Trophic Biomarkers Indicate Coastal Surf Zone Hydrodynamics Affect Resource Assimilation by *Mytilus californianus* Mussels

Carlissa D. Salant[1,2] ⓘ · Alan L. Shanks[2] · Julie B. Schram[2,3] · Aaron W. E. Galloway[2]

Abstract

Surf zone hydrodynamics influences the delivery of coastal phytoplankton and detritus food subsidies to shore. Variation in surf zone hydrodynamics can cause phytoplankton concentrations to be higher at dissipative than reflective surf zones, altering both the quantity and quality of food for intertidal suspension-feeders. To assess if surf zone-dependent food availability influences suspension-feeder diets, we out-planted *Mytilus californianus* mussels for a 5-month common garden experiment at sites with dissipative and reflective surf zones at Cape Arago, Oregon. Stable isotopes, gravimetric lipid weights, and fatty acid trophic biomarkers of extracted abductor muscles were used to examine possible diet differences between mussels grown at dissipative and reflective sites. Both $\delta^{15}N$‰ and $\delta^{13}C$‰ values varied significantly between dissipative and reflective surf zone types, but there was no difference in gravimetric lipid weight between surf zone types. The multivariate fatty acid composition of mussels from dissipative and reflective sites differed; mussels at dissipative sites had a higher proportion of fatty acids indicative of diatoms in their diet (e.g., $16:1\omega7$, $20:5\omega3$), whereas mussels at reflective sites had a higher proportion of fatty acids indicative of dinoflagellates (e.g., $22:6\omega3$). Aspects of our stable isotope and fatty acid data suggest trophic subsidies to mussels were influenced by surf zone hydrodynamics.

Keywords Dissipative · Reflective · Fatty acids · Stable isotopes · Eicosapentaenoic acid · Docosahexaenoic acid

Introduction

The surf zone is a unique marine ecotone, capable of defining the species richness and biomass (Defeo and McLachlan 2005) at both sandy and rocky shores (Morgan et al. 2017). Differences in surf zone hydrodynamics impact delivery of coastal subsidies to intertidal zones and, at some shores, can be more impactful than larger, continental shelf hydrodynamics, such as upwelling (Shanks and Morgan 2019). While upwelling intensity has been correlated with coastal secondary production (Bustamante et al. 1995; Menge et al. 2003), other coastal hydrodynamics are govern subsidies to the shore.

Surf zone hydrodynamics differ based on nearshore, coastal morphology (Woodroffe 2002), and surf zones are characterized along a continuum from dissipative to reflective shores (Wright and Short 1984). Few shores are therefore purely dissipative or reflective. Wider surf zones, gradual shore slopes, fine-grain sand on beaches, and the presence of bathymetric rip currents are characteristics of more dissipative surf zones. Bathymetric rip currents create eddies within surf zones as water from the inner shelf enters the surf zone over the shoals between rip channels and then exits back out to the inner shelf via the rip current (MacMahan et al. 2010). Narrower surf zones, steep shore slopes, coarse-grain sand on beaches, and the notable lack of bathymetric rip currents are characteristics of more reflective shores. While topographic and transient rip currents can be present at reflective shores (Castelle and Coco 2013), these rip currents do not set up persistent eddies characteristic of bathymetric rip current systems seen at dissipative shores (Reniers et al. 2010) and may in fact eject floating objects out of the surf zone. Variation in surf zone hydrodynamics between dissipative and reflective shores has led to observed differences in phytoplankton

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✉ Carlissa D. Salant
cdsalant@mun.ca

1 Ocean Sciences Centre, Memorial University of Newfoundland, 0 Marine Lab Rd, Logy Bay, NL A1K 3E6, Canada

2 Oregon Institute of Marine Biology, University of Oregon, PO Box 5389, Charleston, OR 97420, USA

3 Soboleff Building, University of Alaska Southeast, 11067 Auke Lake Way, Juneau, AK 99801, USA

resources available for intertidal consumers between dissipative and reflective shores (Morgan et al. 2016; Shanks et al. 2017c; Salant and Shanks 2018). The particulate trophic subsidies to suspension feeders are largely comprised of diatoms, flagellates, dinoflagellates, detritus, micro-zooplankton, and other particulate organic matter (POM). Certain phytoplankton taxa, including some diatoms, are adapted to living within more dissipative surf zones (Talbot and Bate 1987). However, the surf zone can also be dominated by coastal phytoplankton. Salant and Shanks (2018) found that the surf zone specialist diatom *Asterionellopsis* spp. accounted for only ~0–7% of phytoplankton in more dissipative zones throughout a year-long study; four genera (*Chaetoceros* spp., *Pseudo-nitzschia* spp., *Thalassiosira* spp., and *Skeletonema* spp.), typical coastal phytoplankton, accounted for ~88–96% of phytoplankton. Moreover, dissipative shores retained significantly higher concentrations of mostly coastal phytoplankton, and not surf zone specialized diatoms, within the surf zone compared to reflective shores. Overall, these studies found that phytoplankton concentrations were an order of magnitude higher at dissipative shores compared to reflective shores (Morgan et al. 2016; Shanks et al. 2017c). This difference in phytoplankton concentration correlated with far higher reproductive output and growth rates of intertidal suspension feeders along the coast of Oregon (Salant and Shanks 2018). It is therefore possible that the available dietary resources, and thus tissue composition, of consumers are related to surf zone type as well and will vary between dissipative and reflective sites.

