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RESEARCH ARTICLE

Kelp and sea urchin settlement mediated by biotic interactions with benthic coralline algal species

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Abstract

Species interactions can influence key ecological processes that support community assembly and composition. For example, coralline algae encompass extensive diversity and may play a major role in regime shifts from kelp forests to urchin-dominated barrens through their role in inducing invertebrate larval metamorphosis and influencing kelp spore settlement. In a series of laboratory experiments, we tested the hypothesis that different coralline communities facilitate the maintenance of either ecosystem state by either promoting or inhibiting early recruitment of kelps or urchins. Coralline algae significantly increased red urchin metamorphosis compared with a control, while they had varying effects on kelp settlement. Urchin metamorphosis and density of juvenile canopy kelps did not differ significantly across coralline species abundant in both kelp forests and urchin barrens, suggesting that recruitment of urchin and canopy kelps does not depend on specific corallines. Non-calcified fleshy red algal crusts promoted the highest mean urchin metamorphosis percentage and showed some of the lowest canopy kelp settlement. In contrast, settlement of one subcanopy kelp species was reduced on crustose corallines, but elevated on articulated corallines, suggesting that articulated corallines, typically absent in urchin barrens, may need to recover before this subcanopy kelp could return. Coralline species differed in surface bacterial microbiome composition; however, urchin metamorphosis was not significantly different when microbiomes were removed with antibiotics. Our results clarify the role played by coralline algal species in kelp forest community assembly and could have important implications for kelp forest recovery.

KEYWORDS
biofilm, bottom-up effects, calcified algae, kelp forest, kelp spores, macroalgae, restoration, sea urchin metamorphosis, seaweed, urchin barren

INTRODUCTION

Community dynamics are influenced by both positive and negative species interactions (Baskett & Salomon, 2010; Bertness & Callaway, 1994; Scheffer & Carpenter, 2003). Understanding key interspecific interactions may help clarify mechanisms in fundamental ecological processes, such as the maintenance or shifts in stable ecosystem states. Kelp forests are important and productive coastal ecosystem, having

Abbreviations: ASV, amplicon sequence variant; bp, base pair; CGEB, Centre for Comparative Genomics and Evolutionary Bioinformatics; IMR, Integrated Microbiome Resource; morpho-groups, morphologically identified groups; PCR, polymerase chain reaction; rRNA, ribosomal RNA.

net primary production rates up to 10 times higher than average coastal phytoplankton productivity (Pessarrodona et al., 2022). These coastal marine forests provide an array of ecosystem functions such as nutrient cycling, food, and habitat for many marine species (Mann, 1973; Smale et al., 2013; Teagle et al., 2017). Worryingly, kelp forests are vulnerable to a number of abiotic drivers, such as temperature, and biotic top-down drivers, such as declines in key predators, which have resulted in declines around the globe (Krumhansl et al., 2016; Rogers-Bennett & Catton, 2019; Wernberg et al., 2013). Most notably there have been regime shifts from healthy kelp forest ecosystem states, which support high levels of biodiversity, to alternate urchin-barren ecosystem states that are devoid of any kelp species and have reduced ecosystem services (Filbee-Dexter & Scheibling, 2014; Mann & Breen, 1972; Sala et al., 1998). The increased prevalence of these barren states has resulted in renewed efforts to further understand regime shifts and how to actively promote kelp forest recovery (e.g., Eger et al., 2022; Layton et al., 2020). Despite these efforts, one important aspect often overlooked in these nearshore ecosystems is the bottom-up role that other macroalgal species could play in shaping these kelp forest and barren regime shifts.

Calcifying coralline algae are one such group of macroalgae that are diverse and abundant in kelp and barren ecosystems and are known to play important ecological roles (McCoy & Kamenos, 2015; Nelson, 2009). For example, coralline algae provide settlement/metamorphic cues for invertebrate species, act as habitat for grazing and cryptofaunal species, influence the settlement of other macroalgae, and prevent erosion by stabilizing reefs (Bosence, 1983; O'Leary et al., 2017; Piazzi et al., 2022; Tebben et al., 2015; Vermeij et al., 2011). Complicating matters is the fact that the ecological roles played by coralline algae have been shown to be species-specific (e.g. Villas Bôas & Figueiredo, 2004, O'Leary et al., 2017, Méndez Trejo et al., 2021), and coralline algal species can be difficult to identify morphologically (Hind et al., 2014; Twist et al., 2020). Moreover, ongoing molecular work has revealed many cryptic species with far greater diversity than previously thought in this group of algae (e.g., Hind & Saunders, 2013; Mills et al., 2022; Twist et al., 2019), and the importance of coralline algal diversity in key ecological processes remains unclear (Hind et al., 2019). Past studies that relied on morphological identification alone may have drawn incomplete or incorrect conclusions if they inadvertently grouped several species with different ecological functions together (see Twist et al., 2020).

In recent years, the use of molecular identification methods has increased our understanding of coralline algal ecology (e.g., Deinhart et al., 2022; Hind et al., 2019; O'Leary et al., 2017). For example, molecular-assisted identifications have shown that coralline abundance, diversity, and species composition differ between healthy kelp forest and urchin-barren habitats (Hind et al., 2019), likely resulting from top-down grazing control of coralline species dominance and community composition (Paine, 1984, 1990; Steneck et al., 1991). Differences in benthic coralline algal communities between stable ecosystem states raises questions about the facultative role coralline algal species may play in the recovery of kelp forests and the persistence of urchin barrens. Here, we examined whether coralline algal diversity, through interspecific species interactions, affects key ecological bottom-up processes that may help maintain or shift stable ecosystem states. Specifically, our aim was to test whether the metamorphosis of sea urchin larvae and the settlement of kelps differed between different molecular-identified coralline algal species observed in alternate stable states of kelp and barren habitats and whether these effects were mediated by surface bacterial microbiota.

