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LETTER

Sea urchin microbiomes vary with habitat and resource availability

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Scientific Significance Statement

Characterizing patterns in the microbial communities that dwell within key species is critical to understanding the role of microbes in shaping fundamental aspects of marine ecosystems. Sea urchins are key grazers in coastal seas, where they can survive a variety of conditions and diets, enhancing their ecological impact on kelp forests. Here we provide the first evidence that the two dominant sea urchin species in southern California have distinct gut microbiomes that vary with habitat. The taxonomic composition of the urchin microbiomes suggests that they may facilitate digestion of food and be a source of nutrition themselves. More work is needed to understand the extent to which their microbiome is the key to sea urchins' ecological success.

Abstract

Sea urchins are key grazers in coastal seas, where they can survive a variety of conditions and diets, enhancing their ecological impact on kelp forests and other ecosystems. Using 16S rRNA gene sequencing, we characterized bacterial communities associated with guts of the two dominant sea urchin species in southern California, the red urchin *Mesocentrotus franciscanus*, and the purple urchin *Strongylocentrotus purpuratus*. Our results show that the two urchin species have distinct gut microbiomes that vary with habitat. The taxonomic composition of their microbiomes suggests that they may facilitate digestion of food and be a source of nutrition themselves. These results highlight the role of microbiomes within macroorganisms as an extended ecological trait, and suggest that microbes may be crucial to resource use and partitioning in co-occurring species.

Sea urchins are key grazers in coastal marine ecosystems worldwide (Steneck 2020). When abundant, urchins can overgraze temperate reefs and kelp forests, creating urchin barrens where algae and other sessile organisms are sparse (Filbee-Dexter and Schiebling 2014). Modulated by predators, disease, or disturbance, urchin populations can therefore drive phase shifts in subtidal ecosystems from algal to coral states in the tropics, or from kelp to barren states on temperate reefs (Steneck 2020).

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Data Availability Statement: Data and metadata are available are available through the Environmental Data Initiative (https://doi.org/10.6073/pasta/201751391a4164340ed9a1c128dbab95). Sequence data are available in the Sequence Read Archive data repository through NCBI (https://www.ncbi.nlm.nih.gov/sra/PRJNA643824).

Additional Supporting Information may be found in the online version of this article.

Although generally considered herbivores, sea urchins are omnivores with catholic diets that can include sessile invertebrates (Elahi and Sebens 2012). Some urchin species can survive for long periods on very low or even no food, ceasing growth and reproduction and adsorbing their tissues (Lares and Pomory 1998). Living for decades, even over a century (Ebert and Southon 2003), urchins can survive extensive variability in their food supply. This tenacity can facilitate the long-term maintenance of extensive barrens on temperate reefs, as urchin populations persist even after resources are used up, preventing macroalgal recovery (Filbee-Dexter and Scheibling 2004). Despite the importance of these ecological processes, the mechanisms by which urchins persist in such conditions are not well understood.

Microbes, particularly bacteria, are profuse in sea urchin guts, where they have been hypothesized to break down macromolecules, aiding digestion and assimilation of food (Lawrence et al. 2013). The gut microbiome may facilitate digestion of recalcitrant material, allow the use of dissolved organic matter, and contribute key metabolites to the host (Apprill 2017). Although the urchin microbiome may be critical to the ecological success of these important and widespread marine grazers, we know little about it.

Two sea urchin species dominate southern California coastal waters, the red urchin *Mesocentrotus franciscanus*, and the purple urchin *Strongylocentrotus purpuratus* (Harrold and Reed 1985; Graham 2004). Here we compare the microbiomes of these two urchin species across three rocky reef habitats that differed in the types and availability of food resources: kelp forests, urchin barrens, and a hydrocarbon seep. Microbial community composition varied between species and among habitats and were most diverse and compositionally distinct in barren-dwelling urchins. This work provides new insights into phylogenetic and habitat-driven patterns of the urchin microbiome and how it may affect the ecology of these important grazers.

Methods

Site description

We sampled urchins from both urchin barrens and kelp forests at two reefs, Naples Reef (34°25′N, 119°57′W) and Arroyo Quemado (34°28′N, 120°07′W), from the kelp forest at Mohawk Reef (34°23′N, 119°43′W), which had no barren, and from Jackpot Seep (34°24′N, 119°52′W), at depths of 10–14 m from February 2016 to April 2016. Urchin barrens lacked giant kelp, and had sparse macroalgae and high densities of urchins. Jackpot Seep is a rocky reef with active hydrocarbon seeps (Ding and Valentine 2008) and little algae, where we observed urchins grazing on seep-associated microbial mats.