One approach for teasing apart which dietary resources are assimilated into the tissues of consumers is through analysis of stable isotopes (SI). The isotopes of both carbon (^{13}C‰) and nitrogen (^{15}N‰) can help assess the trophic source and food web position of consumers (herein referred to as δ^{13}C and δ^{15}N) (Bergamino et al. 2011; Docmac et al. 2017). The δ^{13}C values of consumers in the marine environment can indicate whether the dietary resources are from typically offshore (e.g., dominated by phytoplankton) or nearshore (e.g., strongly influenced by macrophytes and terrestrial detritus sources) (Peterson and Fry 1987). For example, the δ^{13}C values of particulate organic matter (POM) positively correlate to total chlorophyll and diatom content of a diet (Lowe et al. 2014), with lower δ^{13}C values indicating a diet of coastal origin. The δ^{15}N values in consumers provide an estimation of their position in the food web (Caut et al. 2009), with enriched δ^{15}N values indicating assimilation of resources at relatively higher trophic levels (Peterson and Fry 1987).

The combination of SI and fatty acid (FA) biomarkers can provide a more nuanced analysis into the diet and resource assimilation of a consumer than just one type of biomarker (Hebert et al. 2006). FAs are molecules that play diverse roles in the physiology of organisms, and as such are important to the health and vitality of organisms within marine food webs (Kainz et al. 2004). Composed of carbon chains and ethylenic

bonds, FAs are commonly designated as saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) based on carbon chain length and number and position of the ethylenic bonds. The idea that certain FAs can act as trophic markers is based on the fact that producers, including phytoplankton, have diagnostic FA compositions (Galloway and Winder 2015), which are often conservatively transferred up the food web via the tissues of consumers (Kelly and Scheibling 2012; Parrish 2013). In the marine environment, phytoplankton are a major source of FAs, and many of the abundant phytoplankton FA can be used as trophic biomarkers within marine food webs (Dalsgaard et al. 2003). For example, diatoms tend to contain significant amounts of eicosapentaenoic acid (EPA, 20:5ω3) and palmitoleic acid (16:1ω7), while dinoflagellates tend to contain significant amounts of docosahexaenoic acid (DHA, 22:6ω3) (Budge et al. 2001). Nearshore macroalgal detritus is characterized by arachadonic acid (ARA, 20:4ω6), stearidonic acid (18:4ω3), EPA, and oleic acid (18:1ω9) (Galloway et al. 2012; Kelly and Scheibling 2012). Because most FA are not synthesized by only one source, and the conservative transfer of FA up the food chain is not guaranteed (Galloway and Budge 2020), trophic inference using FA is enhanced with additional tracers (e.g., SI) and experimentation. FA and SI profiles can therefore be used as a means of differentiation between organisms which have theoretically consumed different food subsidies.

Here, we investigate whether the dietary resources assimilated by a common benthic suspension feeder varied with surf zone hydrodynamics. The diet of the mussel, *Mytilus californianus*, may consist of phytoplankton, zooplankton, and detritus, in a mixture of POM, so we used SIs, gravimetric lipid weights, and FA biomarkers as metrics by which to differentiate possible diet differences between dissipative and reflective surf zones. Mussels were experimentally translocated in the spring of 2016 to nine sites around Cape Arago, Oregon with varied surf zone hydrodynamics. In past work (Shanks et al. 2017c), we observed far higher concentrations (order of magnitude higher) of phytoplankton subsidies at sites with dissipative surf zones than at the sites with reflective surf zones. We hypothesized that the observed differences in subsidies would translate into differences in resource assimilation, as determined by biomarkers (i.e., putative differences in diets), between mussels experimentally placed at sites with more dissipative and reflective surf zones.

Materials and Methods

Surf Zone Classification

One of the key characteristics of surf zones is their width; more reflective surf zones are narrow while more dissipative

are wider (Wright and Short 1984); hence, surf zone width can be used as a proxy to characterize the surf zone hydrodynamics present at a site. In 2016, we sampled nine sites with surf zone widths varying from 10 to 300 m (e.g., more reflective to more dissipative), spanning ~7 km of shore around Cape Arago, Oregon (Fig. 1).