Species-specific metamorphosis on different coralline algae has been shown for some urchin species (Huggett et al., 2006; Taniguchi et al., 1994) although this has not been supported by any molecular-assisted species identifications. Additionally, Baskett and Salomon (2010) theorized that elevated recruitment facilitation by coralline algae in urchin-barren habitat is a potential mechanism for positive feedback loops. There has been wide debate, however, about whether invertebrate larval metamorphosis cues originate from the coralline alga itself or the associated surface bacterial microbiome (Huggett et al., 2006; Johnson & Sutton, 1994; Pearce & Scheibling, 1991). Recent studies have shown differences in surface microbiome communities between closely related macroalgal species (Lemay et al., 2021) and the increasingly interconnected role microbiomes play in invertebrate larval settlement (Quinlan et al., 2019). We, therefore, hypothesized based on these prior studies that urchin larval metamorphosis would differ between coralline algal species, and specifically, that metamorphosis would be greater on coralline algal species that are more abundant in urchin barren than in kelp forest habitats. Further, we hypothesized that differences in associated surface microbiomes would contribute to these trends, and we expected differences in the percentage metamorphosed when experimentally manipulating the microbiota. In addition to influencing invertebrate metamorphosis, coralline algae have also been shown to inhibit overgrowth of other macroalgal species even in the absence of grazing, often thought to be due to either chemical inhibition or sloughing of the surface layers of cells (Johnson & Mann, 1986; Suzuki et al., 1998). However, the extent of inhibition has been shown to vary widely among macroalgal species (Kim et al., 2004). Therefore, we hypothesized that for kelp

species central to kelp forest succession, sporophyte development would be inhibited by coralline species found in urchin-barren habitats, but not by those located in kelp forests, which would help maintain alternative ecosystem states.

We tested these hypotheses using a series of laboratory experiments examining differential metamorphosis of urchin larvae and settlement of kelp spores on several coralline algal species (identified using molecular methods) abundant in either kelp or urchin-barren habitats along the coast of British Columbia (BC), Canada. Specifically, we examined red sea urchin (*Mesocentrotus franciscanus*) metamorphosis rates and the settlement of the two largest canopy kelps, the giant kelp (*Macrocystis pyrifera*) and bull kelp (*Nereocystis luetkeana*), and the subcanopy five-ribbed kelp (*Costaria costata*) in the presence of several coralline algal species. Given the vulnerability of coralline algae to ocean acidification and warming (Cornwall et al., 2019; Guenther et al., 2022), understanding the ecological services provided by coralline algae and their influence on kelp forest dynamics is becoming increasingly important.

METHODS

Coralline algal identification and relative abundance

To examine the effect of coralline algal species on urchin larval metamorphosis and kelp settlement various coralline algal specimens were collected from kelp forest and urchin-barren habitats around Calvert Island, BC (51°39′15.1″ N, 128°07′50.0″ W), placed into morphologically identified groups (morpho-groups), aided by prior extensive molecular identifications of coralline algal species from the collection sites, and later identified using molecular methods (see Appendix S1 in the Supporting Information: Methods A). In addition, to determine if settlement and metamorphosis were higher on coralline algae found in greater abundance in urchin barren compared with kelp forest habitats, the relative abundances of coralline algal species in these two habitats was calculated using data from Hind et al. (2019; see calculations in Appendix S1: Methods B).

Microbiome community

To examine if microbiome differences occurred across coralline algal species and bare rock, surface bacterial samples were taken by swabbing the surfaces of several coralline algal species in the field. Due to constraints of collections at the time, no samples were taken of articulated coralline algae, which were later used in the metamorphosis experiment. All microbial samples were taken from coralline algae on boulders from one site, located off the northeast side of Rattenbury Island near Calvert Island (51°42′03.6″ N, 128°04′27.4″ W), to avoid differences that may occur across sites. Boulders were lifted from the seafloor (4–5 m deep) using a fish net and lift bag onto a boat. Microbiome samples were taken either directly on the boat or in the laboratory. Each coralline algal species or bare rock was rinsed with 0.22-μm filtered seawater in order to remove non-host associated environmental microbes and was then swabbed for 10 s for microbial communities using a sterile swab. Swabbing was done on an approximate 1–2 cm area of crust or bare rock, with all coralline algal crusts that looked different being swabbed across 35 boulders. We sampled microbial communities from seawater samples taken at the surface of the sampling site using sterile 500-mL plastic containers ($n = 5$), filtered using 0.22-μm membrane filters. Additionally, swabs were taken to control for contamination from the nets used to lift the boulders ($n = 2$) and hands used by the divers ($n = 1$). Finally, a small tissue sample of each of the coralline algal samples that were swabbed was taken and placed in silica gel for later molecular identification (see Appendix S1: Methods A). Only swabs from coralline algae with a confirmed molecular identification were used in the microbiome analysis.

