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Sea otter effects on trophic structure of seagrass communities in southeast Alaska

Wendel W. Raymond[1,3,*], Julie B. Schram[2,4], Ginny L. Eckert[1], Aaron W. E. Galloway[2]

[1]College of Fisheries and Ocean Sciences, University of Alaska Fairbanks, 17101 Point Lena Loop Rd, Juneau, AK 99801, USA
[2]Oregon Institute of Marine Biology, University of Oregon, 63466 Boat Basin Rd, Charleston, OR 97420, USA

[3]*Present address:* Friday Harbor Laboratories, University of Washington, Friday Harbor, WA 98250, USA
[4]*Present address:* University of Alaska Southeast, 11066 Auke Lake Way, Juneau, AK 99801, USA

ABSTRACT: Previous research in southeast Alaska on the effects of sea otters *Enhydra lutris* in seagrass *Zostera marina* communities identified many but not all of the trophic relationships that were predicted by a sea otter-mediated trophic cascade. To further resolve these trophic connections, we compared biomass, carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope (SI), and fatty acid (FA) data from 16 taxa at 3 sites with high and 3 sites with low sea otter density (8.2 and 0.1 sea otters km^{-2}, respectively). We found lower crab and clam biomass in the high sea otter region but did not detect a difference in biomass of other seagrass community taxa or the overall community isotopic niche space between sea otter regions. Only staghorn sculpin differed in $\delta^{13}C$ between regions, and *Fucus*, sugar kelp, butter clams, dock shrimp, and shiner perch differed in $\delta^{15}N$. FA analysis indicated multivariate dissimilarity in 11 of the 15 conspecifics between sea otter regions. FA analysis found essential FAs, which consumers must obtain from their diet, including 20:5ω3 (EPA) and 22:6ω3 (DHA), were common in discriminating conspecifics between sea otter regions, suggesting differences in consumer diets. Further FA analysis indicated that many consumers rely on diverse diets, regardless of sea otter region, potentially buffering these consumers from sea otter-mediated changes to diet availability. While sea otters are major consumers in this system, further studies are needed to understand the mechanisms responsible for the differences in biomarkers between regions with and without sea otters.

KEY WORDS: Food web · Fatty acid · Stable isotope · Apex predator · Trophic cascade

1. INTRODUCTION

Seagrass ecosystems support diverse communities that are considered to be largely structured by top-down forces (Duffy et al. 2014). The consumption of seagrass epiphytes by invertebrate epifauna is, on average, stronger than the bottom-up forces of nutrients, leading to positive indirect effects of herbivores to seagrass (Hughes et al. 2004, Valentine & Duffy 2006, Heck & Valentine 2007). In communities of the seagrass *Zostera marina*, the presence of top-down forcing from invertebrate epifauna appears relatively consistent around the world but can vary in strength and with epifauna community composition, suggesting at least some commonality in structuring forces regardless of geography (Duffy et al. 2015). Such strong interactions are not limited to lower trophic levels. Invertebrate epifauna may be consumed by mesopredators, such as fishes and crabs, which can modulate their top-down effect on epiphytes (Duffy et al. 2005, Douglass et al. 2007, Lewis & Anderson 2012). In systems with dominant apex predators, yet another level of trophic control can be added. Apex predators can regulate mesopredator abundance,

*Corresponding author: wraymond@uw.edu

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which can cascade down the food web and lead to overall positive indirect effects from apex predators to seagrass (Moksnes et al. 2008, Baden et al. 2010, Hughes et al. 2013). Previous inferences on top-down control in seagrass food webs have usually relied on relative biomass or abundance comparisons to describe these trophic controls. While it is an intuitive and time-tested metric of trophic ecology, biomass may mask weak interactions among seagrass-associated taxa, therefore skewing our perspective of trophic structure.

Trophic biomarkers such as stable isotopes (SIs) and fatty acids (FAs) can provide an alternative perspective on trophic structure by focusing on energy transfer from primary producers to consumers. Biomarkers can reflect the diet of a consumer and can reveal ecosystem dynamics not necessarily evident from biomass measures or abundance data alone. Investigations into the trophic structure of communities can use trophic biomarkers to assess the dietary resources and trophic level of a given species or a community at large (Peterson et al. 1985, Peterson 1999, Dalsgaard et al. 2003, Kelly & Scheibling 2012). For example, the combination of SI and biomass data from eutrophic and non-eutrophic seagrass communities showed both a difference in biomass of some primary producers and consumers and a difference in overall trophic structure as measured by isotopic niche space (Thormar et al. 2016). The ratio of the stable isotopes of carbon, ^{13}C to ^{12}C, is commonly used to reflect the ultimate energy source of consumers at a coarse resolution and is assumed to change little with trophic level (Peterson & Fry 1987). Conversely, the ratio of the stable isotopes of nitrogen, ^{15}N to ^{14}N, increases with each level of consumption, making it a useful measure of the relative trophic position of species and food chain length (Cabana & Rasmussen 1996, Layman et al. 2007).

FAs are particularly informative biomarkers in benthic aquatic ecosystems because many aquatic primary producers have distinct FA signatures (Galloway et al. 2012, Kelly & Scheibling 2012, Galloway & Winder 2015). Certain FAs, including certain ω-3 polyunsaturated FAs (PUFAs) are only synthesized de novo in biologically relevant amounts by primary producers, and consumers must obtain them from their diet (Brett & Müller-Navarra 1997, Kelly & Scheibling 2012). Therefore, these and other 'essential' FAs (EFAs) can serve as dietary tracers in benthic food webs and can help reveal trophic relationships that may not be apparent by biomass comparisons alone. Ecosystem-scale availability of long-chain EFAs (LCEFAs), including eicosapentaenoic acid (20:5ω3; EPA) and docosahexaenoic acid (22:6ω3; DHA), can be tightly linked to growth and survival of marine fishes and have been used as indicators of trophic structure in marine ecosystems (Müller-Navarra et al. 2000, Litzow et al. 2006, Budge et al. 2014). Furthermore, many C_{18} PUFAs are found in relatively high proportions in benthic sources of primary production, such as seagrasses and brown and green algae (Galloway et al. 2012). The tight link of specific biomarker FAs and EFAs to certain primary producers can provide taxonomic detail on the diets of consumers not possible with SIs. The combination of relatively coarse-resolution SI and finer-resolution FA biomarkers in the same study can build a more comprehensive understanding of energetic pathways, especially in systems with many possible food sources (e.g. Jankowska et al. 2018). This approach has been used in a variety of ecosystems, including nearshore planktonic communities (Lowe et al. 2014), nearshore suspension-feeder diets (Allan et al. 2010), kelp-dominated benthic communities (Galloway et al. 2013), and seagrass communities (references in the next paragraph).

SI and FA trophic biomarkers have been used to investigate the trophic structure of seagrass communities in a variety of regions. These studies highlight food web complexity, where many consumers show evidence of diverse ultimate energy sources, including macroalgae, epiphytes, and bacteria (Kharlamenko et al. 2001, Alfaro et al. 2006, Jaschinski et al. 2008, 2011, Jephson et al. 2008, Douglass et al. 2011, Thormar et al. 2016, Jankowska et al. 2018). Both SI and FA data support the top-down control hypothesis that epifauna are important for controlling epiphytic and ephemeral macroalgae, and that seagrass biomarkers are only found in very small amounts or not at all in epifauna or higher consumers (Alfaro et al. 2006, Jaschinski et al. 2008, 2011, Jephson et al. 2008, Thormar et al. 2016, Jankowska et al. 2018). SI analyses on entire seagrass communities indicate that trophic structure can vary with abiotic conditions (Thormar et al. 2016) or top-down forces (Jephson et al. 2008), and that mesopredators, such as crabs and fishes, often consume a diverse diet (Douglass et al. 2011). Many common seagrass community consumers, such as gastropods, bivalves, and crabs, have high proportions of FAs that are common in bacteria, suggesting consumption of detrital food sources (Kharlamenko et al. 2001, Alfaro et al. 2006, Jankowska et al. 2018). Taken together, these biomarker studies indicate that the trophic structure of seagrass communities may be affected by top-down control, bottom-up control, or a combination of the two. Given the complexity in dietary sources and structuring forces, any single met-

ric of trophic structure may inadvertently omit underlying trophic pathways and food sources essential for the functioning of seagrass communities.

The role of higher order predators in structuring seagrass communities has recently gained attention. Hughes et al. (2013) documented how an increasing population of sea otters *Enhydra lutris* in Elkhorn Slough, CA, triggered cascading top-down effects that altered seagrass community structure. Other top predators in seagrass communities, such as cod *Gadus morhua* in the northeast Atlantic, can also drive trophic cascades (Jephson et al. 2008, Moksnes et al. 2008, Baden et al. 2010, 2012). These studies describe how, when apex predators returned to eutrophic seagrass ecosystems, their predation pressure reduced mesopredator abundance, releasing seagrass epifauna that were then able to graze off harmful epiphytes from seagrass, leading to increased seagrass biomass. Raymond et al. (2021) tested the generality of these patterns in southeast Alaska using a natural experiment of varying sea otter presence. Using biomass data, Raymond et al. (2021) found a positive correlation between sea otters and seagrass (Pearson correlations) and evidence of the predicted direct relationships among epifauna, epiphytes, seagrass, and nitrate (linear regression). However, Raymond et al. (2021) did not find evidence of a relationship between crabs or fish and epifauna, an essential step in the trophic cascade described in Elkhorn Slough and the northeast Atlantic which links higher order consumers to epifauna, seagrass epiphytes, and seagrass. These results suggest that while some of the forces present in the linear trophic cascade are present at some level, other relationships such as changes in diet may also be present in southeast Alaska seagrass communities that may influence community and trophic structure and could be revealed by SI and FA analysis.

Here, we used a suite of metrics to describe and compare the trophic structure of seagrass *Z. marina* communities in southeast Alaska in regions of high and low sea otter density. This approach allowed us to quantify the seagrass community trophic structure from both the species abundance (biomass) and energetic pathway (biomarkers) perspectives. Specifically, we evaluated whether biomass, SIs of carbon (^{13}C) and nitrogen (^{15}N), whole FA profiles, and specific classes of FAs of the primary producers and consumers differed within conspecifics between regions of high and low sea otter density. Biomass data provided a classic ecological metric to compare to previous studies (Raymond et al. 2021) and to contextualize the biomarker data. SI and FA data were used to compare the primary carbon sources, relative trophic level, food chain length, and primary dietary sources of conspecifics.

Our overall goal was to compare trophic structure between high and low sea otter regions by directly comparing the biomass, SIs, and FAs of conspecifics. While diet can be inferred from these data, our goal was not to estimate or model diets of all of the consumers in the system. We hypothesized that differences in conspecific biomass would follow results from Raymond et al. (2021) and from other studies on the effect of sea otters on clam and crab communities (Kvitek et al. 1992, Hughes et al. 2013, Hoyt 2015), where sea otters have a positive relationship with seagrass and a negative relationship with clam and crab biomass. Following the sea otter–seagrass trophic cascade hypothesis discussed above, we expected the negative effect of sea otters on crabs to cascade down the food web where a reduction in crab biomass leads to increased epifauna biomass, decreased epiphyte biomass, and increased seagrass biomass. We hypothesized that conspecific carbon (^{13}C) and nitrogen (^{15}N) SI and FA values would not differ between sea otter regions because we did not expect sea otters to drive variation in primary producer biomarkers. We focused our FA analyses on EFAs because of the importance of these molecules in marine ecosystems. We asked if sea otters alter the EFA equilibrium in seagrass communities. We hypothesized that if sea otters did have an effect on EFAs, this would be evident in differences within conspecifics between high and low sea otter density regions. Alternatively, if we did observe conspecific differences in SI, FA, and EFA values, we hypothesized that 3 factors could lead to these differences. (1) Conspecific primary producer biomarkers may differ between sea otter regions, leading to differences in consumers. (2) The presence of sea otters could alter the diet of consumer conspecifics between regions. For example, if sea otters greatly reduce clam abundance, that may reduce or eliminate that food source to other consumers such as crabs. (3) Any differences could be the result of natural variability in conspecific biomarkers as a result of variability in individual diets.