Closely spaced sites were used to minimize and control for the effect of offshore hydrographic conditions and alongshore variation in phytoplankton (Salant and Shanks 2018); the primary difference between sites was surf zone width. Further, sites within bays and coves were avoided due to the altered hydrodynamics of these areas. Surf zone width was measured in Google Earth images (Salant and Shanks 2018). Nine to ten images of each study site were available from 1994 to 2015 (Supplementary Material; Tables S1 & S2). Surf zone width was measured immediately seaward of each sample site as the distance from the most seaward breaking wave to the swash line. While some sites were sandy beaches with rock outcrops, the majority of sites were rocky shores, sites typically studied by intertidal ecologists. Imagery of the shoreline was sufficient to estimate our independent variable. Fieldwork was conducted weekly over a year (described in Salant and Shanks 2018), and it was observed that although there was a range in the surf zone widths at each site due to daily weather

Fig. 1 Locations of surf zones sampled around Cape Arago, Oregon. The average distance between sites was 0.83 km and the distance between the northernmost and southernmost site was 7 km. See Supporting Material for exact coordinates of locations and site name abbreviations. Wider surf zones, gradual shore slopes, and the presence of bathymetric rip currents are characteristic of more dissipative surf zones, while narrower surf zones, steep shore slopes, and lack of bathymetric rip currents are characteristic of more reflective shores

conditions and time of year, surf zone widths remained close to the averages obtained from Google Earth images; sites with narrow surf zones remained narrow and wide surf zones remained wide. Sites with average surf zone widths >50 m, sites with bathymetric rip currents, were considered dissipative (Fig. 1; sites 1, 2, 3, and 5), while sites with narrower surf zones (<50 m) were considered reflective (Fig. 1; sites 4, 6, 7, 8, and 9). Herein, we refer to these dissipative and reflective sites as "surf zone types" (as opposed to differing surf zone widths since width was not correlated to any response variables in this study).

Mussel Common Garden Experiment

Common garden experiments are often used to obtain a more mechanistic understanding of processes in the field, by putting organisms from one place, the common garden, into different field conditions. We performed a common garden experiment using trophic biomarkers, including SI, lipids from the adductor muscles (gravimetric mass), and FA composition of *Mytilus californianus* following 5 months of experimentation at 9 sites. In March 2016, 800 ~3 cm long mussels were collected from the intertidal zone at Yoakam Pt (Fig. 1, site 3), a site with a dissipative surf zone. To determine the initial conditions, twenty mussels from this sample population were immediately taken to the laboratory and the adductor muscle is dissected from the mussels and frozen at −20°C ($n = 20$ initial condition). Shells of additional mussels were marked with Floy Tags® attached with Splash Zone Epoxy® and by filing a groove (< 1 mm) into the posterior shell edge so shell length and weight could be determined as metrics for growth rates in the other closely related study, Salant and Shanks (2018). Five days after initial collection, 80 mussels were transplanted to each site at +1.4 ± 0.1 m above mean lower low water (MLLW), into already established mussel populations. This was done to eliminate possible tidal height differences at sites (i.e., wave action or feeding pressure). Tidal elevation was determined using standard clinometer techniques. An observer with a sighting level stood where the transect line was laid at each site and a second person holding a surveyor's rod stood at the water line. Using the sighting level, the observer measured height on the surveyor's rod. Knowing the tidal height at the time the measurement was taken and using trigonometry, the tidal height of the transect line was determined. Mussels ($n = 20$ per cage) were placed within cages ($n = 4$ per site) made from PVC tubes covered with 1-cm plastic mesh. Cages were held to the rock by Splash Zone Epoxy®. This design allowed mussels to move beneath the mesh during high tide, encouraging byssal thread attachment. Average spacing between cages was ~10 m, and mussels were placed within mussel beds to control for density-dependent competition. After 4 weeks, mussels had attached to the rock and cages were removed. In September 2016, after
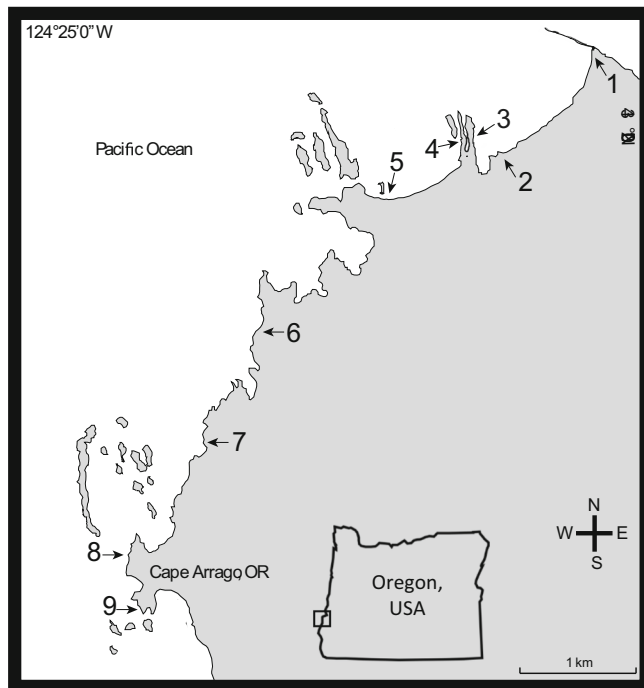
~5 months, mussels were collected from the transplant common garden experiment, immediately placed on ice, and taken to the laboratory. The adductor muscle was immediately dissected out of each mussel and placed in a freezer at −20°C. The adductor muscle from each mussel was used for (1) one SI sample ($n = 20$ initial, $n = 40$ dissipative; $n = 50$ reflective), where ten mussels came from each of the four dissipative sites and ten from the five reflective sites, (2) one lipid sample ($n = 10$ initial, $n = 24$ dissipative; $n = 30$ reflective), where six mussels came from each of the four dissipative sites and six from the five reflective sites, and (3) one FA sample ($n = 10$ initial, $n = 24$ dissipative; $n = 30$ reflective), where six mussels came from each of the four dissipative sites and six from the five reflective sites.