DNA was extracted from swabs and filters using the MoBio PowerSoil-htp 96-well DNA extraction kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer's protocol. Extracted DNA was sent to the Integrated Microbiome Resource (IMR), Centre for Comparative Genomics and Evolutionary Bioinformatics (CGEB, Dalhousie University, Halifax, Nova Scotia, Canada) for PCR amplification and library construction. Primers F515 and R926 targeted the V4–V5 region of the 16S rRNA gene for bacteria and archaea (Quince et al., 2011). Amplicon library preparation and sequencing with Illumina MiSeq using paired-end (2 × 300 bp) v3 chemistry was performed at the IMR CGEB.

Primers from raw sequences were first trimmed using Cutadapt 2.9 (Martin, 2011) with the parameters e = 0.1 and m = 100. Trimmed sequences were then processed using the R package dada2 (Callahan et al., 2016). Reads were quality filtered using the filterAndTrim dada2 R function (with parameters maxEE = 2, truncQ = 2, and truncLen = 270 and 210 for forward and reverse reads, respectively) and merged using the mergePairs function; chimera were removed using the removeBimeraDenovo function. We assigned the taxonomy for each amplicon sequence variant (ASV) using the IDTaxa algorithm implemented in the R package DECIPHER (Wright, 2016) with the SILVA (version 138) database (Glöckner et al., 2017). We only kept bacterial ASVs and removed all ASVs not assigned to a family and assigned to mitochondria or chloroplast or with length > 380 bp. Sequencing depth varied from

1131 to 46,006 reads per sample (median = 15,491). Amplicon sequence variant tables were rarefied to 1000 reads per sample for all analyses in order to keep all the samples while having enough sequencing depth. Non-rarefied ASV tables were also used with compositionally aware methods to determine robustness of results (see below for further details).

Urchin metamorphosis experiment

A single piece (~2.5 cm^2) of each coralline algal morpho-group (later identified using molecular methods, Appendix S1: Methods A) was placed in a 90-mL plastic jar with 50 mL of 1-μm filtered seawater in a randomized block design with one replicate from each morpho-group in each block. "Blank" jars with no coralline algae were included as controls to account for "natural" metamorphosis without any coralline algal cue. Along with the control, we compared settlement on 12 coralline algal morpho-groups and a fleshy red algal crust. There was a total of eight replicate jars for each algal morpho-group or control treatment, with the exception of two coralline morpho-groups that each had six replicate jars (Figure 1a, Appendix S2 in the Supporting Information: Table S1).

In addition, a further eight replicates of a subset of eight coralline algal morpho-groups and the control were treated with antibiotics to reduce/remove the biofilm on the coralline algal surface (see antibiotic methods below) and were placed in the jars in every second block. For the control treatment without coralline algae, antibiotics were added to the jar to test if they had a lingering effect on the jar surface, which affected sea urchin larval metamorphosis. This resulted in a total of 180 jars split into 16 blocks (eight blocks of antibiotic treatment and eight untreated) with one individual from each morpho-group (or control) in each block (Figure 1a).

To experimentally reduce the microbiome, an antibiotic treatment was applied in which coralline algae were soaked in 30 mL of a sterile (autoclaved at 100°C for 1 h) seawater antibiotic solution in 90-mL plastic jars for 72 h using the antibiotics chloramphenicol (50 $mg{\cdot}L^{-1}$), tetracycline (30 $mg{\cdot}L^{-1}$), streptomycin (30 $mg{\cdot}L^{-1}$), and penicillin (18 $mg{\cdot}L^{-1}$ or 30,000 units) following modified protocols by Johnson and Sutton (1994) and Gómez-Lemos et al. (2018). Following this treatment, the jars and coralline specimens were rinsed thoroughly with sterile seawater to remove any residual antibiotics and dislodge any dead cells and were subsequently placed back into the jar with fresh 1 μm filtered seawater for the urchin metamorphosis experiment (see above). To test the effectiveness of the antibiotics in reducing the microbiome on the surface of the corallines, swabs from before and after the antibiotic treatment were taken for a subsample of the antibiotic-treated corallines (three paired samples from each of the eight morpho-groups). Coralline algal specimens were rinsed with sterile seawater prior to taking the swabs to remove non-host associated environmental microbes and residual antibiotics. Twelve randomly selected swab samples across morpho-groups were then extracted using DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) following the manufacturer's protocols and