2. MATERIALS AND METHODS

2.1. Study area

Our study took place on the west coast of Prince of Wales Island in southern southeast Alaska, USA (Fig. 1). Southeast Alaska contains over 10 000 km of seagrass shoreline (Harper & Morris 2004, NOAA

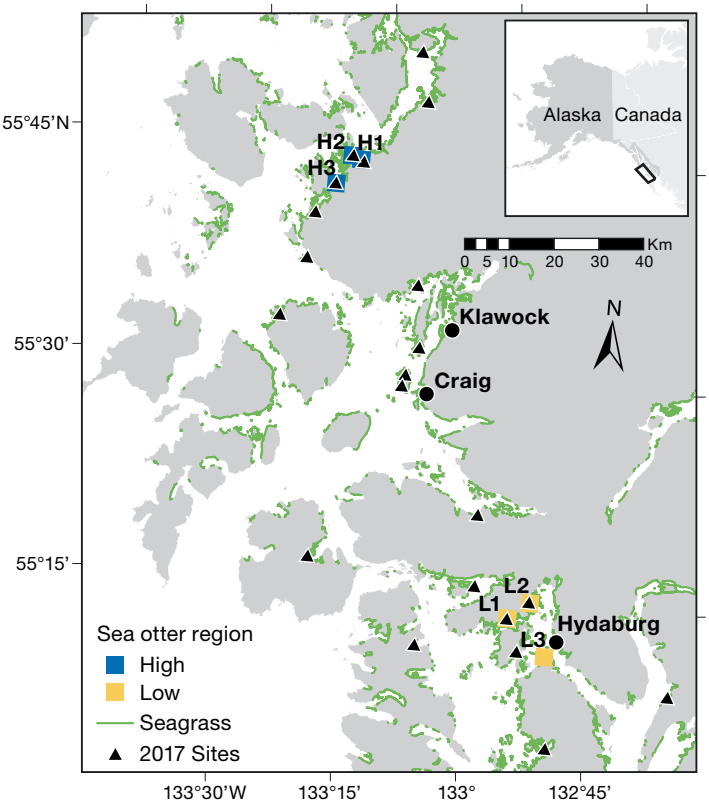

Fig. 1. Study area, including sites sampled for this study in high (blue) and low (gold) sea otter density regions and seagrass shoreline (Harper & Morris 2004, NOAA 2019). Locations of additional environmental data collected in 2017 are from Raymond et al. (2021) (triangles)

2019), primarily in protected, soft-sediment shorelines composed of seagrass *Zostera marina*, surfgrass *Phyllospadix serrulatus*, and a mixture of the two (Stephens et al. 2019). Seagrass beds in the region are often found in close proximity to other habitats, such as canopy and understory kelp forests, sand flats, and estuaries, creating a mosaic of nearshore habitats (O'Clair et al. 1997, NOAA 2019). Southeast Alaska is home to a large sea otter population which, after near extinction from the maritime fur trade, has expanded to an estimated population of over 25 000 individuals (USFWS 2014, Tinker et al. 2019). Sea otters are unevenly distributed across southeast Alaska, including Prince of Wales Island, creating a heterogeneous seascape of sea otter occupation time, abundance, and population status with respect to estimated carrying capacity (USFWS 2014, Tinker et al. 2019). We identified 6 study sites, 3 in a region of high sea otter occupation and 3 in a region of low sea otter occupation (described further in Section 2.2), and all sites had similar seagrass bed size, underlying substrate, and exposure. At each site, we measured the biomass of major seagrass community taxa and collected tissue samples for SI and FA analysis (Fig. 1, Table S1 in the Supplement at www.int-res.com/articles/suppl/m674p037_supp.pdf).

2.2. Sea otter occupation

We used US Fish and Wildlife Service aerial sea otter surveys (USFWS 2014) paired with a recent analysis of these survey data (Tinker et al. 2019) to identify areas of high and low sea otter density. Sea otters have been present in the high region since at least 1975, and it has an estimated sea otter density of 3.663 km^{-2}, which is 89.5% of its estimated carrying capacity (Tinker et al. 2019). The low sea otter region has had sea otters present since at least 1999 and an estimated sea otter density of 0.163 km^{-2}, which is 2.1% of its estimated carrying capacity (Tinker et al. 2019). Due to the coarse spatial resolution of the above sea otter population measures and our personal observations of sea otter movement in the region, we supplemented these data with 2 replicate boat-based sea otter surveys at each site. These surveys were conducted following methodology described in Raymond et al. (2021). Briefly, we counted all sea otters within a 3.4 km radius (over water distance) of each site twice between June and August 2018. Sea otter counts were converted to density based on the total water area surveyed for each site.

2.3. Environmental sampling

We collected environmental parameters on 24 July 2018 at low sea otter sites and on 25 July 2018 at high sea otter sites. We used a YSI Pro2030 meter to measure water temperature (°C), salinity (ppt), and dissolved oxygen (mg l^{-1}) at 1 and 5 m depths. With a LI-COR LI-193R spherical quantum sensor, we measured photosynthetically active radiation (PAR; as µmol s^{-1} m^{-2}) at the surface (air) and in 1 and 5 m water depth. We calculated the percent of light transmittance as the percent of PAR measured at 5 m divided by PAR measured at 1 m. We collected 50 ml of seawater at 1 and 5 m depths for nitrate and phosphate concentration analysis. Seawater was immediately filtered through a 0.4 µm Whatman GF/F filter into sample vials and then frozen at −20°C for approximately 1 mo and then at −80°C until nutrient analyses were conducted on 28 March 2019 using an Astoria Pacific Analyzer at the University of Alaska Southeast. We also compiled environmental data

from previous research in the region in similar seagrass habitats to compare to our data from this study (Raymond et al. 2021). These data consisted of samples from 21 sites collected from 28 April to 22 August 2017 and covered the same parameters measured for this study. The data are divided into 2 types: (1) samples collected at all 21 sites during seagrass community sampling for Raymond et al. (2021) which span the entire date range and ~100 km of shoreline; and (2) samples collected at all 21 sites during a coordinated sampling effort on 14 August 2017 that was aimed to capture spatial patterns in environmental parameters. Locations and type of environmental sampling are summarized in Fig. 1.

2.4. Biomass

In July 2018, we surveyed the seagrass community at each site, recording biomass measurements of seagrass, seagrass epiphytes, seagrass-associated invertebrate epifauna, clams, crabs, and fishes (Tables 1 & S1). Sampling of seagrass and associated epiphytes and epifauna followed similar published methods (Hughes et al. 2013, Raymond et al. 2021). At each site, we placed one 100 m transect in the seagrass meadow at least 5 m linear distance below the upper edge of the continuous seagrass meadow and at least 10 m from a vertical edge of the meadow at approximately −0.6 m mean lower low water (MLLW) tidal elevation (mean annual tidal range: 2.4 m). Along the transect, we counted seagrass shoot density in eight 0.5 × 0.5 m evenly spaced quadrats. We characterized the primary sediment type in each quadrat using a qualitative scoring system ranging from soft to hard substrates (1 = mud, 2 = sandy mud, 3 = muddy sand, 4 = sand, 5 = coarse sand, 6 = pebble, 7 = gravel, 8 = cobble, 9 = boulder, 10 = bedrock). Scores were averaged across the transect at each site. Adjacent to each quadrat, we conducted a grab sample to collect seagrass and associated epifauna. The grab was accomplished by affixing a 400 µm mesh bag measuring approximately 28 × 60 cm to a 0.018 m^2 circular metal ring. The ring and bag were carefully lowered over the seagrass, and the seagrass was cut at the sediment interface. The bag was inverted and brought to the surface, taking care to avoid loss of seagrass or associated grazers. Collection bags were placed in coolers until further processing later that day.

We processed seagrass collection bags in the laboratory to quantify biomass of seagrass, epiphytes, and epifauna. Collection bags were emptied into trays and gently rinsed with fresh water to release epifauna. Epiphytes consisted almost entirely of diatoms (Class Bacillariophyceae), consistent with previous studies in the region (Raymond et al. 2021). Other seagrass epiphytes such as red algae are occasionally observed in the region but only at sites closer

Table 1. Key to names and abbreviations of collected taxa and summary of differences in the presence of sea otters. FA: fatty acid; EFA: essential fatty acid; –: comparison not conducted

	Scientific name	Abbreviation	Biomass	$\delta^{13}C$	$\delta^{15}N$	FA shift	Sum EFA contribution to dissimilarity
Primary producers							
Seagrass	*Zostera marina*	SG				Y	36.5
Seagrass epiphytes	Class Bacillariophyceae	EP			↓	N	18.4
Rockweed	*Fucus distichus*	FU			↑	Y	30.4
Sugar kelp	*Saccharina lattisima*	SK			↑	N	27.3
Sea lettuce	*Ulva* spp.	UL				Y	32.3
Primary consumers							
Seagrass isopod	*Pentidotea rascata*	IDO				Y	27.8
Seagrass limpet	*Lottia pelta*	LMP		–	–	Y	22.2
Butter clam	*Saxidomus giganteus*	BUT	↓		↓	Y	24.1
Macoma clam	*Macoma* spp.	MAC					19.9
Secondary consumers							
Dock shrimp	*Pandalus danae*	DSH			↑	Y	26.9
Helmet crab	*Telmessus cheiragonus*	HEL				Y	41.2
Graceful crab	*Metacarcinus gracilis*	GRC				Y	28.3
Red rock crab	*Cancer productus*	RRC	↓	–	–	–	–
Shiner perch	*Cymatogaster aggregata*	SHN			↑	N	33.5
Snake prickleback	*Lumpenus sagitta*	SNK				Y	40.2
Staghorn sculpin	*Leptocottus armatus*	STG		↓	↑	Y	48.2

to the outer coast (W. W. Raymond unpubl. data). Epiphytes from individual seagrass leaves were collected on pre-dried and weighed cotton pads. Epifauna were grouped into the following taxonomic groups: isopods *Pentidotea resecata*, limpets *Lottia pelta*, and all other epifauna including gammarid amphipods (Suborder Gammaridea), caprellid amphipods (Family Caprellidae), and other gastropods (primarily *Lacuna* sp. and *Littorina* sp.). Seagrass leaves, epiphytes, and epifauna from each grab sample were dried for at least 24 h at 60°C and weighed to the nearest 0.0001 g. For analysis, seagrass leaf mass was converted to biomass per square meter by multiplying biomass from our grab samples by 55.55. Epiphyte and epifauna mass were converted to epiphyte and epifauna load, defined as grams of epiphyte or epifauna per gram of seagrass in each grab sample, and averaged for each site. For analysis, epifauna were grouped as total epifauna load, *Pentidotea* load, and limpet load.

We measured clam abundance, species composition, and biomass by digging eight 0.25 × 0.25 × 0.25 m pits evenly spaced along a 100 m transect at approximately 0 m MLLW at each site (Table S1). Clams and sediment were passed through a 1 cm sieve, and all retained clams were identified to species and measured to the nearest mm. Clam lengths were converted to biomass using species-specific conversion coefficients (Table S2) using Eq. (1), where m is clam width and a and b are conversion factors. Clam density (g m^{-2}) was calculated for each species and for the total clam assemblage and averaged for each site.

$$\text{mass(g)} = a \times m_{\text{taxa}}\,(\text{cm})^{b} \quad (1)$$

We found a variety of clam species but focused our analyses on total clam mass and mass and tissue samples (below) of butter clams *Saxidomus giganteus* and *Macoma* spp. clams, which were the most dominant taxa by biomass (butter clams mean: 30.5 ± 21.4%; *Macoma* spp. clams: 50.5 ± 29.2% by weight across all sites).

We measured crab abundance, species composition, and biomass by setting 4 strings of 2 crab pots each at approximately −3 m MLLW for 24 h at each site (Table S1). Each string consisted of one 61 × 61 × 33 cm pot with 10 × 10 cm wire mesh and four 20 cm openings, and one 'fukui' type pot measuring 60 × 45 × 20 cm with 1 × 1 cm fabric mesh with two 20 cm openings. All pots were baited with approx. 500 g of chopped herring. Upon collection, all crabs were identified to species, and we measured carapace width to the nearest mm. Carapace widths were converted to biomass using species-specific conversion factors (Table S2) using Eq. (1) above, where m is carapace width. Total and species-specific crab biomass were summed for each string and then averaged across the 4 strings at each site.