Stable Isotopes, Total Lipids, and Fatty Acid Analysis

For SI analysis, tissue samples were removed from the freezer and dried within 4 months of collection (9 months for the initial population). After drying at 70°C for 48 h, samples were ground to a fine powder, stored at room temperature until 10 mg was removed, and sent to Washington State University Stable Isotope Core Laboratory for analysis. Samples were converted to N_2 and CO_2 with an elemental analyzer (ECS 4010, Costech Analytical, Valencia, CA), separated with a 3-m gas chromatograph (GC) column, and analyzed with a continuous flow isotope ratio mass spectrometer (Delta PlusXP, Thermofinnigan, Bremen; Brenna et al. 1997). $\delta^{13}C$ and $\delta^{15}N$ values were calculated using a multi-point normalization by fitting a regression line through acetanilide standards provided by the Stable Isotope Core Laboratory. Total lipids were determined gravimetrically; approximately 100 μl of sample extracted in chloroform was transferred to an 8.0-mL vial, allowed to evaporate overnight, and the difference between initial and final mass of the vial was determined for the calculation of lipid concentration of extracted tissues (Schram et al. 2018). For FA analysis, approximately 20 mg of powdered tissue was placed in chloroform, sealed under nitrogen flow, and stored at −80°C for later analysis. Within 3 months, fatty acid methyl esters (FAMEs) were prepared by transesterification using sulfuric acid, H_2SO_4, and a modified Folch procedure (Schram et al. 2018). A known amount of C19:0 was added as a methyl ester internal standard. FAMEs were analyzed on a gas chromatograph mass spectrometer (GCMS; Model QP2020, Shimadzu, OR, USA) and a DB-23 column (30 × 0.25 mm x 0.15 um, Agilent, Santa Clara, CA, USA), using the same program described in Schram et al. (2018). FA were identified using relative retention times, FAME standards (GLC standard mixture 566C, Nu-Chek Prep, Elysian, MN, USA), specific ions, and quantified using the peaks of major ions. Chromatograms were integrated using GCMS Solutions (Shimadzu). We did our analyses on only FA that made up >1.0% of the total FA. Gravimetric lipid

weight and SI values ($\delta^{13}C$ and $\delta^{15}N$) were compared between the initial population, and dissipative and reflective sites using a one-way analysis of variance (ANOVA) and post-hoc comparisons (Tukey's HSD) using Minitab (Version 17). Of the 34 identified FA, 13 individual FA were, on average, present in proportions > 1% in the mussel samples.

For the mussel common garden experiment, 12 FA, excluding biomarker summations and ratios, were included in multivariate analyses. All data were log (x + 1) transformed prior to analyses and a Bray-Curtis matrix of similarity was used to create a resemblance matrix. A similarity percentage (SIMPER) pairwise test was used to determine which FA contributed most to the differences seen between dissipative and reflective groups. ANOVAs and Tukey's HSDs were then used to test for differences between those FA that contributed to the differences seen between the initial population and dissipative and reflective sites. Non-metric multidimensional scaling (nMDS) plots, which use ranks of similarities, were utilized to visualize the relationship between surf zone hydrodynamics and FA composition of the out-planted mussels. A one-way permutational multivariate analysis of variance (PERMANOVA; Type III SS, 999 permutations) was performed using a Bray-Curtis matrix of similarities to examine both main and interactive effects of site, experiment, and surf zone type on FA composition. Multivariate analyses were performed using PRIMER v6 +PERMANOVA (Primer-E Ltd.) (Clarke and Gorley 2006; Anderson et al. 2008).

Results

Surf Zone Classification

The average surf zone width calculated from 9 to 10 images (5/1994, 8/2000, 6/2003, 4/2004, 8/2005, 6/2007, 11/2011, 8/2012, 5/2013, 5/2015) (Supplementary Material; Tables S1 & S2) represented both high and low tides and a range of sea states. Seasonal surf zone widths could not be calculated as the majority of the images were from spring and summer. Bastendorff N., Bastendorff S., Yoakam Pt., and Lighthouse (Figure 1; Sites 1, 2, 3, 5) were characterized by wide surf zones (average width 235 ± SE 26 m) with bathymetric rip currents; these sites were considered dissipative. At Mussel Pt., Shore Acres, Pack Trail, Cape Arago, and Middle Cove (Figure 1; sites 4, 6, 7, 8, 9) surf zones were narrow (average width 21 ± 4 m), and there were no bathymetric rip currents; these sites were considered reflective. While the images used only represent a snapshot in time, the surf zone width of reflective, narrower surf zones consistently stayed below 50 m; dissipative, wider surf zones remained wider than 50 m. In a survey of 40 intertidal sites, bathymetric rip currents were absent at surf zones narrower than 50 m (Shanks et al. 2017a), and bathymetric rip currents were not observed at the