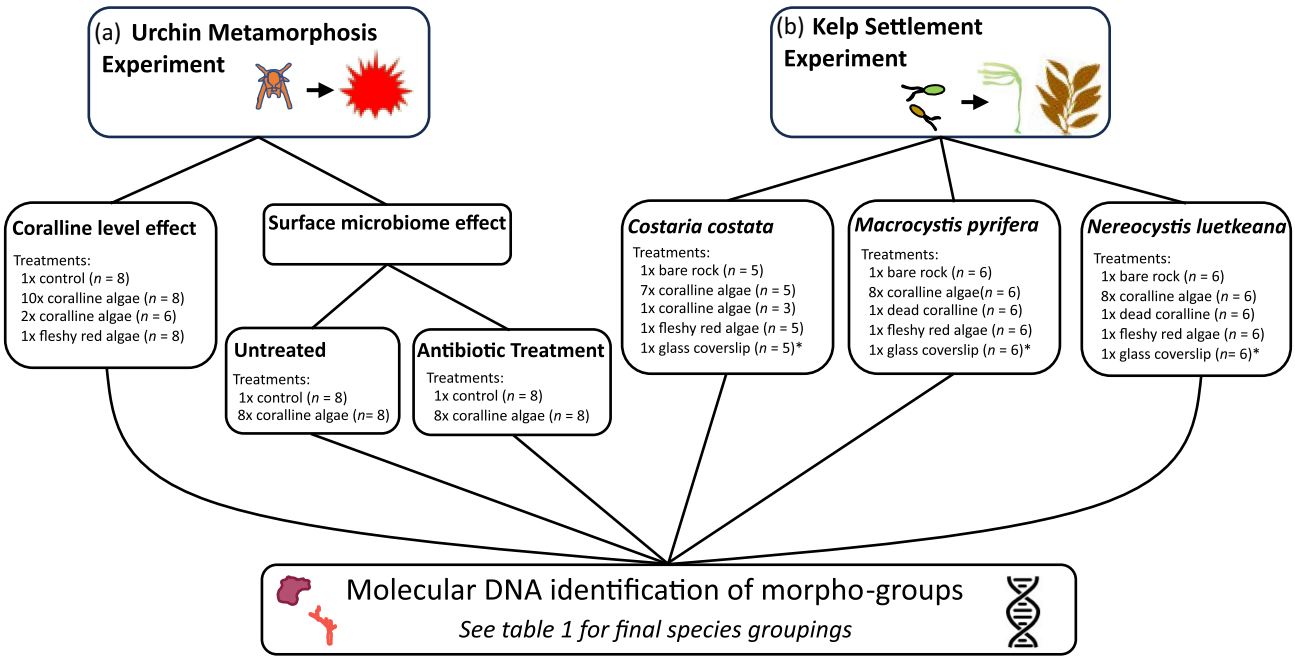

FIGURE 1 Schematic diagram of the experimental design used in manipulations of coralline algae to test (a) urchin metamorphosis and (b) kelp settlement. Treatments are given as number of groups/morpho-groups and the number of replicates (*n*). *Glass cover slips were used to track changes in kelp gametophyte/sporophyte development and not used in analysis.

then amplified (98°C for 10 s, 30 cycles of 98°C for 1 s, 50°C annealing for 5 s, 72°C extension for 24 s, followed by 72°C final extension for 1 min) in the V4 region of 16S rRNA gene using the primers F515 and R806 (Caporaso et al., 2011). The PCR samples were then cleaned using a Mag-Bind Total Pure NGS magnetic bead kit (Omega Bio-Tek, Norcross, GA, USA), and along with raw DNA samples, were quantified using a dsDNA (HS) assay on a Qubit 2.0 fluorometer. The values from the Qubit readings from before the antibiotic treatment were then compared with those from after to determine if there was a reduction in DNA and PCR product, indicating the reduction of the load of the associated microbiome.

Prior to adding the urchin larvae to the experiment, the seawater was replaced with fresh seawater after antibiotic treatment (including in the untreated jars). Competent red urchin larvae (20 per jar) were released and reared in the laboratory (see methods in Appendix S1: Methods C). Larvae were then transferred from holding containers into each of the coralline jars (80 mL final volume) and maintained at 12°C on a 12:12 h fluorescent light:dark cycle. After 24 and 48 h, larvae were counted under a dissecting scope and were classified as completely metamorphosed (i.e., tube feet present) or unmetamorphosed, working in order from the first block to the last, thus enabling statistical accountability of the time effect (~8 h). A total of eight data points were removed: four due to the number of metamorphosed individuals being higher at 24 than at 48 h (and thus considered to be miscounts) and a further four as multiple algal species were observed in one jar after closer examination of the algal specimens under the microscope.

Kelp settlement experiments

Spore solutions released from reproductive tissue (see methods in Appendix S1: Methods D) from three kelp species, canopy species *Macrocystis pyrifera* and *Nereocystis luetkeana* and subcanopy species *Costaria costata*, were pipetted into six-well (3.5-cm diameter Thermo Scientific™ BioLite™ Microwell Plates) plates containing coralline algal specimens (~2.5 cm^2) and 10 mL of sterile seawater to achieve a final density of 20,000 spores·mL^{-1} (Figure 1b). For *C. costata*, the settlement experiment included seven coralline algal morpho-groups with five replicates each. Settlement experiments with the other two kelp species included eight coralline algal morpho-groups with six replicates each. Clean microscope coverslips were used as controls for spore settlement in the absence of coralline algae in all three experiments and to track gametophyte development under the compound microscope. In addition, bare rocks (collected from the same sites as the coralline algae), dead coralline algae (specifically dead *Crusticorallina* spp.), and fleshy red algal crusts (later primarily identified as *Hildenbrandia* spp. through genetics and microscopy) were also used in all kelp settlement experiments using the same number of replicates indicated above. The algal and control treatments were arranged in a randomized block design with the six-well plates, where one replicate of each treatment was sequentially randomly assigned to each plate so that no one plate had more than one replicate of each treatment. Immediately after the spore solution was added to the wells, the plates were wrapped in foil to reduce light levels to zero in order to promote settlement of the kelp spores. Subsequently, the foil was removed after 24 h, and the plates were stored in an incubator at 10°C with 15–25 μmol of fluorescent light on a 12:12 h day:night cycle. Seawater was changed weekly, which involved removing specimens from the well plate, scrubbing the wells clean with freshwater to prevent growth of biofouling on the sides of the wells, adding clean sterile seawater, and replacing the specimens into the appropriate wells. After the first week, F/2 nutrients (Andersen, 2005) and 0.66 mg·L^{-1} germanium dioxide were added to autoclaved seawater to facilitate kelp gametophyte growth and prevent diatom growth, respectively. After 5–6 weeks, when gametophytes were well developed (observed on the control coverslips), the light levels were increased to 40 μmol on a 16:8 h day:night cycle, and the temperature was increased to 12°C to facilitate sporophyte development.