We measured fish abundance and species composition in terms of numbers and biomass following methods described in Johnson et al. (2012) (Table S1). Fish were captured using a 37 m variable-mesh beach seine. Outer panels were 10 m sections of 32 mm mesh, intermediate panels were 4 m sections of 6 mm mesh, and the center panel was a 9 m section of 3.2 mm mesh. The seine tapered from 5 m tall at the center to 1 m tall at the ends to conform to the shape of the beach slope when set. The seine was set as a round haul by holding one end on the beach while backing around the other end in a small boat to the beach approx. 18 m from the start. Once the seine was pulled onshore the catch was sorted, identified to species, counted, and a subsample (up to 30 fish) of each species was measured to the nearest mm (fork length). For species with more than 30 individuals, we counted all remaining members of that species. These unmeasured fish were assigned lengths in proportion to the size–frequency distribution of measured fish of that species at the same site. Fish lengths were converted to biomass using species-specific length–weight conversions coefficients (Table S2) and Eq. (1) above, where m is fork length. We searched the FishBase (www.fishbase.org) database and other literature for published length–weight conversion coefficients. Species that did not have any published values and/or species that we were only able to identify to genus or family in the field were assigned conversion factors first based on the congeners or, if not available, other species with similar overall body plans (e.g. fusiform). Once all coefficients were compiled, we calculated the mean of each factor for each species, if applicable, and assigned a single a and b (Eq. 1) value to each species.

2.5. Biomarker sampling and processing

SI and FA values can vary across relatively small spatial and time scales (Guest et al. 2010, Dethier et al. 2013); therefore, our tissue sample scheme was designed to (1) collect tissue samples within the smallest time window possible, especially for a given taxon, (2) balance capturing biomarker variability within and among sites (e.g. Galloway et al. 2013), and (3) balance logistical constraints in each sea otter density region. This approach allowed us to control for time and within-region space-induced variation

of taxa in biomarker samples. Our goal was to obtain 2 replicate tissue samples for each taxon from each site in each sea otter region, for a total of 6 tissue samples in each sea otter region and 12 tissue samples for each taxon. For all biomarker comparisons, we did not test for site effects, rather our emphasis was on any difference between sea otter regions. Due to logistical constraints and low abundance, we were not always able to meet this sample size goal, leading to an imbalance in sample sizes within some taxa (Table S1).

We collected tissues for biomarker sampling that represented organisms from different trophic guilds found in southeast Alaska seagrass meadows, are relatively common, and are important components of the hypothesized sea otter — seagrass trophic cascade at each site described above (Tables 1 & S1). These taxa tended to be the dominant species by mass in their trophic guild in this region (Raymond et al. 2021). We selected 16 taxa that fell into 3 general categories: primary producers, primary consumers, and secondary consumers. Whenever possible, we collected the same taxa at all sites. For primary producers, we collected seagrass *Z. marina*, seagrass epiphytes consisting primarily of diatoms (Class Bacillariophyceae), rockweed *Fucus distichus* (hereafter *Fucus*), sugar kelp *Saccharina latissima*, and sea lettuce *Ulva* sp. (hereafter *Ulva*). For primary consumers, we collected the isopod *Pentidotea rascata* (hereafter *Pentidotea*), seagrass limpets *Lottia pelta*, and butter *Saxidomus giganteus* and *Macoma* spp. clams. For secondary consumers, we collected dock shrimp *Pandalus danae*, helmet crabs *Telmessus cheiragonus*, graceful crabs *Metacarcinus gracilis*, red rock crabs *Cancer productus*, shiner perch *Cymatogaster aggregata*, snake prickleback *Lumpenus sagitta*, and Pacific staghorn sculpin *Leptocottus armatus*. Names and abbreviations of collected taxa can be found in Table 1. The tissue or tissues sampled from each taxa varied with regard to the organism's size and body plan. Seagrass and macroalgae tissues were collected distal from meristematic regions, and avoided relatively older or fouled tissues. Animal tissues included all soft body tissues (excluding shell) of limpets, foot muscle tissue from clams, whole *Pentidotea*, leg muscle tissue from crabs, and dorsal muscle tissue from fish. Tissue samples were frozen at −20°C for 1 mo and then at −80°C for 5 mo. Samples identified for biomarker analysis were lyophilized for 48 h and stored at −80°C until further processing. For both SI and FA analysis, lyophilized tissues were ground to a fine powder with a mortar and pestle. This process also served to homogenize pooled samples (Table S1).

We determined carbon and nitrogen SI ratios using the procedures of the NOAA Auke Bay Laboratories–Fisheries Recruitment Energetics and Coastal Assessment Chemistry Laboratory in Juneau, Alaska. Subsamples of ground tissues (approx. 1.0 mg) were weighed with a microbalance and placed into tin capsules for analysis. SI analysis was performed using a FlashSmart elemental analyzer coupled to a Delta V continuous-flow isotope ratio mass spectrometer (Thermo Scientific). SIs are reported in delta (δ) notation as the per mille of the ratio of heavy to light isotope relative to international standards of Vienna Pee Dee Belemnite for carbon and atmospheric nitrogen for nitrogen. The instrument was calibrated using certified reference materials from the International Atomic Energy Agency and the US Geological Survey. Internal laboratory standards (purified methionine and homogenized Chinook salmon muscle) were used as quality controls and yielded long-term precision estimates of ±0.12‰ for carbon and ±0.13‰ for nitrogen.

FAs were extracted and analyzed with gas chromatography coupled with mass spectrometry from ground tissues following methods found in Yoshioka et al. (2019), which are modified from Taipale et al. (2013, 2016). All FA analyses were conducted at the Oregon Institute of Marine Biology (OIMB) in Charleston, Oregon. Following lyophilization, samples of each tissue were homogenized, lipid extracted, and transesterified to produce FA methyl esters (FAMEs) for analysis (Taipale et al. 2016). During the initial lipid extraction, we added C19:0 as an internal standard to each sample. To extract total lipids, homogenized tissue samples were digested in a 4:2:1 chloroform:methanol:0.9% NaCl solution twice. From the resulting pooled organic layers, 1 ml was removed for transesterification, evaporated under N_2 gas flow, and the organics were re-suspended in a toluene and 1% sulfuric-acid methanol solution and maintained at 90°C for 90 min to transesterify FAMEs, which were then neutralized with 2% $KHCO_3$, diluted with hexane, vortexed, and centrifuged before carefully transferring the FAME layer to 2 ml glass vials for gas chromatography. FAMEs dissolved in hexane were analyzed, identified, and quantified using gas chromatography mass spectrometry following Taipale et al. (2016). We quantitatively measured FAME concentrations using a serial dilution of a mixed external FA standard (Nu-chek Prep 566C) and calculated relative proportions of each identified FA from the area under each sample peak.

2.6. Statistical analyses

To test for a difference in trophic structure, we compared biomass and SI and FA data between regions with high and low sea otter density for 14 taxa (Table S1). We were not able to obtain tissues from red rock crabs in the high sea otter region, and therefore are unable to present conspecific comparisons of red rock crab SIs and FAs. We present only biomass and FA data for limpets because we were unable to obtain realistic and consistent SI values from those tissues based on available literature (e.g. Pfister et al. 2011). Finally, we did not obtain biomass estimates of dock shrimp across sites; however, they were abundant in beach seines at all sites.

We compared conspecific biomass using linear mixed effects models with a fixed effect of sea otter region, with the high sea otter region as the reference group and site as a random effect. We chose this approach as our primary objective was to test the effect of sea otter region on biomass; however, we wanted to separate sea otter region-induced variation from other variation associated with site. As only one beach seine set was conducted per site, we did not include a random effect of site for total and species-specific fish biomass. Biomass data were transformed such that the residuals of linear models were approximately normal. We natural-log transformed seagrass biomass and total fish biomass. We square-root transformed epiphyte load, epifauna load, and helmet crab biomass; cube-root transformed limpet load, total clam density, and red rock crab biomass; and fourth-root transformed *Pentidotea* load, *Macoma* spp. density, total crab, and graceful crab biomass, and shiner perch, snake prickleback, and staghorn sculpin biomass. Butter clam density was not transformed. We note that biomass responses for limpets, butter clams, helmet crabs, graceful crabs, and red rock crabs included multiple measures of zero biomass; however, our transformations were aimed at normalizing positive values. Given that the inclusion of zeros resulted in minor normality violations and that zeros were not distributed across all sites and sea otter regions, we proceeded to fit standard linear mixed effects models. All models were fit and assessed in R v.3.5.1 (R Core Team 2018).

We analyzed $\delta^{13}C$ and $\delta^{15}N$ data in 2 ways. First, we compared the overall seagrass community isotopic niche space between the 2 sea otter regions (Layman et al. 2007) using the 'convexhull' function in the 'siar' package in R (Parnel & Jackson 2013, Thormar et al. 2016) on mean $\delta^{13}C$ and $\delta^{15}N$ values for each taxon and by plotting $\delta^{13}C$ and $\delta^{15}N$ values in a bi-plot. This analysis provided an overall comparison of the breadth of carbon source and food chain length of each seagrass community. Second, we directly compared mean $\delta^{13}C$ and $\delta^{15}N$ values of conspecifics between high and low sea otter regions using *t*-tests. Unlike biomass analyses, we did not fit mixed effects models with a random site effect due to a lack of site-level replication for some taxa (above) leading to model convergence issues, reflecting an inability to separate among-site variability from within-site variability. This analysis would indicate differences in ultimate carbon source through $\delta^{13}C$ and relative trophic position through $\delta^{15}N$. We recognize that performing multiple *t*-tests on our SI data set may increase the probability of Type I errors; however, we elected to not correct for multiple comparisons and present *t*-statistics and p-values in their raw form (Moran 2003).

Before FA analyses, we filtered all FAs identified from gas chromatography to those unique FAs with a mean proportional peak area ≥0.5% (chosen to balance capture of informative FAs but exclusion of noise) for a given taxon. This resulted in 42 unique FAs which we selected for each taxon on which we conducted proportional FA analyses. First, we used permutational multivariate analysis of variance (PERMANOVA) to test the effect of sea otter region on whole FA profiles of each taxon except red rock crabs, which we did not have in sufficient numbers for comparison. PERMANOVAs of FAs were performed on Euclidean distances of arcsine-square-root transformed proportional FA data, as is common for such data sets (e.g. Raymond et al. 2014, Yoshioka et al. 2020). PERMANOVAs were conducted using the 'adonis2' function in the 'vegan' package in R (Oksanen et al. 2020) using 9999 permutations. Second, we conducted SIMPER analysis on all taxa. SIMPER ranked individual FAs on their contribution to multivariate dissimilarity between sea otter density regions. SIMPER analysis was performed on untransformed FA proportions using the 'simper' function in the 'vegan' package in R (Oksanen et al. 2020) using 9999 permutations. We summarized SIMPER output by (1) reporting the mean dissimilarity and contribution to dissimilarity for each FA and (2) plotting the total sum of dissimilarity for all EFAs (ω-3 and ω-6 PUFAs with ≥18 carbon atoms), LCEFAs (ω-3 and ω-6 PUFAs with ≥20 carbon atoms), EPAs, and DHA. This approach allowed us to evaluate which FAs contribute the most to dissimilarity and the relative degree to which EFAs are responsible for overall dissimilarity. Third, we summarized the proportion of 8 FAs or FA groups commonly used to

describe trophic relationships and dietary variability among consumers. We compared the mean proportion of these FAs and FA groups for each taxon between sea otter regions using Mann-Whitney *U*-tests, which are robust to non-normal distributions such as the distribution of proportion data. Finally, we tested for the effects of taxon and sea otter density region on the concentration of essential ω-3 FAs EPA and DHA using 2-way ANOVA. We square-root transformed EPA and DHA concentration so that model residuals were approximately normal. This was followed up by pairwise comparisons of EPA and DHA concentration of conspecifics between sea otter regions using Mann-Whitney *U*-tests. All analyses were performed in R v.3.5.1 (R Core Team 2018).