Table 1 Summary of T-tests

Common garden experiment—*Mytilus californinanus*			
	Beginning vs. end of experiment		
	df	T	p
Dissipative δ^{13}C‰	1	10.5	< 0.002
Dissipative δ^{15}N‰	1	42.47	< 0.001
Dissipative gravimetric lipid	1	11.3	0.0018
Reflective δ^{13}C‰	1	0.14	0.709
Reflective δ^{15}N‰	1	2.5	0.118
Reflective gravimetric lipid	1	7.25	0.011
	End of experiment		
	Dissipative vs. reflective sites		
δ^{13}C‰	1	23.26	< 0.001
δ^{15}N‰	1	48.35	< 0.001
Gravimetric lipid	1	0.67	0.418
Saturated fatty acids (SFAs)	1	20.93	< 0.0001
Mono-unsaturated fatty acids (MUFAs)	1	18.83	< 0.0001
Poly-unsaturated fatty acids (PUFAs)	1	1.64	0.206

Sites are averaged into dissipative (BN, BS, YP, LH) and reflective (MP, SA, PT, CA, MC) groups

reflective study sites during a year-long, weekly visitation experiment at the sites used in this study (Salant and Shanks 2018).

Mussel Common Garden Experiment

Stable Isotopes

Mytilus californianus mussel SI values revealed differences between surf zone type and both δ^{13}C and δ^{15}N values (Fig. 2a, Table 1). SI values from mussels at the reflective surf zone sites were not significantly different from the initial population collected at a dissipative site. In contrast, SI values from

mussels out-planted at the more dissipative surf zone sites were significantly more depleted in δ^{13}C and less enriched in δ^{15}N compared to the initial population (Fig. 2a, Table 1).

Lipids and Fatty Acids

The gravimetric lipid weight of *M. californianus* did not differ between surf zone types after the 5-month out-planting (Fig. 2b, Table 1). Mussels significantly increased their gravimetric lipid weight during the experiment at both dissipative and reflective surf zone sites (Fig. 2b, Table 1) compared to the initial population. Of the 34 identified FA, 12 were present on average, across all samples and above 1% of the total possible

Fig. 2 Average bulk nitrogen and carbon isotopic values (**a**) and average gravimetric lipid per dry mass (**b**) (95% CI) for *Mytilus californianus* mussels from the 5-month common garden experiment grouped into dissipative (BN, BS, YP, LH) and reflective (MP, SA, PT, CA, SN) categories (initial $n = 10$, dissipative $n = 24$, reflective, $n = 30$)
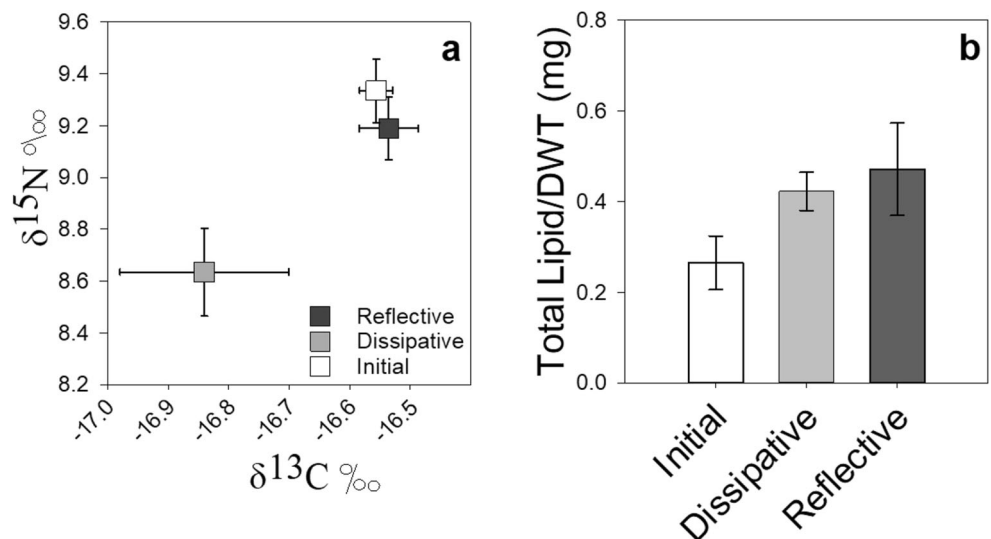
Table 2 Summary of fatty acids shown constitute, on average, $\geq 1\% \pm$ SE of total fatty acids analyzed