The number of sporophytes on the coralline algae and control surfaces were counted under a dissecting microscope (≤4.5× magnification) when sporophytes had developed to a size large enough to effectively count (i.e., 50–200 μm in length). This occurred after 7–9 weeks, depending on the kelp species. When the density of sporophytes was too high to count for the entire replicate, three randomly selected areas were counted and averaged. The sporophyte density per cm^2 was calculated for each coralline alga and control, based on the total area of the coralline (or control) as measured in ImageJ. A second count of the number of sporophytes was made 1 week after the first count and the highest density from the two timepoints was used in the analysis in order to account for slight delays in the development of kelp sporophytes that could have occurred among different coralline species.

Statistical analysis

All analyses were conducted with the statistical program R (v 4.2.1, R Core Team, 2022). Differences in microbiome community composition between coralline algal species were visualized using non-metric multidimensional scaling (nMDS) on Bray-Curtis dissimilarities between samples. A PERMANOVA (9999 permutations) was used to test for the effect of substrate type and host species on microbiome community structure using

the adonis2 function in the R package Vegan (Oksanen et al., 2017). We reported omega2 as effect sizes of the PERMANOVA models (equivalent to adjusted R^2) and calculated type II sums of squares (marginal effects) when jointly estimating the effect of host species and boulder identity on community composition. To measure the robustness of the microbe community composition results, we also used an alternative compositionally aware method (Quinn et al., 2018): the Aitchison distance metric and PCoA calculated on non-rarefied data.

To examine the differences in the percentage urchin larval metamorphosis across substrate type, independently for both 24- and 48-h observation periods, two one-way ANOVAs were undertaken with substrate (control, fleshy red crust, or coralline algal species) as the dependent variable. The assumption of equal variance was violated for both ANOVAs, which transformations did not help; therefore ANOVAs were run using heteroskedasticity-consistent standard errors using white-adjusted ANOVAs with the ANOVA function in the R package Car (Fox & Weisberg, 2019; White, 1980). In addition, to test if differences in urchin larval metamorphosis occurred for given coralline algal species (or the control) with the microbiome reduced (antibiotic-treated) compared with those with the microbiome intact (untreated), a series of paired *t*-tests, with a Bonferroni correction for multiple comparisons, was conducted.

Similarly, to examine differences in the sporophyte densities of the three kelp species (*Costaria costata*, *Macrocystis pyrifera*, and *Nereocystis luetkeana*) across substrate type (control, rock, fleshy red crust, or coralline algal species) a series of one-way ANOVAs were undertaken. The sporophyte densities for all three kelp species were log transformed prior to analysis to meet the assumption of normality. After transformations, the *M. pyrifera* dataset still violated the assumption of equal variance, so again, the ANOVA was run using heteroskedasticity-consistent standard errors (white-adjusted ANOVA).

For all ANOVAs, post hoc comparisons were made using the emmeans R package using Tukey contrasts with a heteroskedasticity-consistent covariance matrix estimation (vcov = vcovHC) from the package sandwich, which is deemed a robust method under unbalanced designs that may have some heteroskedasticity to the data structure (Herberich et al., 2010; Zeileis, 2004).

RESULTS

Coralline algal species identity and condition

Coralline algae and non-calcified fleshy red crusts swabbed for microbiome analysis were identified using molecular methods as eight species of coralline algae and one fleshy red algal crust (*Peyssonnelia* sp.), with replicates ranging from two to nine individuals per species (Appendix S2: Table S2).

Out of the 12 coralline algal morpho-groups in the urchin metamorphosis experiment, only nine molecular-identified species with three or more replicates were identified (Table 1a and Appendix S1: Table S1). The two *Crusticorallina* and two *Lithothamnion* morpho-groups turned out to be mostly comprised of one species each: *Crusticorallina painei* and *Lithothamnion glaciale*, respectively. The two *Lithophyllum* morpho-groups were comprised of three different species; however, only one species (*Lithophyllum* sp.1) had sufficient replication (≥3 individuals) to be included in the statistical analysis.

For all the kelp settlement experiments the number of coralline algal morpho-groups matched the molecular-identified species, with seven for the *Costaria costata* experiment and eight each for the *Macrocystis pyrifera* and *Nereocystis luetkeana* experiments (Table 1b and Appendix S2: Table S1). The two *Crusticorallina* morpho-groups both had a mix of *Crusticorallina muricata* and *Crusticorallina painei*, and there were enough replicates of both species to include in the analyses. The *Lithophyllum* morpho-group for the kelp *C. costata* experiment was comprised of two species, but only one (*Lithophyllum* sp.1) had three or more replicates and was retained in the statistical analysis.

Coralline algae remained healthy through all urchin and kelp experiments with no evidence of bleaching. During the urchin experiment no epiphytic growth of fleshy algal species was observed over the coralline algal species. In contrast, due to the longer conditions in culture and the addition of nutrients to aid gametophyte development, some epiphytic growth of fleshy algae species was observed on coralline algal specimens, which may actually reflect natural conditions.