Sampling

Twelve adult sea urchins of each species were collected in each of the three habitats, totaling 36 per species (Supporting Information Table S1). To characterize the microbial communities of potential food for the urchins, we also collected giant kelp (*Macrocystis pyrifera*) blades (n = 6, three each from Mohawk Reef and Arroyo Quemado), and bacterial mats (n = 15 from Jackpot Seep). Giant kelp is considered the preferred food of both urchin species examined here (Leighton 1966; Foster et al. 2015).

Before dissection urchins were washed with autoclaved seawater and placed in 70% ethanol for 10 min to reduce external contaminants. Urchins were dissected in sterile petri dishes with sterilized tools; after removing the digestive system an approximately 5 cm region including the stomach and beginning of the intestine was placed in a sterile 1.5 mL tube, homogenized using a micropestle and frozen at −20°C.

DNA extraction

A Qiaamp Fast DNA Stool mini kit (Qiagen 51604) was used for DNA extraction of urchin gut, kelp, and microbial mat samples following the standard protocol, with modifications to remove inhibitors and improve DNA quality. In brief, rather than adding gut contents directly to the Inhibitex buffer in step 2, we transferred ~ 100 mg to a sterile 2 mL microcentrifuge tube containing 500 mL of phosphate buffered saline, ground them further, vortexed for 10 min, and transferred 200 μL to a new tube with 1 mL of Inhibitex buffer, and the final incubation time was increased to 40 min. Samples were quantified using a Qubit (3.0) fluorometer, diluted in TE buffer (pH 8.0) to 2 ng μL^{-1} and stored at −20°C for library preparation.

Library preparation and sequencing

The V3-V4 region of the 16S ribosomal gene was amplified with primers U341f (5′-CCTACGGGRSGCAGCAG-3′) and 785r (5′-GACTACHVGGGTATCTAATCC-3′) modified for the dual-index sequencing strategy for MiSeq. The 16S rRNA amplicon libraries were amplified in one round of polymerase chain reaction (PCR) in a thermocycler (Eppendorf Mastercycler Nexus gradient) using Phusion Flash Master Mix (#F-548L) and 8 ng of DNA. The temperature cycles were 98°C for 10 s, followed by 98°C for 1 s, 62°C 5 s, 72°C for 15 s repeated 30X, and 72°C for 60 s 1X. Two microliters of final product was run on a 1% agarose gel to confirm that only one band of ~ 465 bp was present, the expected length of the V3-V4 region. Amplicons were successfully prepared for 33 purple and 35 red sea urchins, 4 giant kelp, and 10 bacterial mat samples. Five negative controls containing reagents were included. All PCR products were normalized using SequalPrep Normalization plates (Invitrogen A1051001) and 10 μL of each was pooled for concentration in Amicon Ultra Filters (Ultra 0.5 mL UFC503096). The purified libraries were sequenced on an Illumina Miseq (MCS version 2.5.0.5) by the UC Davis Genome Center DNA Technologies Core.

Sequence analysis

MiSeq sequences were processed using the 16S QIIME Single-End pipeline implemented in Nephele using QIIME 1.9.1. The R1 forward read quality was significantly better than the R2 reverse read, so only R1 reads were used (Jovel et al. 2016). Operational taxonomic units (OTUs) were determined using the pick_open_reference_otus.py pipeline (Kopylova et al. 2016). OTUs were filtered to remove chimeras and any with relative abundance < 0.005%. Taxonomic assignments were made against Greengenes 13_8 reference database at 97% similarity (Caporaso et al. 2012). The OTU table was filtered for contaminants using the negative control samples ($n = 5$). Only one contaminant, *Pseudomonas*, was present in significant numbers in negative controls, and 39 OTUs out of 51 in this genus were removed. After filtering, the total sequences remaining were 1.4×10^6, the median sequence read count was 13,872 with a minimum of 3581 and maximum of 71,170.