FAs and FA groups (Table S3) were based on previous studies of benthic community FA markers and similar seagrass trophic structure studies. FA composition of seagrass-associated species that indicate high proportions of FAs known to be produced by bacteria suggest that bacterial/detrital energy pathways may be an important aspect of seagrass food webs (Kharlamenko et al. 2001, Alfaro et al. 2006, Jaschinski et al. 2008, 2011, Jankowska et al. 2018). The monounsaturated FA 16:1ω7 (palmitoleic acid, PAL) is considered a marker for diatoms (Dalsgaard et al. 2003, Kelly & Scheibling 2012). The C_{18} PUFAs 18:3ω3 (alpha-linolenic acid, ALA) and 18:2ω6 (alpha-linoleic acid, LIN) are highly abundant in vascular plants including *Z. marina*, and therefore may serve as a marker for the consumption of seagrass (Kharlamenko et al. 2001, Alfaro et al. 2006, Kelly & Scheibling 2012, Galloway et al. 2012, Jankowska et al. 2018). The proportion of the PUFA 20:4ω6 (arachidonic acid, ARA) was examined, as it is considered a marker for brown and red algae (Kelly & Scheibling 2012, Galloway et al. 2012) and could suggest alternative dietary sources among consumers compared to the seagrass, bacteria, diatoms, and dinoflagellates. EPA and DHA are considered biomarkers for diatoms and dinoflagellates, respectively. It is important to note that most of these PUFAs discussed above are present in varying levels in multiple producer groups and are not truly discreet source biomarkers. Diatom mats and films are common in southeast Alaska seagrass beds and are the predominant seagrass epiphyte. Furthermore, diatoms can be a predominant food source to seagrass community epifauna and clams (Kharlamenko et al. 2001, Alfaro et al. 2006, Jephson et al. 2008, Thormar et al. 2016, Jankowska et al. 2018). Alternatively, dinoflagellates can be consumed by filter feeders and planktivorous fishes that are common in southeast Alaska seagrass beds.

3. RESULTS

3.1. Sea otter presence

Boat-based sea otter counts indicated higher sea otter densities in concordance with USFWS surveys (USFWS 2014) and estimated sea otter density in the region (Tinker et al. 2019) (Table S4). Mean (±SD) boat-based sea otter density at high sites was 8.3 ± 4.2 ind. km^{-2} compared to 0.01 ± 0.02 ind. km^{-2} at low sites. Estimated sea otter density from historical USFWS surveys in the vicinity of high sites was 3.633 km^{-2} and 0.163 km^{-2} for low sites (Tinker et al. 2019) (Table S4). Sea otter densities measured for this study were similar to those measured 1 yr earlier (2017) in the same region (Raymond et al. 2021).

3.2. Environmental parameters

Environmental parameters varied little between sea otter regions and were similar to previous studies in the region (Raymond et al. 2021). Since our environmental sampling consisted of point measurements at each site, replication within each sea otter region was only 3 for each parameter. Therefore, we elected not to conduct statistical comparisons of environmental parameters and to report data in full here (Table S4, Figs. S1 & S2). Environmental parameters were similar across sites and regions, noteworthy considering the dynamic nature of nearshore ecosystems, with the exception of nitrate and water clarity. Water nitrate concentration was greater in the high sea otter region with a mean ± SD of 0.025 ± 0.017 µmol l^{-1} at 1 m and 0.032 ± 0.027 µmol l^{-1} at 5 m, compared to a mean of 0.007 ± 0.008 µmol l^{-1} at 1 m and 0.015 ± 0.004 µmol l^{-1} at 5 m across low sea otter region sites (Fig. S2d). Nitrate concentrations in the present study were lower than those observed by Raymond et al. (2021) in the region (0.08–2.79 µmol l^{-1}), who measured nitrate concentration across 21 sites on the west coast of Prince of Wales Island (Fig. S2). Across other parameters, measures in the present study are within ranges previously recorded in the region (Fig. S2) in a study by Raymond et al. (2021) spanning a greater spatial and temporal coverage.

3.3. Biomass

Biomass differed between sea otter regions for total clam biomass, butter clam biomass, total crab bio-

mass, and red rock crab biomass (Table S5, Fig. 2). Total clam and butter clam biomass were lower in the high sea otter region ($p = 0.007$ and 0.032, respectively; Fig. 2f,g). Similarly, total crab and red rock crab biomass was lower in the high sea otter region ($p = 0.004$ and $p < 0.001$, respectively; Fig. 2i,l). We found no evidence of an effect of regions differing in sea otter densities on seagrass biomass, epiphyte load, epifauna, *Pentidotea*, and limpet load, *Macoma* spp. biomass, graceful and helmet crab biomass, total fish, staghorn sculpin, shiner perch, and snake prickleback biomass (Table S5).

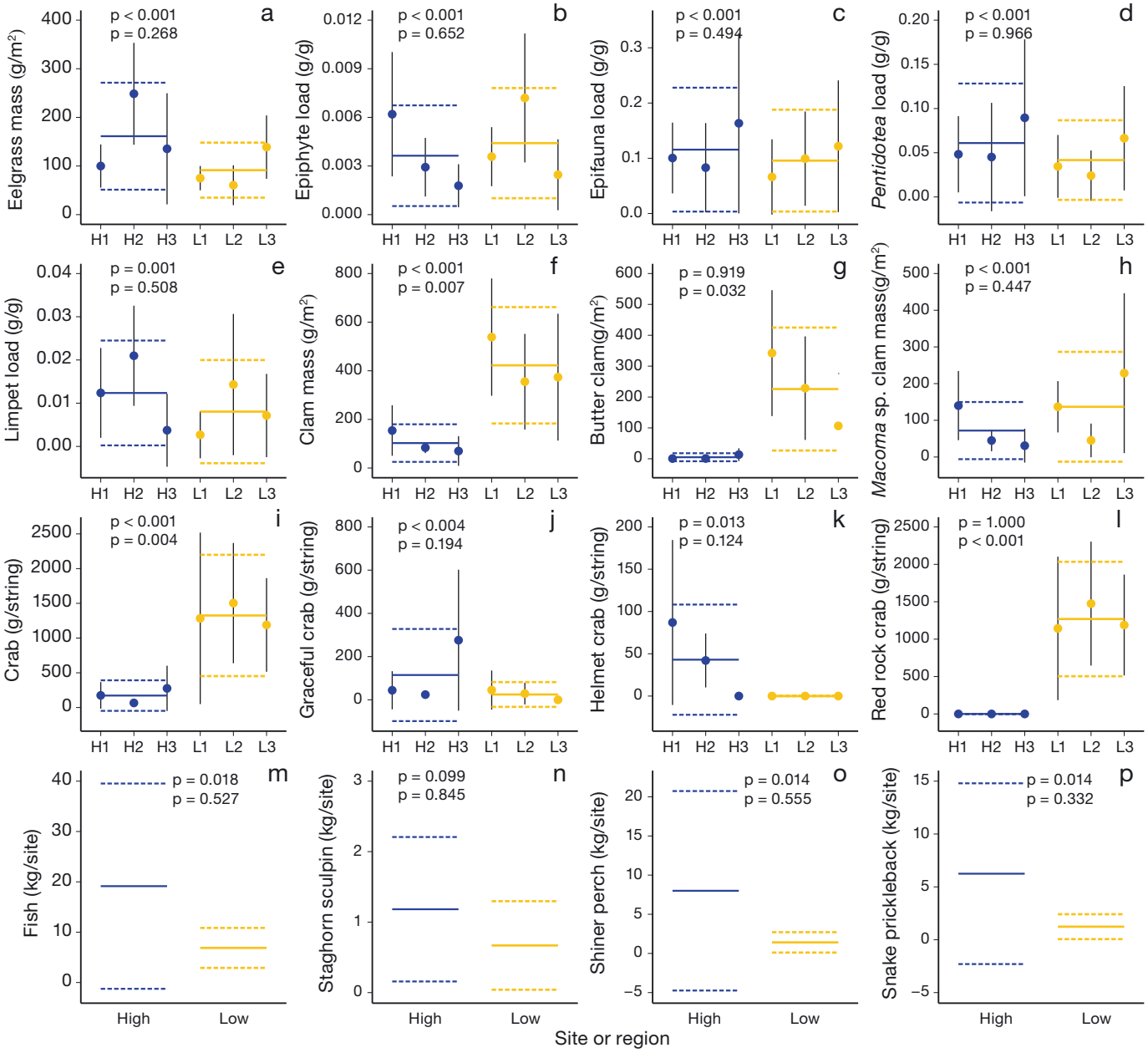

Fig. 2. Mean biomass (solid lines) and error (SD, dashed lines) of sampled seagrass community taxa in high (blue) and low (gold) sea otter regions. Points represent site level means with vertical error bars (SD), for (a) seagrass, (b) epiphyte load, (c) total epifauna load, (d) *Pentidotea* load, (e) limpet load, (f) total clam density, (g) butter clam biomass, (h) *Macoma* spp. biomass, (i) total crab biomass, (j) graceful crab biomass, (k) helmet crab biomass, (l) red rock crab biomass, (m) total fish biomass, (n) staghorn sculpin biomass, (o) shiner perch biomass, and (p) snake prickleback biomass. Since one beach seine was used at each site, only regional fish biomass is presented. All biomass data are presented in g except for fishes (m–p), which are presented in kg. String is the replication unit for crab data. Upper p-values indicate intercept significance and lower p-values indicate significance of high vs. low sea otter region from generalized linear mixed models. Full model results are presented in Table S5

3.4. Stable isotopes

Convex hull isotopic niche area in the high sea otter region was 40.3 compared to 46.5 in the low sea otter region. This represented a 13.3% greater isotopic niche area in the low sea otter region than the high sea otter region (Fig. 3a). $\delta^{13}C$ values of primary producers ranged from −17.8 to −7.5‰ in the high sea otter region and from −19.6 to −8.2‰ in the low sea otter regions, suggesting a similar breadth of dietary sources between the 2 regions. $\delta^{15}N$ of all taxa ranged from 6.2–14.0‰ in the high sea otter region and from 5.7–13.5‰ in the low sea otter region, suggesting similar food chain length between the regions. We found conspecific differences in $\delta^{13}C$ and $\delta^{15}N$ between sea otter regions for multiple taxa (Table S6, Fig. 3b,c). Among primary producers, we found no significant differences in $\delta^{13}C$ values (Fig. 3b). There were no differences in $\delta^{13}C$ among primary consumers, but we did find lower $\delta^{13}C$ in the secondary consumer staghorn sculpin in the high sea otter region ($p = 0.021$; Fig. 2b). We found differences in $\delta^{15}N$ in 7 of our 14 taxa, ranging from primary producers to secondary consumers (Table S6, Fig. 3c). There were lower $\delta^{15}N$ values in the low sea otter region in *Fucus*, sugar kelp, dock shrimp, and shiner perch ($p = 0.031$, 0.010, 0.044, and 0.015, respectively) and higher $\delta^{15}N$ values in the low sea otter region in seagrass epiphytes and butter clams ($p = 0.054$ and $p < 0.001$, respectively).

3.5. FAs

The multivariate FA profiles between sea otter regions differed in 11 out of 15 taxa, ranging from primary producers to secondary consumers (Table S7, Fig. 4). FA composition differed between sea otter regions for *Fucus*, limpet, dock shrimp, and graceful crab ($p < 0.01$; Fig. 4, see Table S7) and for seagrass, *Ulva*, *Pentidotea*, butter clam, helmet crab, snake prickleback, and staghorn sculpin ($p < 0.05$; Table S6, Fig. 3). We did not find evidence of an effect of sea otter region on the FA composition for seagrass epiphyte and *Macoma* spp. ($p < 0.1$; Table S7), or for sugar kelp and shiner perch ($p > 0.1$; Table S7).