Microbiome

Bacterial partial 16S rRNA gene sequences were obtained for 52 samples (35 coralline samples, five *Peyssonnelia* sp. samples, four rock samples, five water samples, and three control samples of divers hands and of the nets used to lift boulders). We observed clear and significant compositional differences among these substrates (Appendix S2: Figure S1, PERMANOVA models, $n = 52$, BrayCurtis: omega2 = 0.25, $F_{(5,46)} = 4.56$, $p < 0.001$; Aitchison: omega2 = 0.24, $F_{(5,46)} = 4.36$, $p < 0.001$), suggesting that microbiomes associated with coralline algae and *Peyssonnelia* sp. differed from those associated with the environment (rock, water) and sampling tools. Among coralline algae and *Peyssonnelia* sp. samples, microbiome composition clustered by host species (Figure 2 and Appendix S2: Figure S2, PERMANOVA

TABLE 1 Number of replicates for each of the settlement substrates for (a) the urchin metamorphosis experiment and (b) the kelp settlement experiment.

		a. Urchin metamorphosis experiment		b. Kelp settlement experiment		
Species group	**Morphology**	**Untreated replicates**	**Antibiotic replicates**	***Costaria costata***	***Macrocystis pyrifera***	***Nereocystis luetkeana***
Bare rock				5	6	6
Dead *Crusticorallina*	Crustose			2[a]	6	5
Bossiella schmittii	Articulated	8	8	5	6	6
Bossiella orbigniana	Articulated				6	6
Calliarthron tuberculosum	Articulated	8	8	5	6	6
Chiharaea bodegensis	Crustose	8	7	5	6	6
Crusticorallina muricata	Crustose	1[a]		3	8	8
Crusticorallina painei	Crustose	7	8	7	4	4
Lithophyllum sp.1	Crustose	14	5	3	6	6
Lithophyllum sp.2	Crustose		3[a]	2[a]		
Lithophyllum sp.3	Crustose	2[a]	2[a]			
Lithothamnion glaciale	Crustose	12	16	5	6	6
Lithothamnion phymatodeum	Crustose	1[a]				
Hapalidiales sp.1	Crustose	5				
Hapalidiales sp.2	Crustose	4				
Hildenbrandia spp.	Fleshy red	6		3	3	
Peyssonnelia spp.	Fleshy red	1[a]		2[a]	1[a]	2[a]
Lithothamnion sp.1	Rhodolith	8				

[a]Indicates where sample size was too small to use in formal statistical analysis and was not included in the analyses.

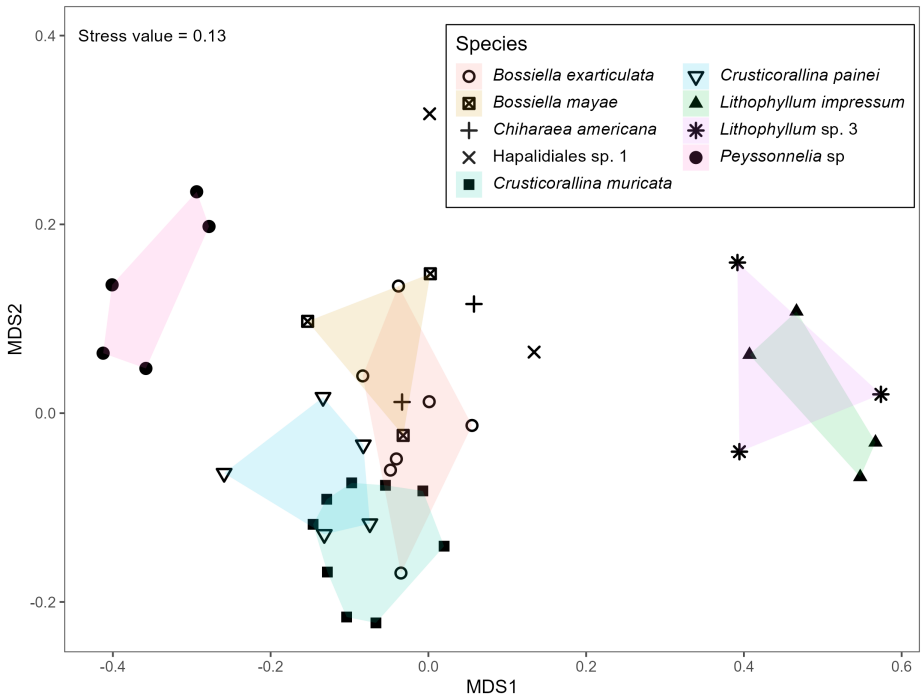

FIGURE 2 Non-metric multidimensional scaling (nMDS) ordination plot showing microbiome community composition on different coralline algal species and fleshy red algal crusts (*Peyssonnelia* sp.) with shapes linking samples from the same species when the sample size is greater than two.

models, $n = 39$, BrayCurtis: omega2 = 0.31, $F_{(8,31)} = 3.44$, $p < 0.001$; Aitchison: omega2 = 0.19, $F_{(8,31)} = 2.17$, $p < 0.001$), with *Peyssonnelia* sp. samples being significantly different from the coralline algal species (PERMANOVA model, BrayCurtis: omega2 = 0.15, $F_{(1,8)} = 2.76$, $p < 0.01$). These results were robust to the use of an alternative distance metric that is compositionally aware (i.e., Aitchison distance—Appendix S2: Figures S1B and S2). Evident from the nMDS plot, four distinct microbial communities formed with species belonging to the same genus (i.e., in *Lithophyllum*, *Crusticorallina*, and *Bossiella*) having similar microbiome composition compared with species from different genera (Figure 2). Three separate clusters formed between the coralline algal species, the two *Lithophyllum* species clustered together, the crustose provisional species Hapalidiales sp.1 clustered by itself, and all the other remaining coralline algal species clustered together. The fleshy red algal crust (*Peyssonnelia* sp.) also clustered by itself and was significantly different from all coralline algal species in its surface microbiome community.