	Mytilus californianus		
	Initial (YP)[a]	Dissipative[b]	Reflective[c]
Sample size	$n = 10$	$n = 24$	$n = 30$
% 12:0	-	-	-
% 14:0	1.9 ± 0.001	5.9 ± 0.4[a,c]	2.6 ± 0.2[a,b]
% 15:0	1.2 ± 0.001	1.0 ± 0.1[a,c]	1.5 ± 0.03[b]
% 16:0	39.3 ± 0.01	39.5 ± 0.6	40.2 ± 0.3
% 17:0	2.0 ± 0.001	1.6 ± 0.1[a,c]	2.2 ± 0.1[b]
% 18:0	15.3 ± 0.01	10.8 ± 0.8[a,c]	15.4 ± 0.4[b]
∑ %SFAd	60.0 ± 1.0[c]	59.0 ± 0.7[c]	62.1 ± 0.3[a,b]
% 16:1ω7	1.7 ± 0.01	5.5 ± 0.6[a,c]	2.6 ± 0.2[b]
% 18:1ω9*t*	1.5 ± 0.002	1.5 ± 0.8	1.7 ± 0.2
% 18:1ω9	1.8 ± 0.001	2.4 ± 0.1[a]	2.0 ± 0.1
% 20:1ω9	4.0 ± 0.002	3.2 ± 0.2[a,c]	4.1 ± 0.2[b]
∑ %MUFAe	8.9 ± 0.3[b,c]	13.0 ± 0.5[a,c]	10.5 ± 0.3[a,b]
% 18:2ω6	1.3 ± 0.001	1.2 ± 0.1	1.4 ± 0.1
% 20:5ω3	15.7 ± 0.004	16.5 ± 0.4[c]	12.5 ± 0.3[a,b]
% 22:6ω3	13.3 ± 0.001	8.5 ± 0.5[a,c]	12.6 ± 0.3[b]
∑ %PUFAf	31.0 ± 0.9[b,c]	28.2 ± 0.6[a]	27.4 ± 0.4[a]

Significant ($p<0.05$) ANOVA Tukey results are indicated by superscripts (Initial[a], Dissipative[b], and Reflective[c]) between groups for the common garden experiment, labeled within the dissipative and reflective columns. A dashed line indicates that particular FA was not detected.

[d] Sum also includes 10:0, 11:0, 12:0, 13:0, 20:0, 22:0, 24:0

[e] Sum also includes 14:1ω5, 15:1ω5, 17:1ω7, 18:1ω11, 18:1ω6, 18:1ω5, 22:1ω9, 24:1ω9

[f] Sum also includes 18:3ω6, 18:3ω3, 18:4ω3, 18:4ω1, 18:5ω3, 20:2ω6, 20:3ω6, 20:3ω3, 22:4ω6, 22:2ω6

Fatty acid values and summations of *Mytilus californianus* mussels are shown from the common garden experiment. Sites are averaged into dissipative (BN, BS, YP, LH) and reflective (MP, SA, PT, CA, MC) groups. Mussel *n* values represent an individual muscle sample. Analysis of variances (ANOVA) results are outlined in Supporting Material; Table S5

FA pool (Table 2). Common garden mussel FA composition consisted of SFA, MUFA, and PUFA with values of 62.4% ± 0.6 (mean ± standard error, SE), 10.1% ± 0.4, and 27.5% ± 0.8, respectively. There was a significant difference between mussels at dissipative and reflective surf zone sites for proportional summations of SFA and MUFA, but not PUFA summations (Table 1).

An nMDS plot of the FA results shows the clear separation of mussels raised at a dissipative surf zone from those raised at a reflective surf zone relative to FA profiles of the initial mussels at the source of the common garden; the vector overlays (> 0.7 Pearson correlation) in the plot show that the FA most strongly correlated with the nMDS axes (Fig. 3). Separate one-way PERMANOVAs showed that mussel FA differed

among sites ($df = 8$, *pseudo-F* = 4.7083, $p = 0.001$) and between surf zone types ($df = 1$, pseudo-F = 21.681, $p = 0.001$). The nMDS plot includes the initial mussel FA profiles for reference, but the PERMANOVA tests did not include the initial samples as that would be a comparison of "time" as well as surf zone type, which was not the focus of this study.