SIMPER analyses identified EFAs as important in FA discrimination of conspecifics between sea otter regions (Table 2). Of the top 5 discriminating FAs for each taxon, a range of 1 (limpet) to 4 (*Ulva* and snake prickleback) of those FAs were EFA. The cumulative contribution, defined as the sum of individual FA dissimilarity from SIMER analysis, of EFAs, LCEFAs,

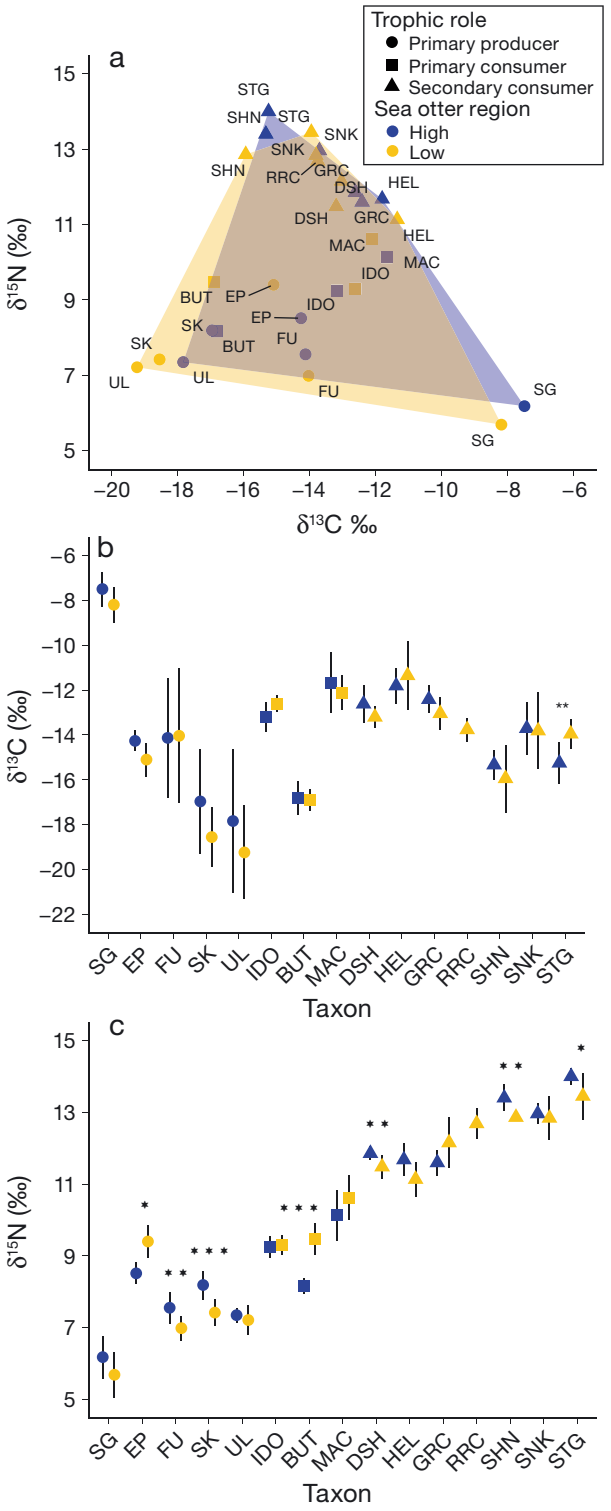

Fig. 3. Stable isotope values for primary producer (circles), primary consumers (squares), and secondary consumers (triangles) in high (blue) and low (gold) sea otter regions. (a) Mean $\delta^{13}C$ and $\delta^{15}N$ biplot of sampled species with convex hull overlay and (b) mean (±SD) $\delta^{13}C$ and (c) $\delta^{15}N$ for sampled taxa between high and low sea otter density regions. Species abbreviations in Table 1. *p ≤ 0.1; **p ≤ 0.05; ***p ≤ 0.01 (from *t*-tests); full statistical results are presented in Table S6

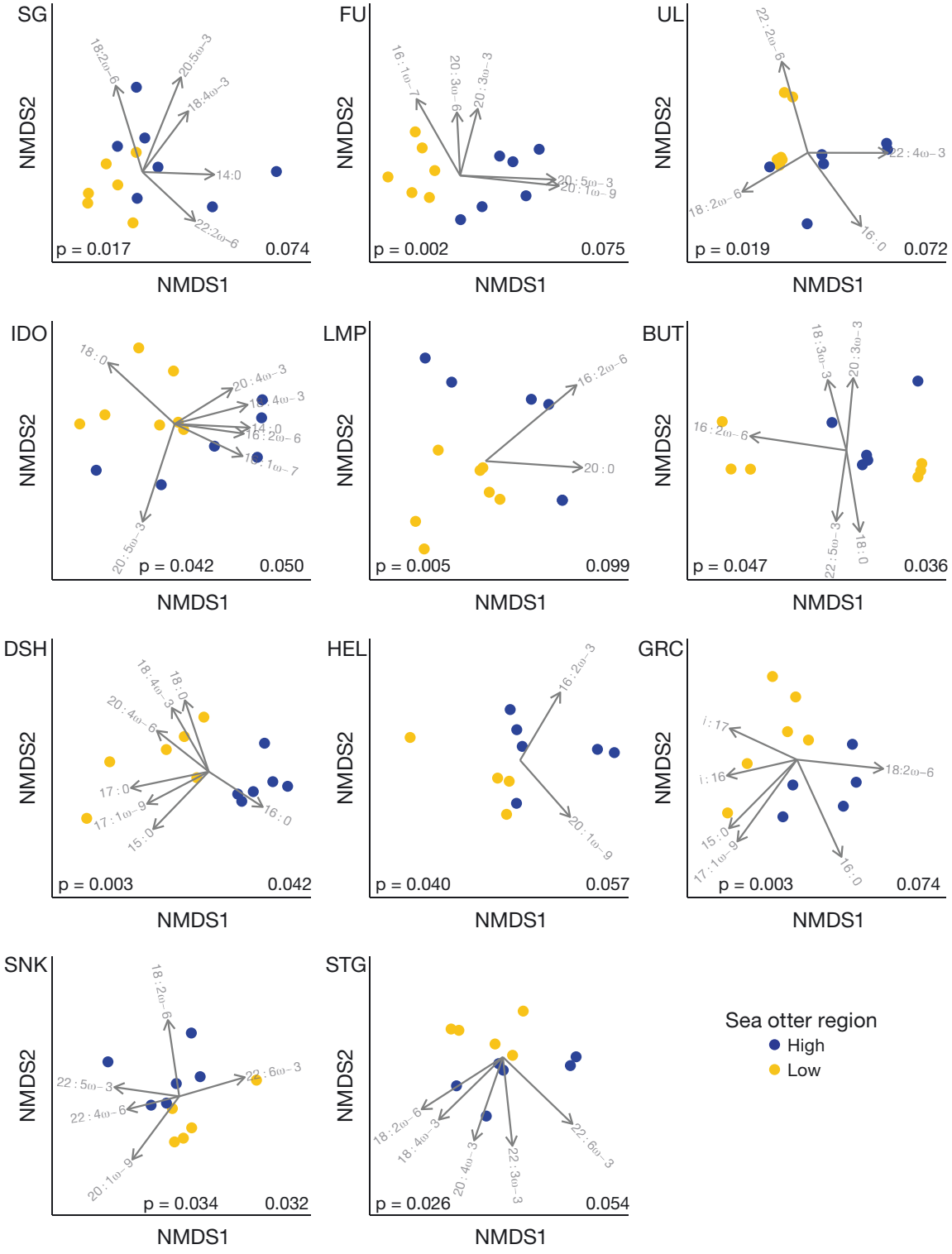

Fig. 4. Non-metric multidimensional scaling (NMDS) plots of fatty acid (FA) profiles for taxa with evidence for a sea otter region effect determined from PERMANOVA analysis ($p \leq 0.05$), including seagrass (SG), *Fucus* (FU), *Ulva* (UL), *Pentidotea* (IDO), limpet (LMP), butter clam (BUT), dock shrimp (DSH), helmet crab (HEL), graceful crab (GRC), snake prickleback (SNK), and staghorn sculpin (STG). Points represent unique tissue samples from high (blue) and low (gold) sea otter density regions. Two-dimensional stress is listed in lower right of each plot along with p-values from PERMANOVA in lower left. Vectors reflect FAs with $r^2 \geq 0.8$ with NMDS axes. Full PERMANOVA results are presented in Table S7

Table 2. SIMPER results on the top 5 fatty acids (FAs) contributing most to multivariate dissimilarity for all taxa. Essential FAs are in **bold**. Full SIMPER results can be found in Table S7

Taxon	Overall dissimilarity (%)	FA	Mean FA %		Mean dissimilarity (%) (SD)	Dissimilarity	Cumulative dissimilarity	p
			High sea otter	Low sea otter				
Seagrass	6.3	**18:3ω3**	**36.0**	**37.6**	**1.2 (1.0)**	**19.1**	**19.1**	**0.378**
		16:0	33.1	32.2	0.9 (0.6)	14.1	33.2	0.394
		16:3ω3	5.7	7.0	0.8 (0.5)	12.4	45.6	0.026
		18:2ω6	**6.2**	**7.0**	**0.8 (0.6)**	**12.3**	**57.9**	**0.482**
		14:0	1.6	0.6	0.5 (0.5)	7.9	65.8	0.420
Epiphytes	11.5	16:0	38.3	34.7	2.0 (1.2)	17.3	17.3	0.071
		18.3ω6	**0.9**	**3.9**	**1.5 (0.5)**	**13.1**	**30.4**	**0.019**
		20:5ω3 (EPA)	**15.0**	**15.1**	**1.3 (0.8)**	**11.0**	**41.4**	**1.000**
		14:0	17.1	19.4	1.2 (0.6)	10.2	51.6	0.036
		16:4ω3	2.9	1.7	1.2 (0.4)	10.1	61.7	0.520
Fucus	4.3	16:0	30.9	31.2	0.7 (0.4)	15.6	15.6	0.42
		18:1ω9	11.3	11.8	0.4 (0.3)	10.2	25.8	0.235
		20:4ω6 (ARA)	**7.3**	**6.5**	**0.4 (0.3)**	**9.5**	**35.3**	**0.032**
		14:0	19.1	19.5	0.4 (0.3)	9.4	44.7	0.354
		20:5ω3 (EPA)	**4.0**	**3.3**	**0.3 (0.2)**	**7.9**	**52.5**	**0.010**
Sugar kelp	9.6	16:0	31.6	31.4	1.7 (1.2)	18.1	18.1	0.976
		18:4ω3	**4.0**	**4.2**	**1.4 (0.9)**	**15.0**	**33.1**	**0.488**
		20:4ω6 (ARA)	**9.5**	**10.6**	**1.0 (0.7)**	**10.3**	**43.4**	**0.282**
		20:5ω3 (EPA)	**8.7**	**10.4**	**1.0 (0.8)**	**10.1**	**53.5**	**0.236**
		14:0	19.6	19.3	0.7 (0.5)	7.3	60.8	0.649
Ulva	14.3	**18:2ω6**	**5.5**	**10**	**3.0 (2.0)**	**21.0**	**21.0**	**0.114**
		16:0	45.6	41.8	2.1 (1.5)	14.9	36.0	0.032
		18:4ω3	**8.9**	**7.3**	**1.2 (1.1)**	**8.5**	**44.5**	**0.412**
		18:3ω3	**15.6**	**15.4**	**1.1 (0.8)**	**7.7**	**52.3**	**0.631**
		16:3ω3	**3.4**	**4.4**	**1.0 (0.6)**	**6.6**	**58.9**	**0.131**
Pentidotea	13.9	14:0	6.4	2.5	2.2 (1.3)	16.1	16.1	0.016
		20:5ω3 (EPA)	**21.9**	**22.2**	**1.9 (1.2)**	**13.6**	**29.7**	**0.387**
		18:0	11.1	13.7	1.7 (1.0)	12.6	42.3	0.059
		20:4ω6 (ARA)	**2.5**	**4.7**	**1.1 (0.6)**	**8.0**	**50.3**	**0.002**
		16:1ω7	4.2	2.5	1.1 (0.7)	7.9	58.1	0.077
Limpet	9.6	**20:5ω3 (EPA)**	**18.4**	**16**	**1.3 (1.1)**	**13.8**	**13.8**	**0.039**
		16:0	26.6	28.5	0.9 (0.5)	9.9	23.7	0.001
		18:0	10.8	9.5	0.8 (0.6)	8.5	32.2	0.051
		16:1ω7	4.7	4.2	0.7 (0.4)	7.0	39.2	0.062
		20:1ω9	2.9	4.2	0.6 (0.6)	6.6	45.8	0.044
Butter clam	13.2	16:0	34.6	31.1	1.7 (0.8)	13.1	13.1	0.002
		22:6ω3 (DHA)	**16.5**	**13.8**	**1.4 (0.9)**	**10.8**	**23.9**	**0.036**
		22:2ω6	**1.2**	**2.3**	**1.2 (1.0)**	**8.8**	**32.7**	**0.353**
		20:5ω3 (EPA)	**9.8**	**8.3**	**1.1 (0.8)**	**8.6**	**41.3**	**0.148**
		22:1ω9	0.1	2.1	1.0 (1.0)	7.7	49	0.136
Macoma spp.	7.7	22:1ω9	6.2	5.4	0.8 (0.9)	10.0	10.0	0.624
		20:5ω3 (EPA)	**9.2**	**9.4**	**0.7 (0.5)**	**9.1**	**19.0**	**1.000**
		22:5ω3	**1.9**	**2.9**	**0.6 (0.6)**	**8.0**	**27.0**	**0.160**
		16:0	27.3	26.3	0.6 (0.4)	7.3	34.3	0.045
		22:1ω7	2.3	2.8	0.5 (0.4)	6.0	40.3	0.363
Dock shrimp	5.7	16:0	38.0	35.8	1.2 (0.7)	20.3	20.3	0.017
		20:5ω3 (EPA)	**19.5**	**18.3**	**0.6 (0.3)**	**10.7**	**31.0**	**0.007**
		18:0	9.4	10.5	0.6 (0.4)	10.1	41.1	0.014
		15:0	0.8	1.8	0.5 (0.5)	8.5	49.5	0.005
		22:6ω3 (DHA)	**11.6**	**10.9**	**0.5 (0.3)**	**8.4**	**58.0**	**0.069**
Helmet crab	5.7	**20:5ω3 (EPA)**	**27.8**	**26.5**	**1.0 (0.6)**	**17.9**	**17.9**	**0.106**
		16:0	28.9	30.4	0.9 (0.7)	15.7	33.6	0.174
		22:6ω3 (DHA)	**11.7**	**10.5**	**0.7 (0.6)**	**11.7**	**45.2**	**0.292**
		18:0	11.1	11.1	0.5 (0.3)	9.4	54.6	0.053
		20:4ω6 (ARA)	**2.9**	**3.3**	**0.3 (0.2)**	**6.1**	**60.7**	**0.643**