Statistical analysis

We estimated microbial diversity using the bias-corrected Chao estimate of species richness (Chiu et al. 2014) and Pielou's index of evenness. Each sample was rarefied to a common 1078 reads prior to analysis (Supporting Information Table S2). We used two-way ANOVA to test for significant differences in microbiome diversity among sea urchin species and habitat types and Tukey tests for pairwise differences. We used negative binomial generalized linear models (Love et al. 2014) on the unrarefied OTU data (Love et al. 2014) to identify which OTUs significantly differed across species and habitat types; a Wald post hoc test and p values were adjusted for multiple comparisons (Benjamini and Hochberg 1995).

To investigate the relationship between compositional variation in the microbial community (beta diversity), urchin species, and habitat types, we modeled the rarefied OTU table as a function of the two urchin species and three habitats using redundancy analysis (RDA). We selected significant variables by forward selection (Blanchet et al. 2008) and tested the significance of the relationship based on 999 permutations (Legendre et al. 2011). We then partitioned the total variation in microbial composition into the relative and unique contribution of habitat types and urchin species (Peres-Neto et al. 2006). Finally, we used an analysis of multivariate homogeneity of group dispersions (Anderson et al. 2006) to quantify variability in microbial composition within each group of samples (species × habitat). We used two ecological distance measures, Hellinger distance (Legendre and Gallagher 2001) and weighted UniFrac distance (Lozupone and Knight 2005) to account for phylogenetic relatedness among OTUs.

All analyses were performed in R 3.4.3 (R Core Team 2017). Alpha diversity was computed using the *estimate_richness* function from the package phyloseq (McMurdie and Holmes 2014). The negative binomial generalized linear models were fitted using the package DESeq2 (Love et al. 2014). We used the *rda*, *varpart*, and *betadisper* vegan package functions for RDA, variation partitioning, and dispersion analysis, respectively. OTU tables and complete metadata are published in the Environmental Data Initiative repository (Miller et al. 2021).

Results

We detected 408 OTUs across all samples, comprising 52 families and 22 classes of bacteria. The two most abundant OTUs across all samples were OTU 393, genus *Achromobacter* (Alcaligenaceae, 27% of assigned reads) and OTU 167, family Campylobacteraceae (13%, Supporting Information Table S3). The two most prevalent OTUs found in *Mesocentrotus franciscanus* were also OTU 167 (30%) and 393 (28%), while *Achromobacter* OTUs 393, 397, and 396 made up 38% of all reads in this species (Fig. 1 and Supporting Information Table S3). In *S. purpuratus*, however, OTUs from Campylobacteraceae were rare (< 1%). The three most prevalent OTUs in this species were also OTU 393, 397, and 396 (35%, 7%, and 6%, respectively) while four OTUs from order Bacteroidales made up 15% of all reads in this species (Fig. 1 and Supporting Information Table S3). OTUs from genus *Achromobacter* were prevalent in all three habitats (Fig. 1 and Supporting Information Table S3).

Microbial taxonomic richness based on the Chao estimate significantly differed across habitat types ($F_{2,6} = 6.469$, $p = 0.003$) but not urchin species ($F_{1,66} = 0.354$, $p = 0.554$), reflecting the higher richness in the guts of barren-dwelling urchins (mean richness = 54.125; Fig. 2) compared to kelp forests (34.055; $p = 0.002$) and seeps (5.255; $p = 0.003$). Richness was also much higher in the bacterial mats (74.131) and kelp blades (72.642). Evenness also significantly differed across habitat types ($F_{2,66} = 6.974$, $p = 0.002$, Fig. 2); barrens exhibited lower evenness (0.054) than kelp forests (0.067; $p = 0.003$) and seeps (0.067; $p = 0.003$). Evenness did not differ across urchin species ($F_{1,66} = 2.804$, $p = 0.010$).

The generalized linear models revealed 45 OTUs that significantly differed across species and habitats (Supporting Information Fig. S1 and Table S4). Eight OTUs were more abundant in *Mesocentrotus franciscanus* than in *S. purpuratus*, including OTU 35 (family Colwelliaceae), 303 (Rhodobacteraceae), and four OTUs in order Campylobacterales. Thirty-six OTUs were more prevalent in barren-dwelling urchins relative to the two other habitats. These included family OM60 of Order Alteromonadales, family Desulfobulbaceae, including genus *Desulfocapsa* and *Desulfotalea*, and seven OTUs in order Bacteroidales.

