lee, and T. Meziane. 2006. Fatty acids as trophic tracers in an experimental estuarine food chain: Tracer transfer. J. Exp. Mar. Biol. Ecol. **336:** 42–53, doi:10.1016/j.jembe.2006.04.004

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. Prog. Oceanogr. **43:** 205–234, doi:10.1016/S0079-6611(99)00015-4

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. Mar. Ecol. Prog. Ser. **351:** 65–76, doi:10.3354/meps07120

Iverson, S. J. 2009. Tracing aquatic food webs using fatty acids: From qualitative indicators to quantitative determination, p. 281–307. *In* M. T. Arts, M. T. Brett, and M. J. Kainz [eds.], Lipids in aquatic ecosystems. Springer.

———, C. Field, W. D. Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: A new method of estimating predator diets. Ecol. Monogr. **74:** 211–235, doi:10.1890/02-4105

———, 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. Mar. Ecol. Prog. Ser. **241:** 161–181, doi:10.3354/meps241161

Kelly, J. R., and R. E. Scheibling. 2012. Fatty acids as dietary tracers in benthic food webs. Mar. Ecol. Prog. Ser. **446:** 1–22, doi:10.3354/meps09559

———, ———, S. J. Iverson, and P. Gagnon. 2008. Fatty acid profiles in the gonads of the sea urchin *Strongylocentrotus droebachiensis* on natural algal diets. Mar. Ecol. Prog. Ser. **373:** 1–9, doi:10.3354/meps07746

Kiørboe, T., H. P. Grossart, H. Ploug, and K. Tang. 2002. Mechanisms and rates of bacterial colonization of sinking aggregates. Appl. Environ. Microbiol. **68:** 3996–4006, doi:10.1128/AEM.68.8.3996-4006.2002

Krumhansl, K. A., and R. E. Scheibling. 2012*a*. Production and fate of kelp detritus. Mar. Ecol. Prog. Ser. **467:** 281–302, doi:10.3354/meps09940

———, and ———. 2012*b*. Detrital subsidy from subtidal kelp beds is altered by the invasive green alga *Codium fragile* ssp. *fragile*. Mar. Ecol. Prog. Ser. **456:** 73–85, doi:10.3354/meps09671

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 Biol. **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. Mar. Ecol. Prog. Ser. **315:** 1–11, doi:10.3354/meps315001

McArthur, J. M., R. V. Tyson, J. Thomson, and D. Mattey. 1992. Early diagenesis of marine organic matter: Alteration of the carbon isotopic composition. Mar. Geol. **105:** 51–61, doi:10.1016/0025-3227(92)90181-G

Miller, R. J., and H. M. Page. 2012. Kelp as a trophic resource for marine suspension feeders: A review of isotope-based evidence. Mar. Biol. **159:** 1391–1402, doi:10.1007/s00227-012-1929-2

———, ———, and M. A. Brzezinski. 2013. δ^{13}C and δ^{15}N of particulate organic matter in the Santa Barbara Channel: Drivers and implications for trophic inference. Mar. Ecol. Prog. Ser. **474:** 53–66, doi:10.3354/meps10098

Mintenbeck, K., U. Jacob, R. Knust, W. E. Arntz, and T. Brey. 2007. Depth-dependence in stable isotope ratio of δ^{15}N of benthic POM consumers: The role of particle dynamics and organism trophic guild. Deep-Sea Res. I **54:** 1015–1023, doi:10.1016/j.dsr.2007.03.005

Müller-Navarra, D. C. 2008. Food web paradigms: The biochemical view on trophic interactions. Int. Rev. Hydrobiol. **93:** 489–505, doi:10.1002/iroh.200711046

Nadon, M. O., and J. H. Himmelman. 2006. Stable isotopes in subtidal food webs: Have enriched carbon ratios in benthic consumers been misinterpreted. Limnol. Oceanogr. **51:** 2828–2836, doi:10.4319/lo.2006.51.6.2828

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. Estuar. Coast. Shelf Sci. **96:** 228–235, doi:10.1016/j.ecss.2011.11.004

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*). Mar. Ecol. Prog. Ser. **2:** 45–59, doi:10.3354/meps002045

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. J. Exp. Biol. **210:** 2999–3014, doi:10.1242/jeb.006007

Peterson, B. J. 1999. Stable isotopes as tracers of organic matter input and transfer in benthic food webs: A review. Acta Oecol. **20:** 479–487, doi:10.1016/S1146-609X(99)00120-4

———, and B. Fry. 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. **18:** 293–320, doi:10.1146/annurev.es.18.110187.001453

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. Annu. Rev. Ecol. Syst. **28:** 289–316, doi:10.1146/annurev.ecolsys.28.1.289

Ramirez-Llodra, E., and others. 2010. Deep, diverse and definitely different: Unique attributes of the world's largest ecosystem. Biogeosciences **7:** 2851–2899, doi:10.5194/bg-7-2851-2010

Sebert, P., M. Theron, and A. Vettier. 2004. Pressure and temperature interactions on cellular respiration: A review. Cell. Mol. Biol. **50:** 491–500.

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

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, doi:10.1111/j.1600-0706.2011.19415.x

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 Sci. Technol. **50:** 146–152.

Van Alstyne, K. L. V., J. J. McCarthy. III, C. L. Hustead, and L. J. Kearns. 1999. Phlorotannin allocation among tissues of Northeastern Pacific kelps and rockweeds. J. Phycol. **35:** 483–492, doi:10.1046/j.1529-8817.1999.3530483.x

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. Front. Ecol. Environ. **10:** 409–415, doi:10.1890/110176

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