Table 2 (continued)

Taxon	Overall dissimilarity (%)	FA	Mean FA % High sea otter	Mean FA % Low sea otter	Mean dissimilarity (%) (SD)	Dissimilarity	Cumulative dissimilarity	p
Graceful crab	9.0	16:0	30.4	27.8	1.3 (0.9)	14.9	14.9	0.029
		18:0	8.8	10.7	1.1 (1.0)	12.5	27.5	0.293
		22:6ω3 (DHA)	**12.5**	**11.2**	**0.8 (0.7)**	**9.0**	**36.5**	**0.148**
		20:4ω6 (ARA)	**2.1**	**3.5**	**0.7 (0.3)**	**7.7**	**44.2**	**0.003**
		20:5ω3 (EPA)	**23.3**	**23.8**	**0.5 (0.4)**	**6.0**	**50.3**	**0.379**
Shiner perch	8.7	**22:6ω3 (DHA)**	**18.8**	**19.8**	**1.9 (1.4)**	**21.5**	**21.5**	**0.371**
		16:0	34.3	37.7	1.7 (1.3)	19.5	41.0	0.025
		18:1ω9	3.7	3.4	0.8 (0.6)	8.7	49.8	0.290
		20:5ω3 (EPA)	**12.6**	**12.1**	**0.6 (0.5)**	**6.9**	**56.7**	**0.430**
		14:0	2	1.8	0.6 (0.4)	6.6	63.2	0.847
Snake prickleback	8.8	**22:6ω3 (DHA)**	**13.6**	**15.5**	**1.9 (1.4)**	**21.6**	**21.6**	**0.558**
		16:0	33.1	34.3	1.0 (0.8)	11.1	32.8	0.389
		20:5ω3 (EPA)	**17.3**	**16.5**	**0.7 (0.6)**	**8.0**	**40.7**	**0.325**
		20:4ω6 (ARA)	**2.6**	**3.3**	**0.6 (0.4)**	**6.7**	**47.4**	**0.204**
		22:5ω3	**2.8**	**2**	**0.6 (0.5)**	**6.5**	**53.9**	**0.578**
Staghorn sculpin	8.1	**22:6ω3 (DHA)**	**17.1**	**21.2**	**2.1 (1.1)**	**26.1**	**26.1**	**0.009**
		20:5ω3 (EPA)	**15.9**	**13.3**	**1.3 (0.7)**	**15.9**	**42**	**0.007**
		16:0	32.5	30.7	1.1 (0.9)	13.7	55.7	0.087
		18:0	14.4	14.1	0.5 (0.3)	6.4	62.1	0.626
		22:5ω3	**4.0**	**3.5**	**0.5 (0.3)**	**5.8**	**67.9**	**0.165**

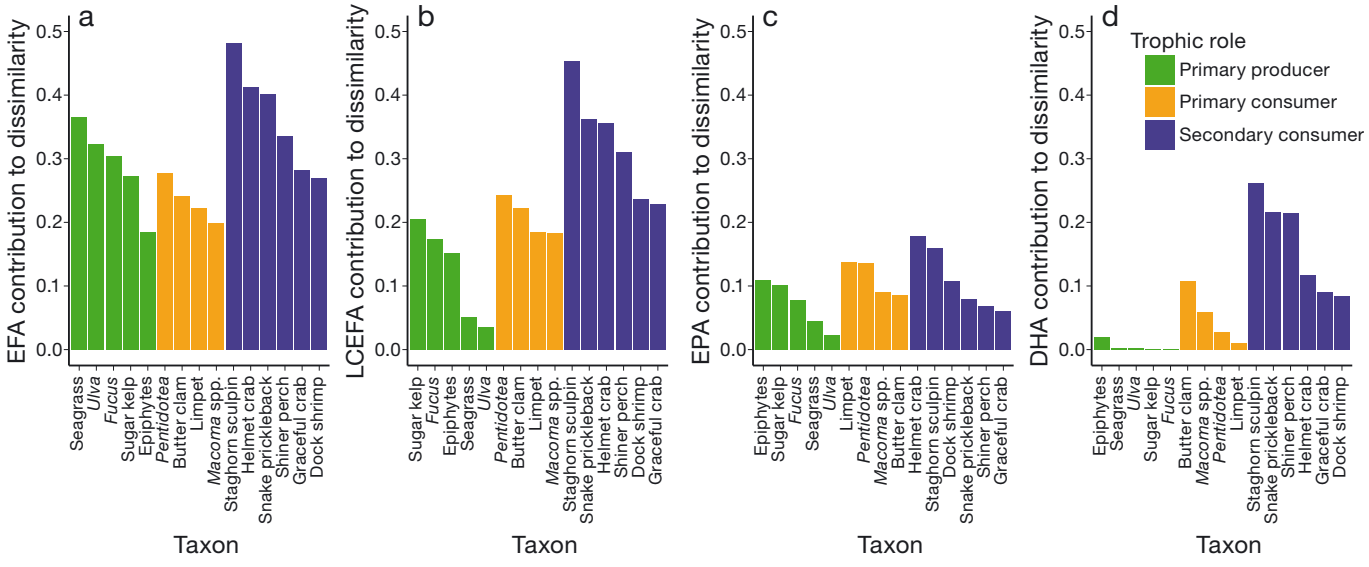

Fig. 5. Essential fatty acid (EFA) results from SIMPER analysis. Summed contribution to dissimilarity of (a) all EFAs, (b) all long-chain EFAs (LCEFAs), (c) eicosapentaenoic acid (EPA), and (d) docosahexaenoic acid (DHA)

EPA, and DHA to dissimilarity ranged across taxa and trophic role, with the greatest contributions in secondary consumers such as staghorn sculpins, snake prickleback, and helmet crabs (Fig. 5). The cumulative contribution of EFAs to dissimilarity ranged from 18.4% (epiphytes) to 48.2% (staghorn sculpin) (Fig. 5a). The cumulative contribution of LCEFAs to dissimilarity ranged from 3.5% (*Ulva*) to 45.4% (staghorn sculpin) (Fig. 5b). EPA ranked in the top 5 discriminating FAs for all taxa except seagrass and *Ulva*, accounting for 2.3% (*Ulva*) to 17.8% (helmet crab) of the total dissimilarity. However, we did not find a pattern of consistently higher or lower EPA proportions across taxa collected from each region (Table 2, Fig. 5c). DHA substantially contributed to FA dissimilarity between regions among secondary

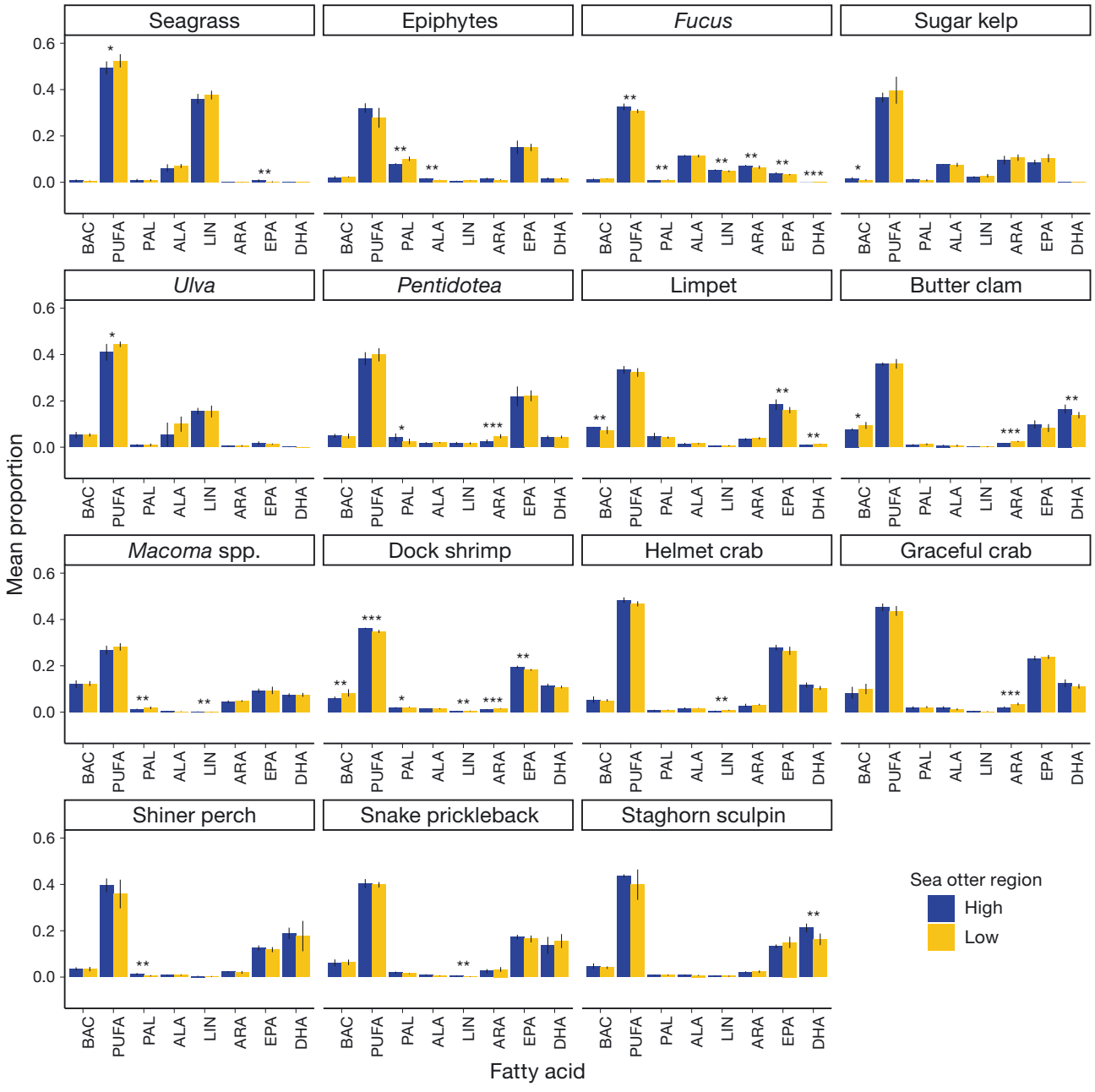

Fig. 6. Mean (±SD) proportions of marker fatty acids (FAs) and FA groups for all taxa measured between high and low sea otter density regions. BAC: bacterial FAs including 15:0, iso-15:0, isto-16:0, 17:0, iso-17:0, anteiso-17:0, 17:1ω9, anteiso-18:0, and 18:1ω7. PUFA: all polyunsaturated FAs including all FAs with ≥2 double bonds. PAL: palmitoleic acid, 16:1ω7; ALA: alpha-linolenic acid, 18:3ω3; LIN: alpha-linoleic acid, 18:2ω6; ARA: arachidonic acid, 20:4ω6; EPA: eicosapentaenoic acid, 20:5ω3; DHA: docosahexaenoic acid, 22:6ω3. *p ≤ 0.1; **p ≤ 0.05; ***p ≤ 0.01: full Mann-Whitney *U*-test results are presented in Table S9