The most abundant bacterial microbiome families sampled on algal surfaces were Flavobacteriaceae and Saprospiraceae (Appendix S2: Figure S3). The *Lithophyllum* species grouping had higher relative abundances of the microbiome families Alteromonadaceae and Flavobacteriaceae but lower relative abundances of Thiotrichaceae than all other groups on their surface. *Peyssonnelia* sp. group had higher relative abundances of the family Saprospiraceae and lower of Arenicellaceae compared with other algal surfaces. The crustose provisional species Hapalidiales sp.1 had a high relative abundance of rare bacterial families on their surface.

Urchin larval metamorphosis

The percentage of urchin larvae that completely metamorphosed showed significant differences among treatments both after 24 h (ANOVA, $F_{(10,78)} = 9.93$, $p < 0.001$) and 48 h (ANOVA, $F_{(10,78)} = 13.90$, $p < 0.001$; Figure 3). This pattern was driven by the significantly higher percent metamorphosis on coralline algal and the non-coralline *Hildenbrandia* spp. treatments compared to the control (a 2.5- to 22-fold increase in mean metamorphosis percentage, compared with the control after 24 h) and not by significant differences among the various coralline algal species and the non-coralline *Hildenbrandia* spp. (Figure 3, Appendix S2: Table S3). Therefore, urchin larval metamorphosis did not significantly differ across coralline algal species.

The highest mean ± *SE* percent metamorphosis values were in response to non-coralline *Hildenbrandia* spp. (44.4% ± 11.3% and 59.5% ± 11.1% for 24 and 48 h, respectively), which is found in higher relative abundance in kelp forests. This was followed by the crustose coralline *Crusticorallina painei* (30.0% ± 4.1% and 40.8% ± 7.1%), which is found in higher relative abundance in urchin barrens. Although not significantly different, mean percent metamorphosis were up to 2.8- and

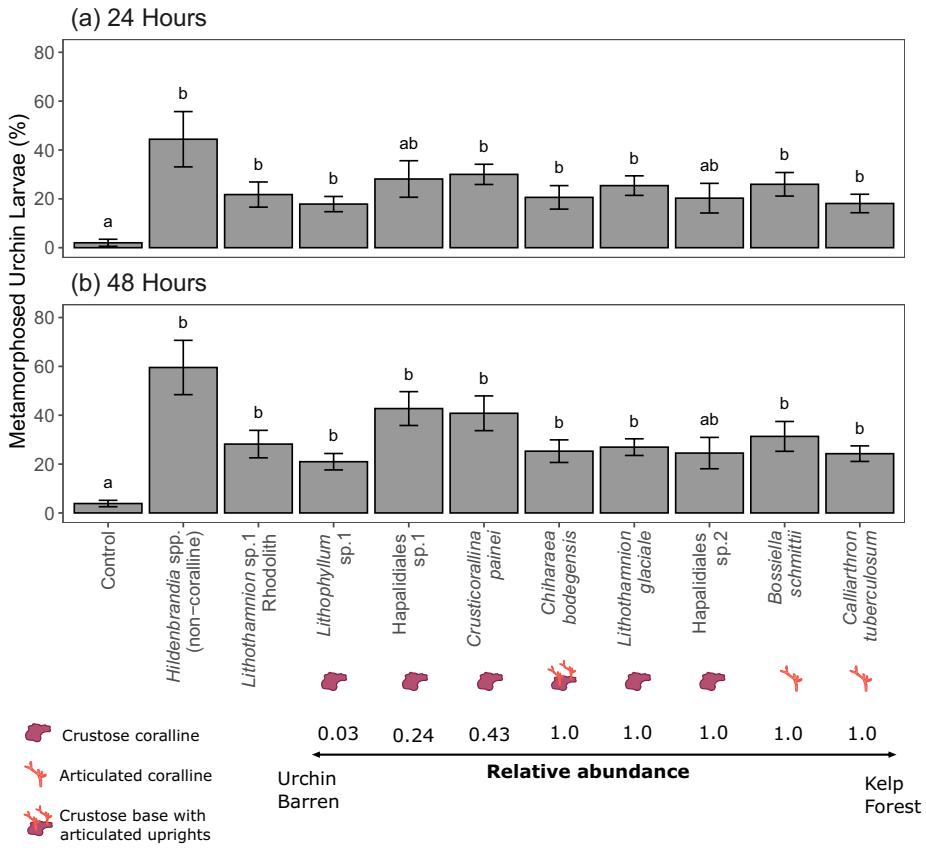

FIGURE 3 Mean (±SE) percentage of red sea urchin (*Mesocentrotus franciscanus*) larvae that underwent metamorphosis on different coralline algal species after (a) 24 and (b) 48 h. Different letters above bars indicate significant differences (Tukey's test, $p < 0.05$). The relative abundance of each coralline algal species in kelp forest compared with urchin barren habitat is shown and is based off data collected by Hind et al. (2019). The relative abundance of the fleshy red *Hildenbrandia* spp. was 0.83 in kelp forests compared with urchin barrens. *Lithothamnion* sp.1 rhodolith is a free-living crustose coralline algae observed on soft sediment habitat and therefore does not have an associated relative abundance. See Table 1a for sample sizes.