Microbial composition significantly varied across species and habitats ($R^2_{adj} = 0.211$, $F_{3,66} = 7.138$, $p = 0.001$; Fig. 3). 11.3% of the total variation was explained by species ($F_{1,66} = 10.578$, $p = 0.001$) and 10.2% by habitat ($F_{2,66} = 5.411$, $p = 0.001$). The first two axes of the canonical relationship

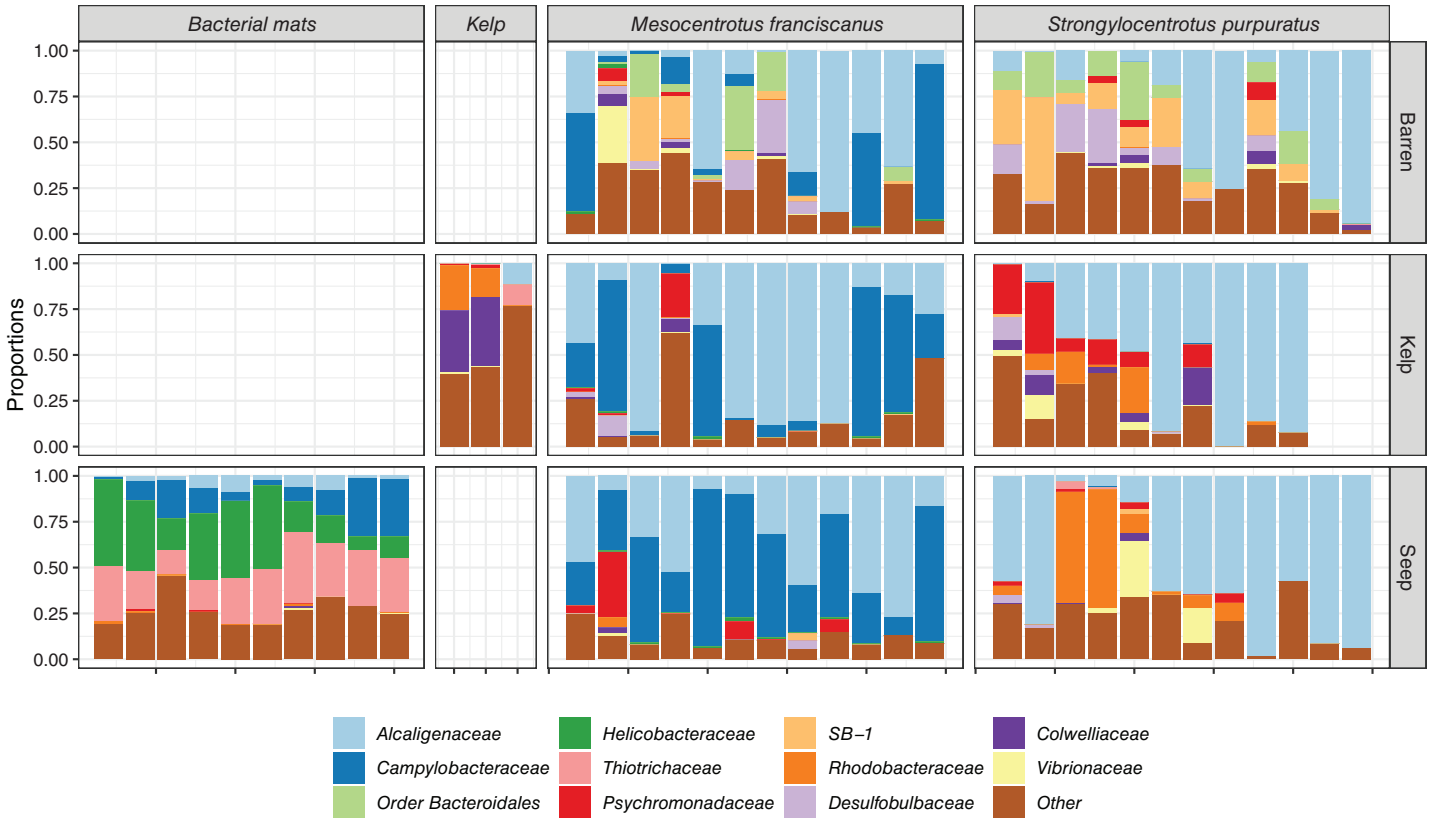

Fig. 1. Stacked bar plot of microbiome community structure within each sample across habitats and sea urchin species. Each bar represents the relative proportion of each of the top 11 families, with remaining families grouped as one category (other).