consumers, including dock shrimp (8.4%), helmet crab (11.6%), graceful crab (9.0%), shiner perch (21.4%), snake prickleback (21.6%), and staghorn sculpin (26.1%), with greater proportions found in the high sea otter region in all secondary consumers except snake prickleback and staghorn sculpin (Table 2, Fig. 5d). Full SIMPER results are shown in Table S8.

Comparison of marker FAs among conspecifics found that all taxa showed at least some evidence of differing FA proportions between high and low sea otter density regions (Table S9, Fig. 6). We also

Table 3. ANOVA results on the effect of sea otter region and taxon on EPA and DHA concentration. **Bold** indicates p < 0.10

FA/factor	df	SS	MS	*F*	p
EPA					
Sea otter region	1	1.200	1.198	1.359	0.246
Taxon	14	918.490	65.606	74.444	**<0.001**
Sea otter region × taxon	14	33.990	2.428	2.755	**0.001**
Residuals	142	125.140	0.881		
DHA					
Sea otter region	1	0.620	0.622	2.074	0.152
Taxon	14	893.450	63.818	212.863	**<0.001**
Sea otter region × taxon	14	14.940	1.067	3.559	**<0.001**
Residuals	142	42.570			

Table 4. Mann-Whitney *U*-tests for differences in EPA and DHA concentration for conspecifics between low and high sea otter regions. Stat: Mann-Whitney *U*-test statistic; –: non-significant difference. **Bold** indicates p < 0.10

	EPA			DHA		
	Region with greater concentration	Stat	p	Region with greater concentration	Stat	p
Eelgrass	High	36	**0.038**	–	13	0.284
Epiphytes	–	6	0.766	–	6	0.766
Fucus	High	33	**0.020**	Low	0	**0.003**
Sugar kelp	–	16	0.927	–	20	0.407
Ulva	–	25	0.298	–	25	0.298
Pentidotea	High	36	**0.038**	–	33	0.100
Limpet	Low	5	**0.027**	Low	5	**0.027**
Butter clams	High	28	**0.022**	High	25	**0.083**
Macoma spp. clam	–	9	0.315	–	9	0.315
Dock shrimp	High	35	**0.008**	High	33	**0.020**
Helmet crabs	–	20	0.110	–	20	0.110
Graceful crabs	High	27	**0.036**	High	30	**0.008**
Shiner perch	–	18	1.000	–	21	0.689
Snake prickleback	High	30	**0.008**	High	26	**0.055**
Staghorn sculpin	–	20	0.810	High	32	**0.031**

found evidence of differences in multiple dock shrimp marker FAs, including the proportion of bacterial FAs, total PUFAs, LIN, ARA, and EPA (all p < 0.05; Table S8, Fig. 6) and no difference in the proportion of PAL (p = 0.093). Across all taxa, we found total PUFAs constituted greater than 20% of total FAs, especially for primary producers (Fig. 5). We found evidence for a difference in total PUFAs between sea otter regions in *Fucus* (p = 0.045) and dock shrimp (p = 0.008). EPA differed in 3 out of 6 primary producers, including seagrass (p = 0.027) and *Fucus* (p = 0.020); however, there was no consistent pattern of higher or lower proportions between sea otter regions. In contrast, DHA was found to be in significantly lower proportion in the low sea otter region in *Fucus* (p = 0.003). EPA differed in 2 out of the 10 consumers, including limpets and dock shrimp, both with lower proportions in the low sea otter region. Similarly, we found lower proportions of DHA in the low sea otter region for limpets (p = 0.018), butter clams (p = 0.036), and staghorn sculpin (p = 0.013).

ANOVA testing for the effect of sea otter region and taxon on EPA and DHA concentration revealed an interaction effect in both EPA and DHA (both p < 0.001; Table 3). As expected, we found an effect of taxon on EPA and DHA concentration (both p < 0.001). However, we did not find evidence for an effect of sea otter region on DHA (p = 0.246) or EPA (p = 0.312) concentration. Post hoc pairwise comparisons of EPA concentration identified differences in concentration between sea otter regions in dock shrimp and snake prickleback (p < 0.01), and seagrass, *Fucus*, limpet, butter clams, and graceful crabs (p < 0.05) (Table 4). Post hoc comparisons of DHA concentration found evidence for a difference in concentration between sea otter regions in *Fucus* and graceful crabs (p < 0.01), limpet, dock shrimp, and staghorn sculpin (p < 0.05), and no evidence in butter clams and snake prickleback (p < 0.1) (Table 4). However, we did not find a consistent pattern of higher or lower DHA concentrations between sea otter regions across taxa (Table 4).

4. DISCUSSION

Of the 3 metrics we used to assess seagrass trophic structure (biomass, SIs, and FAs), FAs provided the most evidence of differing trophic structure between regions with different sea otter densities (Table 1). We found evidence for differences in FA profiles for 11 out of 15 taxa including primary producers, primary consumers, and secondary consumers. These differences appear to be driven in large part by EFAs including EPA and DHA, especially in consumers, and suggest subtle differences in diets of conspecifics between regions with high and low sea otter density. Overall, SI and biomass data provide

little evidence of wholesale differences in trophic structure but do highlight some species-specific effects of sea otter presence. While we did find some differences in conspecific $\delta^{13}C$ and $\delta^{15}N$, our comparison of overall trophic niche space indicated similar total area and range of $\delta^{13}C$ and $\delta^{15}N$ between sea otter regions, suggesting little difference in overall trophic structure. Contrary to our predictions, we did not observe that sea otters conferred the same patterns on seagrass biomass as Raymond et al. (2021) or a similar study in Elkhorn Slough, CA (Hughes et al. 2013). Overall, we found few differences in conspecific biomass between sea otter density regions, suggesting limited indirect effects of sea otters on patterns of seagrass community biomass. However, as expected, there were clear direct negative effects of sea otter density on clam and crab biomass. FA results may suggest that sea otters have a large effect on trophic structure; however, further examination of FA results and our alternative hypotheses suggest that differential patterns in FAs of conspecifics are more likely a function of diet diversity than a sea otter-mediated effect alone.

Essential FAs, including EPA and DHA, were consistent and relatively strong divers of differences in conspecific FAs between sea otter regions, refuting our central hypothesis that FA biomarkers would not differ between sea otter regions. Given that consumers obtain nearly all of their EFAs (including EPA and DHA) from their diet, this result is the strongest evidence of conspecific variation in diet composition (and therefore trophic structure) of the seagrass community. We found EPA to be an important discriminating FA in our study, and this finding supports a growing consensus of EPA as a critical FA in describing trophic variability in ecosystems (Arts et al. 2001, Litzow et al. 2006, Galloway et al. 2013, Budge et al. 2014). While EPA is often considered a biomarker for diatoms, is it also present in elevated proportions in other sources of nearshore primary production including dinoflagellates (Kelly & Scheibling 2012) and brown and red macroalgae (Galloway et al. 2012). Given that we found variable directional patterns of EPA proportions within taxa between the sea otter regions, it may be that even if sea otters have an effect on EPA availability in the community, consumers are buffered from this effect by EPA availability from other diet sources within or outside the seagrass community. DHA also appeared to be an important discriminating FA among secondary consumers, further supporting the importance of DHA as a trophic marker for many species (Arts et al. 2001, Dalsgaard et al. 2003). Generally, these differences in FA profiles in conspecifics could result from differences in diet composition, either unique sources and/or proportions, between sea otter regions. At present, we are unable to evaluate which of these scenarios is occurring in southeast Alaska; however, given the evidence for diverse diets of consumers in this study and other seagrass ecosystems (Kharlamenko et al. 2001, Alfaro et al. 2006, Jaschinski et al. 2008, 2011, Jephson et al. 2008, Douglass et al. 2011, Jankowska et al. 2018), it is likely a combination of different sources and relative contribution of those sources to a given consumer's diet.

In contrast to FA results, our comparison of isotopic niche space suggested little difference in overall trophic structure between sea otter density regions. Other studies on the trophic structure of seagrass communities have reported a 60% difference in isotopic niche space between sites (Thormar et al. 2016). Notably, these differences were largely attributed to differences in nutrient loading in the environment (i.e. bottom-up forces), and the authors suggested that SI values of seagrass community species may be more susceptible to change from bottom-up forces than top-down forces (Thormar et al. 2016). The similarity in $\delta^{13}C$ in nearly all conspecifics in our study is likely due to a lack of observed difference in the $\delta^{13}C$ of primary producers between regions. The only taxa that differed in $\delta^{13}C$ were staghorn sculpins. While this could be due to a direct or indirect sea otter effect, as seen in fishes in other studies (Markel & Shurin 2015), our results could also reflect natural variability of the species. Staghorn sculpins are known generalist predators in the region (Whitney et al. 2017, Duncan & Beaudreau 2019), which could result in a wide range of $\delta^{13}C$ values (Whitney et al. 2018). Furthermore, our results could be reflective of the slightly larger individuals sampled in the low sea otter region, as body size can reflect ontogenetic diet shifts and therefore changes in SIs (Table S1). While we observed more differences between sea otter regions in $\delta^{15}N$, they were not consistent in direction and did not appear to propagate up the food chain. However, regardless of direction, differences in $\delta^{15}N$ suggest that some taxa may occupy different relative trophic positions between sea otter density regions.

Conspecific differences in whole FA profiles, largely driven by EFAs, and FA groups, provide the most evidence of differing trophic structure between sea otter density regions. We identify 3 potential mechanisms for these patterns in southeast Alaska seagrass communities: (1) that primary producers vary in their FA profiles across our study area, which

then propagate to consumers; (2) that the diets of consumers vary across our study area as a function of natural variation in diet composition; or (3) sea otter-induced change in consumer diets. As evidence for Scenario 1, of the 5 primary producers analyzed, 3 differed in their whole FA profile between sea otter regions. These differences may be the result of natural variability in primary sources rather than sea otters because we do not know of a mechanistic link between sea otters and primary producer FAs. However, we did not find overall evidence that these differences in primary producer FAs propagate to their likely consumers which would lead to different consumer FA. Combining PERMANOVA results with our examination of marker FAs and FA groups, we found only 2 instances where FA differences in primary producers correspond to differences in a primary consumer. We found lower proportions of EPA in low sea otter region samples of both seagrass and *Fucus* and in the secondary consumer limpets. The driver of this pattern is unclear, as limpets may not rely on seagrass as a major dietary source (see below) and we have not observed them attached to or consuming *Fucus*. The lack of concurrence between primary producer and likely consumer FAs may also reflect that many consumers rely on diverse diets (Kharlamenko et al. 2001, Alfaro et al. 2006, Douglass et al. 2011, Jankowska et al. 2018) and could obtain FAs from a variety of sources, including many that were not measured in this study.