Because site, along with surf zone type, was significantly different when FA values were compared, SIMPER tests were also run between all sites (Supplementary Material; Tables S3 & S5). Of the 12 identified FA present at > 1% proportions, SIMPER results indicated that six individual FAs were responsible for distinguishing mussels at sites with dissipative surf zone from those at reflective surf zones (> 70%, Supplementary Material; Table S4). The FAs that contributed most to the differences between surf zone types were 14:0, 16:1ω7, 18:0, 20:1ω9, and 22:6ω3 (myristic acid, palmitoleic acid, stearic acid, eicosenoic acid, and docosahexaenoic acid) (Figure 4). Due to its importance as a diatom indicator, 20:5ω3 (eicosapentaenoic acid) was also included.
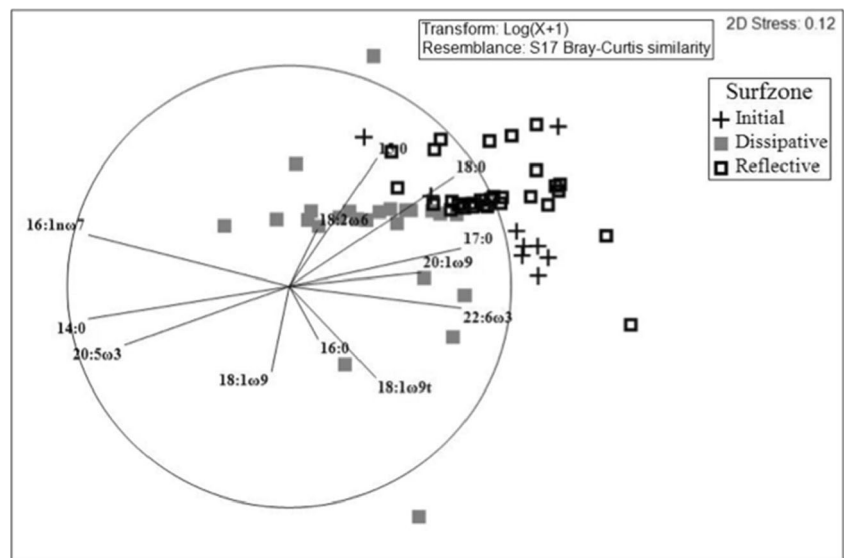
Discussion

We found clear differences in some biomarker metrics of *Mytilus californianus* mussels raised for 5 months at dissipative and reflective surf zone sites, indicating that surf zone type is related to resource assimilation of this ubiquitous coastal suspension feeder. The FAs responsible for these differences align with previous research which suggested that different types of phytoplankton are present at dissipative and reflective surf zone sites (Salant and Shanks 2018). Overall, our results and conclusions presented here add to the body of research showing surf zone hydrodynamics is an important physical component controlling alongshore variation in plankton subsidies (Shanks et al. 2010, 2017a, b, c; Morgan et al. 2016, 2017).

Mussels at the dissipative shores had lower δ^{13}C values, suggesting their food source was more coastal in origin compared to mussels at reflective surf zone sites (Bergamino et al. 2016). We previously found that phytoplankton species at these dissipative surf zone sites were of coastal origin (Salant and Shanks 2018). Phytoplankton abundance was indeed higher at dissipative shores (Fig. 2, Salant and Shanks 2018), so the δ^{13}C values should be interpreted with caution. Increased δ^{15}N values for mussels reared in reflective surf zones suggest that mussels were assimilating food at a slightly higher trophic level, for example, by possibly consuming flagellates, mixotrophic plankton, or zooplankton.

There was no difference in the gravimetric lipid weight of abductor muscles in mussels reared in dissipative surf zones compared with animals in the reflective sites. One hypothesis to explain this result is that differential lipid storage is not

Fig. 3 A non-metric multi-dimensional scaling (nMDS) ordination comparing FA profiles for *Mytilus californianus* mussels from the 5-month common garden experiment (initial $n = 10$, dissipative $n = 24$, reflective, $n = 30$)

occurring in muscle tissue but may be in reproductive tissue; mussels reared at dissipative surf zone sites had 3× more gonad mass compared to mussels at reflective surf zone sites (Salant and Shanks 2018). Therefore, total lipids (as opposed to adductor muscle bulk lipid) or gonad lipid may better reflect differences in surf zone type because that is where the most active growth was occurring. In fact, there was a difference between the gravimetric lipid per DWT of the initial population and that of mussels at dissipative and reflective surf zone sites after 5 months, confirming lipid is important for bivalve growth (Marshall et al. 2010).
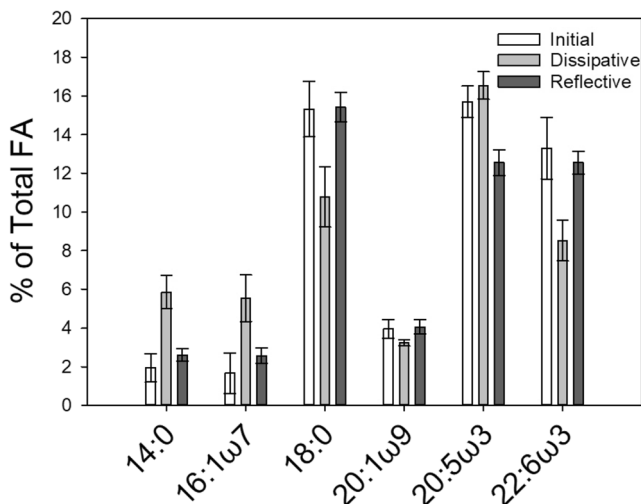

Fig. 4 Average percent fatty acids (95% CI) for *Mytilus californianus* mussels from the 5-month common-garden experiment (initial $n = 10$, dissipative $n = 24$, reflective $n = 30$). Fatty acids graphed were both present in quantities greater than 1% of total fatty acids present, and were found through an ANOVA test to be significantly different between mussels raised at dissipative surf zones (BN, BS, YP, LH and reflective (MP, SA, PT, CA, SN) ($p<0.05$), and contributed to >70% of the differences between mussels raised at dissipative and reflective surf zones (similarity percentages, SIMPER). 20:5ω3 was also included

Mussels at reflective surf zone sites had a significantly higher ratio of SFA, driven primarily by 18:0 (Table 1). A higher ratio of SFAs, especially those between 14 and 18 carbons, in bivalves has been associated with organic detritus-rich environments (Ackman et al. 1968), Indeed, the density of benthic macroalgae is higher at reflective sites and would contribute to the detritus concentration (Conser and Shanks 2019). Additionally, when phytoplankton concentrations are low, detritus makes up a larger part of the mussels' diet (Handå et al. 2012), and phytoplankton concentrations are significantly and often more than 10× lower at the sampled reflective surf zone sites (Salant and Shanks 2018).