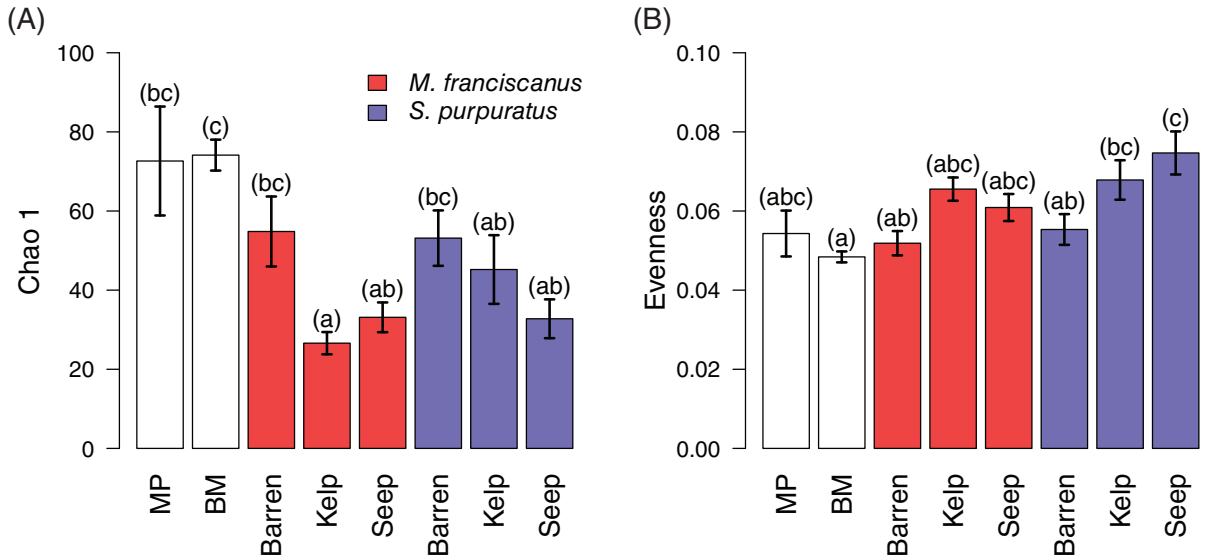

Fig. 2. Microbial diversity across food types (BM, bacterial mat; MP, *Macrocystis pyrifera* blades), species of sea urchins and habitat types, represented as the Chao estimate of species richness (**A**) and the Pielou's index of evenness (**B**). Purple sea urchins (*S. purpuratus*) and red sea urchins (*Mesocentrotus franciscanus*) are displayed in red and purple, respectively. Bars represent ± 1 standard error and letters correspond to significant pairwise differences.