FA results that support our alternative hypotheses that consumer FAs may vary as a function of natural variation in diet composition, an indirect sea otter-induced change in consumer diets, or a combination of the two. For example, graceful crabs, which are consumed by sea otters, may be limited in their foraging and perhaps diet diversity in areas of high sea otter abundance due to fear of predation from sea otters. In contrast, in low sea otter density regions, graceful crabs may not be limited in their foraging and therefore have a more diverse diet, leading to differences in key FA values. More generally, our examination of marker FAs and FA groups provides further evidence of general diet diversity in southeast Alaska seagrass communities. For example, the proportion of bacterial FAs only differed between sea otter regions in limpets and dock shrimp, suggesting that detrital food sources may be an important primary dietary resource in southeast Alaska seagrass communities. The proportion of total bacterial FAs among primary and secondary consumers ranged from 0.027–0.116, which is similar to other seagrass meadows, ranging from approximately 0.02–0.11 in the Sea of Japan (Kharlamenko et al. 2001) and 0.02–0.05 in the Baltic Sea (Jankowska et al. 2018). Our results contrast with higher proportions reported from northern New Zealand, ranging from 0.132–0.146 (Alfaro et al. 2006); however, these high values may be due to a diverse estuarine habitat that included multiple foundational species in a relatively small area.

Differences in DHA proportion and concentration may be partially explained by a coccolithophore bloom observed near one of our high sea otter density sites. DHA occurs in relatively high proportions in coccolithophores (Class Prymnesiophyceae) and can be useful in distinguishing them from other phytoplankton (Dalsgaard et al. 2003, Fiorini et al. 2010, Galloway & Winder 2015). We collected particulate organic matter (POM) samples from all sites but did not include them in the analysis because we were only able to sample POM once per site, and POM biomarkers are known to vary over short time periods (Lowe et al. 2014). However, a single POM FA sample taken from the coccolithophore bloom measured a DHA proportion of 0.176, compared to a mean of 0.082 ± 0.016 (SD) across all other high sea otter sites. Mean DHA proportions at low sea otter density sites were notably lower, at 0.038 ± 0.007. This may indicate a difference in the trophic structure between sea otter regions; however, we believe this is unlikely for 2 reasons. One, the coccolithophore bloom was an ephemeral and unanticipated event and is not necessarily a consistent feature of our study sites. Two, as noted above, POM FA signatures are known to vary in space and time, which was not likely captured by our limited sampling. Similar to our other FA results (EFAs above and FAs in general below), a lack of a mechanistic link between sea otters and DHA concentration may indicate that we have described the natural variability of DHA concentration in seagrass-associated taxa in our study area. Variation in DHA concentration with sea otter regions may be influenced by the spatial scale of our study, as location and water body can affect DHA concentration in freshwater fishes (Williams et al. 2017).

Primary producer SI and FA biomarkers can also vary as a function of environmental conditions, location, and season (Guest et al. 2010, Dethier et al. 2013, Lowe et al. 2014). This variation is especially evident in differences in $\delta^{15}N$ in primary producers between sea otter regions and in that these differences are not consistent in sign. Environmental parameters measured for this study were relatively consistent across sites and sea otter regions, except water nitrate concentrations, which were slightly higher in the high sea otter region. Measurements for

this study were similar to those measured in seagrass meadows in the region in a previous study (Raymond et al. 2021) across a larger spatial and temporal range, further supporting that these environmental parameters vary little across these sites. However, geographic location could affect environmental conditions not captured by our sampling, potentially introducing unaccounted effects on and variation of biomarker values. Given the distribution of sea otters in the region, we were unable to control for geographic location in our sampling regime and therefore rely on the environmental measures at our sites alone. However, we took care to select sites with qualitatively similar geomorphology and seagrass bed size. We also constrained biomarker sampling to the shortest time frame possible for a given taxon, ranging from 1–3 d. Assuming that environmental or temporal factors did not affect primary producer biomarkers, our observed differences could be reflective of natural variability or some sea otter effect. While there is support for location-associated variability in biomarker values in nearshore ecosystems (Dethier et al. 2013), to date there is no evidence of sea otter-mediated effects on biomarker values in primary producers. As FA synthesis of primary producers is a function of a taxon's physiology and environmental setting (Dalsgaard et al. 2003), a mechanistic relationship between sea otters and primary producer biomarker values is unclear.

Sea otters had strong negative effects on total crab and clam biomass and little effect on other species in the hypothesized sea otter–seagrass trophic cascade that was expected based on similar studies of apex predator–seagrass trophic cascades (Moksnes et al. 2008, Baden et al. 2010, 2012, Hughes et al. 2013, Raymond et al. 2021). Notably, we failed to find a positive relationship between sea otters and seagrass, contrary to Raymond et al. (2021) and Hughes et al. (2013). This may be due to the reduced number of study sites and the grouped (ANOVA) versus correlation analysis in this study compared to Raymond et al. (2021). Sea otter density region had the greatest effect on total clam and crab biomass, butter and *Macoma* spp. clams, and helmet and red rock crabs, confirming results from previous research in the region (Hoyt 2015, Raymond et al. 2021). We found lower total crab biomass at high sea otter sites but, interestingly, helmet crabs were not present in traps at low sea otter density sites. However, we were able to easily obtain them via snorkel, confirming their presence. This sampling artefact may be due to interference competition between helmet and red rock crabs. At low sea otter density sites, where red rock crabs were abundant and relatively large (mean carapace width: 150.5 ± 4.7 mm), they may have entered the crab pots first, discouraging entrance by the much smaller helmet crabs (mean carapace width: 40.0 ± 4.0 mm). These observations may provide evidence of interference competition between these 2 species; thus, there is a potential that helmet crabs occupy different trophic niches when red rock crabs are present versus absent. We found good evidence for a difference in FA profile of helmet crabs between regions driven by 3 EFAs — EPA, DHA, and ARA — suggesting differences in diet and supporting a different ecological niche for this species between sea otter regions.

An essential component of the top-down structuring theory in seagrass communities is that epifaunal grazers predominantly consume seagrass epiphytes and other ephemeral macroalgae rather than seagrass itself (Hughes et al. 2004, Heck & Valentine 2007). Our SI and FA results support this hypothesis in southeast Alaska, in line with similar studies using biomarkers (e.g. Jaschinski et al. 2008, 2011). We found little evidence that *Pentidotea* or limpets contain large amounts of the sum of LIN and ALA, which are relatively abundant in *Zostera marina* (Fig. 6). Instead, *Pentidotea* and limpets contained relatively high proportions of ARA and EPA, which are relatively high in *Fucus*, sugar kelp (Fig. 6), and other brown algae (Kelly & Scheibling 2012, Galloway et al. 2012) and DHA, which is relatively high in zooplankton (Kharlamenko et al. 2001, Dalsgaard et al. 2003, Alfaro et al. 2006). Furthermore, the dietary proportions of these FAs correlate well with *Pentidotea* FA composition described in other studies (Galloway et al. 2014), further supporting that *Pentidotea* likely consume a diverse algae diet including macro- and microalgae. The FA profile of gastropods, including limpets (in our study) and snails, follows similar patterns of ARA, EPA, and DHA described in other studies (Kharlamenko et al. 2001, Jankowska et al. 2018) and suggests that seagrass community epifaunal gastropod diets likely consist of a variety of sources. Our FA and SI results contrast McConnaughey & McRoy (1979), who suggested that seagrass itself may make up a large portion of the base of Alaska seagrass community food webs based on $\delta^{13}C$ data. Our data indicate $\delta^{13}C$ values of consumers, especially *Pentidotea*, align more closely with sources of primary production other than seagrass.

Predation by mesopredators, including crabs and fishes, on epifauna is a central element of seagrass trophic cascades that include higher order predators, as described in the northeast Atlantic and Elkhorn

Slough, CA (Jephson et al. 2008, Moksnes et al. 2008, Baden et al. 2010, 2012, Hughes et al. 2013). Previous research in southeast Alaska, however, did not find evidence of an association between mesopredators and seagrass epifauna (Raymond et al. 2021). Our SI and FA comparison of crabs (including helmet and graceful crabs) and fishes (snake prickleback and staghorn sculpin) suggests that these taxa consume diets with a wide variety of ultimate sources, regardless of sea otter influence. While it is possible that fishes and crabs consume seagrass epifauna, FA analysis indicates that these secondary consumers likely consume a variety of other species that lie outside the current hypothesized sea otter–seagrass trophic cascade. In addition to the preceding references on staghorn sculpins diets, seagrass-associated fishes often consume a diverse diet consisting of detritus, epibenthic, and planktonic prey (Adams 1976, Jaschinski et al. 2008, 2011, Jankowska et al. 2018). Seagrass-associated crabs can also exhibit diverse diets, not necessarily tightly linked to seagrass epifauna (Douglass et al. 2011). Overall results from this study and Raymond et al. (2021) may indicate that the assumption that common mesopredators in seagrass communities (crabs and fishes) consume seagrass epifauna is not generalizable and may be a large reason for lack a of relationship between these 2 groups in the region.

5. CONCLUSIONS

No single taxon differed in biomass, $\delta^{13}C$, $\delta^{15}N$, and FA between the 2 sea otter regions. Staghorn sculpin differed in SI and FA biomarker measures but did not differ in biomass, and butter clams differed in biomass, $\delta^{15}N$, and FA but not $\delta^{13}C$ (Table 1). These variable results highlight that while top-down forces from sea otters can have large effects on the biomass of certain taxa, this does not necessarily translate to a wholesale difference in energy flow among other community constituents. However, FA results point to differences in trophic structure not apparent from biomass or SI data alone. This was most pronounced from the perspective of EFAs. The consistency and degree to which EFAs are responsible for driving differences in conspecifics between high and low sea otter regions is a strong indication of differing trophic structure between these regions. However, to date, a mechanistic link between this pattern and sea otter presence is not clear. Rather, we suggest that our results further support the diverse trophic structure of seagrass ecosystems similar to previous studies (Kharlamenko et al. 2001, Alfaro et al. 2006, Douglass et al. 2011, Jankowska et al. 2018). Interestingly, variability from the FA perspective does not appear in our SI results, which found a similar breadth of carbon sources (range of $\delta^{13}C$ values) and food chain length (range of $\delta^{15}N$ values). The variation in conspecific biomarkers that we did find appears to be more of a feature of the complexity of seagrass food webs and natural variability than a sea otter-induced effect. Therefore, we did not find evidence for a sea otter-mediated effect on trophic structure at the biomarker level, such that might come about through sea otter-induced diet switching among all consumers. Future research could identify potential diet shifts through experimental manipulation and feeding trials. Furthermore, our results highlight that regions like southeast Alaska, where communities are relatively open, composed of a mosaic of habitats (O'Clair et al. 1997), and harbor relatively diverse multitrophic assemblages and consumers that appear to utilize diverse diet resources, may be resilient to localized perturbations to the food chains which can lead to linear trophic cascades (McCann 2000, Bellmore et al. 2015, O'Gorman 2021). From this perspective, it may be an over-simplification to consider seagrass ecosystems in southeast Alaska as only influenced by top-down forces, in isolation from other habitats, and characterized by simple linear food chains. This includes the potential effects of other large or top predators that may or may not be correlated with sea otters. To date, the role of large predators such as lingcod *Ophiodon elongates*, salmon *Oncorhynchus* spp., dogfish shark *Squalus suckleyi*, and harbor seals *Phoca vitulina* in southeast Alaska seagrass communities is not well understood. Thus, future research should consider the resources available in adjacent habitats, the flux of those resources among habitats, and other predators.

Data availability. All data are available on the Knowledge Network for Biocomplexity (KNB), https://knb.ecoinformatics.org/profile/CN=Sustainable%20Marine%20Ecosystems%20in%20Alaska%20Lab,DC=dataone,DC=org

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