Changes in mussel lipid and FA composition have also been linked to phytoplankton availability (Fernández-Reiriz et al. 2015). Mussels at dissipative surf zone sites had a significantly higher proportion of MUFA, resulting from increased values proportionally in the phytoplankton diatom indicator 16:1ω7. Since *Mytilus* mussels can selectively digest diatoms in the gut compared to flagellates (Rouillon and Navarro 2003), it may be that mussels at dissipative surf zone sites are digesting more diatoms as these are not only more abundant (Salant and Shanks 2018) but also more important for successful recruitment and metamorphosis of larvae (Pettersen et al. 2010). The enrichment in δ^{15}N at reflective surf zone sites is also consistent with this hypothesis. Mussels raised at dissipative surf zone sites also had significantly lower proportions of 20:1ω9 than that of the initial population and in those animals reared at reflective surf zones (Fig. 3), indicating food origin higher on the food web, perhaps in the form of copepods (Graeve et al. 1997) or other zooplankton (Alfaro et al. 2006). Mussels reared at dissipative and reflective surf zone sites were not significantly different in overall PUFA proportionally, but did exhibit differences in essential FAs; mussels reared at dissipative surf zones had elevated

proportions of 20:5ω3, and mussels at reflective surf zone sites had elevated 22:6ω3 (Fig. 4). This further suggests that mussels reared at dissipative surf zone sites were enriched with diatom biomarkers and those at reflective sites with dinoflagellate markers (Jaschinski et al. 2011).

The initial population was statistically indistinguishable from the mussels reared at the reflective surf zone sites, with the exception of 20:5ω3 (Table 2; Fig. 4). This is interesting given that the initial population was from Yoakam Pt. (YP), a dissipative site (Fig. 1). However, after 5 months, the biochemical profile of Yoakam Pt mussels was more similar to the other dissipative sites (Table 1; Supplementary Material Table S5). One potential explanation of these patterns is that winter phytoplankton populations are more similar to that of phytoplankton populations retained in reflective shores; the initial population was sampled in the in the winter, and phytoplankton concentrations were low in the winter (Salant and Shanks 2018, Fig. 2).

The biomarker results presented here should be considered within the context of the previous work at the same sites with the same species by Salant and Shanks (2018); our trophic biomarker results presented here indicate that surf zone type causes variations in subsidies for filter feeders, possibly contributing to those previously observed differences in growth rates and reproductive potential. Although we did not sample the biomarkers of phytoplankton itself in this study, weekly water samples were taken during the common garden growth experiment and the phytoplankton cells were enumerated in these samples. The high concentration of phytoplankton found in dissipative surf zones compared to reflective surf zone sites was mainly due to coastal diatom genera, and reproductive output and growth rates of mussels varied with phytoplankton concentration (Salant and Shanks 2018). Future studies should focus on sampling populations that have grown at these sites for years, and also sample additional intertidal filter-feeders. An addition of seston analysis would also have greatly enhanced our interpretation of the results, but was not available at the time.

FA biomarkers are rarely specific to one primary producer source alone; for example, kelps and red macroalgae, common members of coastal subtidal and intertidal rocky shore communities, are also rich in EPA (Galloway et al. 2012). Moreover, interpretation of FA biomarkers can be confounded by a general lack of understanding of whether dietary FAs are indeed transferred conservatively to consumers, a core but usually untested assumption of much of the biomarker literature (Galloway and Budge 2020); it is therefore important to approach biomarker analysis with a weight of "evidence" approach, combining interpretation of biomarkers with other lines of evidence, including other biomarker such as SI, growth, lipids, natural history knowledge gleaned from field experiments, and when available, controlled feeding experiment results and mixing model analysis (Budge et al. 2008).

In conclusion, we found that surf zone hydrodynamics influenced diet quality and composition in mussels in a 5-month common garden experiment. Generally, FAs indicative of diatom consumption were proportionally higher at dissipative sites (16:1ω7 and 20:5ω3), whereas FAs indicative of dinoflagellate consumption (22:6ω3) and organisms higher in the trophic food chain (20:1ω9) were proportionally higher at the reflective sites. However, both the SI and FA results may be limited to tissue type (only the adductor muscle was sampled), mussel age, and season (only sampled during the upwelling season). Overall, variation in diet in *M. californianus* mussels as indicated by SI and FA appears to be related to surf zone hydrodynamics, which, in turn, influences subsidies of food to the intertidal filter feeder community.

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