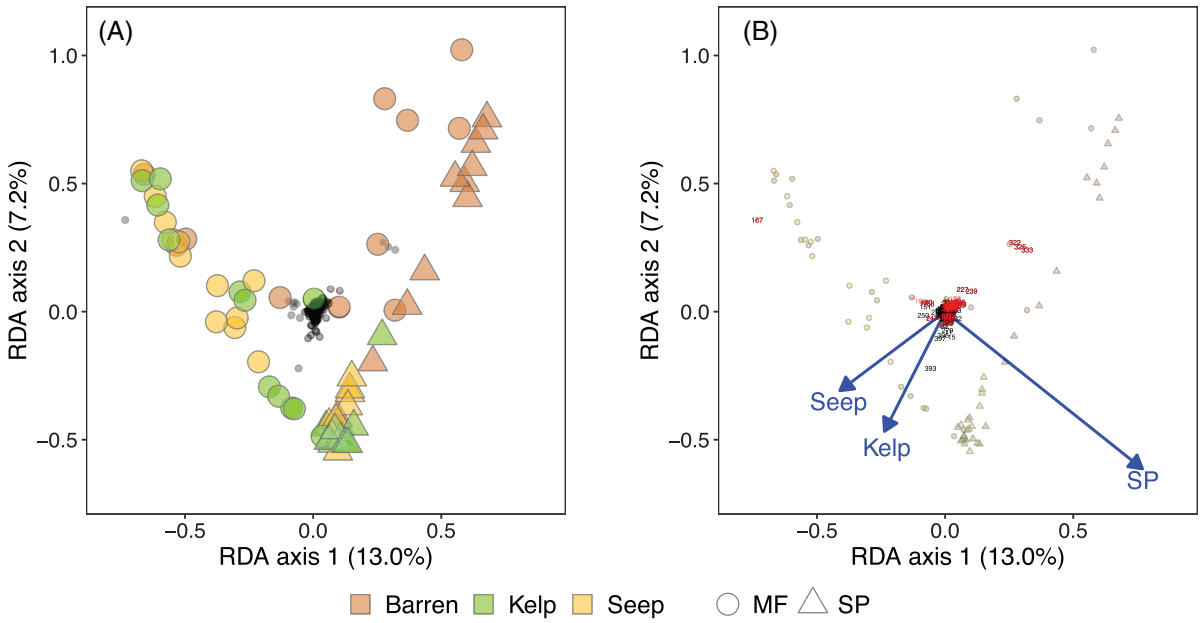

Fig. 3. RDA biplots illustrating the relationship between microbial composition across species of sea urchin and habitat types. (**A**) Samples are colored by habitat types and shapes correspond to species. OTUs are outlined in the background as gray dots. (**B**) Explanatory variables and OTUs: explanatory variables are represented by vectors; vector length indicates the relative weight of a given variable in the ordination, and direction indicates the correlation of that variable with each axis.

were significant. The first axis (R^2_{adj} = 0.130, $F_{1,66}$ = 13.176, p = 0.001) captured significant differences between the two species of urchins (Fig. 3). In particular, samples were clustered into an *Mesocentrotus franciscanus* group on the left and an *S. purpuratus* group on the right side of the RDA biplot (Fig. 3). In agreement with the generalized linear model, the *Mesocentrotus franciscanus* cluster was associated with higher counts of OTU 167 from family Campylobacteraceae. In *S. purpuratus*, three OTUs in the order Bacteroidales including the family SB-1 (OTU 333) were most abundant. The second axis of the RDA (R^2_{adj} = 0.072, $F_{1,66}$ = 7.278, p = 0.001) explained more complex differences between habitats. In particular, this axis separated *S. purpuratus* individuals by habitat. Barren-dwelling individuals were associated with higher abundance of OTUs 322, 333, and 325 from the order Bacteroidales. Further habitat-driven differences were revealed by separate analyses of *Mesocentrotus franciscanus* (R^2_{adj} = 0.097, $F_{2,33}$ = 2.871, p = 0.002) and *S. purpuratus* (R^2_{adj} = 0.162, $F_{2,31}$ = 4.202, p = 0.001, Supporting Information Fig. S2). In both species, barren-dwellers were associated with higher abundances of the three Bacteroidales OTUs. Adding bacterial mats and giant kelp blades increased the variation explained by our canonical relationship to 30.8% ($F_{5,77}$ = 8.299, p = 0.001: Supporting Information Fig. S3) and the RDA biplot illustrates how distinct the microbial composition of these samples was. Using UniFrac distance did not change our results as species (R^2_{adj} = 0.071, $F_{1,66}$ = 6.459, p = 0.001) and habitat types (R^2_{adj} = 0.060, $F_{2,66}$ = 3.5320, p = 0.002) explained about the same amount of the microbial compositional variation, suggesting that phylogenetic relatedness among OTUs did not influence the results. Finally, variability in microbial composition, based on dispersion analysis, did not vary within sea urchin species ($F_{1,68}$ = 0.960, p = 0.331) or habitat type ($F_{2,67}$ = 0.486, p = 0.617).

Discussion

In this first characterization of the gut microbiome of the two dominant shallow water sea urchin species of California, we found significant interspecific differences in microbiome community composition as well as differences between urchins living in different habitats, including urchin barrens. Red and purple sea urchins are often considered to be ecologically equivalent grazers in kelp forest ecosystems, even though they may have different feeding preferences, activity patterns, and competitive relationships (Rogers-Bennett 2013). Our results demonstrate that *S. purpuratus* and *Mesocentrotus franciscanus* also have distinct gut microbiomes, suggesting systematic differences in feeding ecology and gut physiology, and reinforcing the view that the microbiome is an important ecological trait (Scott et al. 2020).

Urchins of both species in food-poor barrens hosted a different, more diverse microbiome than their congeners living in kelp forests. Urchin barrens are dominated by coralline algae, with poor food resources for urchins (Steneck 2020). Their microbiome could help digest recalcitrant material such

as coralline algae and detritus, and transfer nutrients from suspended particulates or dissolved organic matter to the urchins, either through bacterial exudation or digestion of excess or senescing cells (Thomas et al. 2011; Apprill 2017).

In kelp forests, sea urchins tend to thrive on detrital kelp (Harrold and Reed 1985). Nevertheless, urchins also consume many other species, including algae and sessile invertebrates (Elahi and Sebens 2012). Little information is available regarding the feeding preferences of these two species. It is often assumed that both prefer kelp, particularly *Macrocystis pyrifera*, based on laboratory experiments (Leighton 1966; Foster et al. 2015), though growth may be equally sustained on other algal species (Foster et al. 2015). In the field, urchins may consume a more varied diet. For example, purple urchins in a kelp forest primarily consumed geniculate coralline algae (Kenner 1992). Red urchins consumed mainly drift kelp inside a kelp forest, and foliose red algae outside the forest (Mattison et al. 1976).

Campylobacteraceae were abundant in the guts of red urchins, but not purple urchins, across habitats. This may be consistent with the dependence of red urchins on detrital material if Campylobacteraceae is prevalent on detritus as it was in bacterial mats. The differences we found in the microbiome of these two urchin species across habitats suggests that differences in diet are typical, and more information is needed to quantify the diet of these key grazers.

Sea urchins near active hydrocarbon seeps had a gut microbiome distinct from those in other rocky reef habitats. Abundant microbial mats grow near hydrocarbon seeps off Coil Oil Point (Ding and Valentine 2008), including Jackpot Seep, where we observed urchins feeding on the mats. A diverse community forms these mats, including bacterial methanotrophs and sulfur-oxidizers as well as eukaryotic phototrophs, and they contain abundant fatty acids (Ding and Valentine 2008; Paul et al. 2017).

More metagenomic data and experimental studies are needed to elucidate the function of gut microbes in marine organisms, including sea urchins. Gut microbes have been thought to facilitate food digestion and absorption in marine herbivores (Scott et al. 2020). Fong and Mann (1980) showed that gut flora of the green sea urchin, *Strongylocentrotus droebachiensis*, synthesized and transferred amino acids to the host, increasing the nutritional value of consumed kelp. Some of the abundant taxa we found in urchin guts have also been found in other marine species. For example, members of the family Vibrionaceae were found to differentiate herbivorous fish (Scott et al. 2020), and have been implicated in nitrogen fixation in sea urchin guts (Guerinot and Patriquin 1981). The family Campylobacteraceae, a dominant member of the *Mesocentrotus franciscanus* microbiome, was also dominant in the microbiome of the sea urchin *Lytechinus variegatus*, and predictive metagenomics suggested that these microbes were metabolizing carbohydrates, amino acids, and lipids (Hakim et al. 2016). We found that Campylobacteraceae were sparse in *S. purpuratus*, but a previous examination of the microbiome of three individual *S. purpuratus* from a tide pool in Oregon found abundant Campylobacteraceae (*Arcobacter*, Hakim et al. 2019), underscoring the possibility of habitat-driven differences in gut microbiomes of urchins.

The genus *Achromobacter* was abundant in both urchin species, and much less abundant in the food sources (Fig. 1, Supporting Information Table S1). Many strains of this genus are known for production of biosurfactants (Deng et al. 2016). They are also common in the guts of wood-eating termites, where they break down cellulose (Femi-Ola and Oyebamiji 2019). Most intriguingly, Ehsani et al. (2019) showed that *Achromobacter* became highly abundant in enriched hydrogen-oxidizing microbiomes, where they converted mineral nitrogen and carbon dioxide directly into microbial biomass. This autotrophic production is a promising source of protein production since hydrogen-oxidizing microbes have elevated levels of high-quality protein with desirable amino acid profiles (Ehsani et al. 2019). The potential benefit of such production to sea urchins is clear, particularly in food-poor habitats.

Our data set revealed novel interspecific and habitat-related patterns in the gut microbiome of *S. purpuratus* and *Mesocentrotus franciscanus*, the two dominant sea urchin species on subtidal rocky reefs off southern California. While the mechanisms driving these patterns are yet unclear, they point to likely functional differences related to feeding, and possibly even autotrophic production. If the latter proved true, sea urchins might, via their microbiome, have the capacity for mixotrophy, an invaluable tool in a world of variable nutritional resources.

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