1.9- fold higher than the crustose coralline *Lithophyllum* sp.1, also found in higher relative abundance in urchin barrens, with the lowest mean percent metamorphosis (17.9%±3.1% and 21.0%±3.3%) besides the control (2.0%±1.4% and 3.9%±1.3%). However, these mean percent metamorphosis values were similar to species found only in kelp forest habitats (relative abundance of 1.0) including *Calliarthron tuberculosum* (18.0%±3.8% and 20.3%±3.2%) and Hapalidiales sp.2 (24.3%±6.1% and 24.5%±6.4%). The latter of these species did not significantly induce metamorphosis at a higher level than the background controls after either 24 or 48 h despite a 12.1- and 6.4-fold increase compared with the control after 24 and 48 h, respectively. Additionally, Hapalidiales sp.1, observed in a higher relative abundance in urchin barrens, did not induce metamorphosis at a level significantly higher than the control after 24 h (28.1%±7.5%), although it did have a significantly higher level compared with the control after 48 h (42.7%±6.9%).

The application of antibiotics to the coralline algae caused a reduction in the surface microbiome as indicated through a reduction in DNA concentration of PCR product and extracted DNA compared with that before the antibiotic treatment (mean fold reduction in concentration of 7.5 for DNA and 2.3 for PCR product, Appendix S2: Figure S4; Table S4). However, coralline exposure to antibiotics had no significant effect on urchin metamorphosis; there were small nonsignificant differences in the percentage of larvae metamorphosed between coralline algal species that had different microbiomes (Figures 1 and 2; Appendix S2: Table S3) and there was no significant difference (small fold changes ranging from 1.01 to 2.74 in mean percentage metamorphosed) in percent metamorphosis between coralline algae (and control) with the microbiome intact and with the microbiome experimentally reduced with antibiotics after both 24 and 48 h (Figure 4; Appendix S2: Table S5).

Kelp settlement

Significant differences in kelp sporophyte densities were observed among control and algal treatments for the subcanopy *Costaria costata* (ANOVA, $F_{(8,32)} = 24.03$, $p < 0.001$) and the canopy *Macrocystis pyrifera*

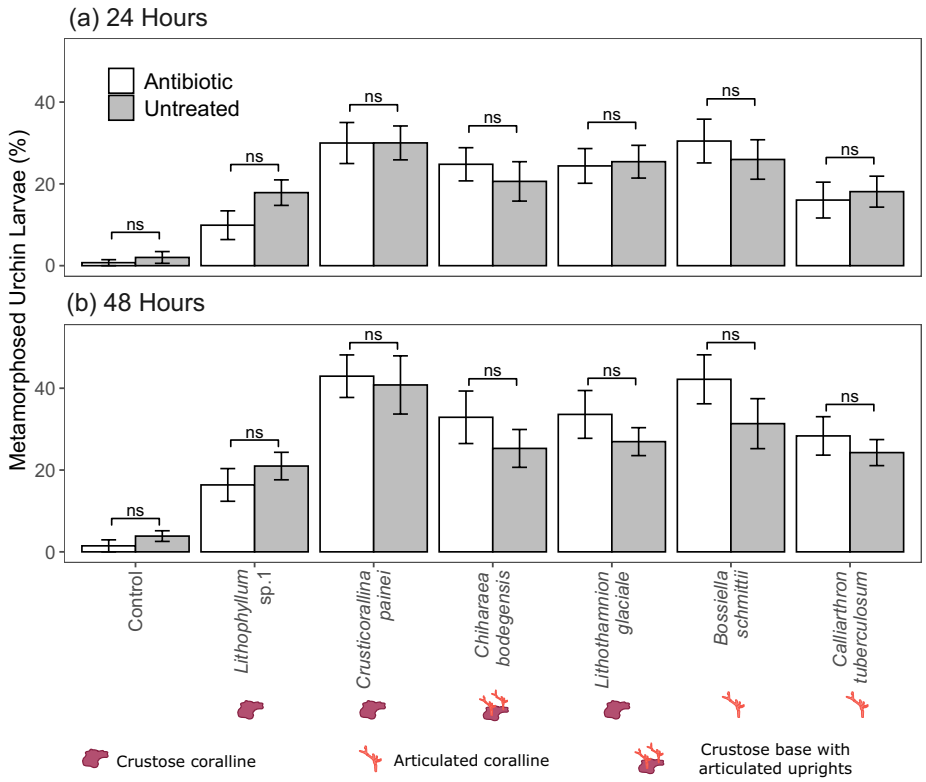

FIGURE 4 Mean (±*SE*) percentage of red sea urchin (*Mesocentrotus franciscanus*) larvae metamorphosed on different crustose and articulated coralline algal species with their microbiome reduced (Antibiotic) and those with their natural microbiome (Untreated) after (a) 24 and (b) 48 h. Paired *t*-test results (corrected with Bonferroni correction for multiple comparisons) shown for each coralline algal species. ns = not significant ($p > 0.05$). See Table 1a for sample sizes.

(ANOVA, $F_{(10,52)} = 6.52$, $p < 0.001$) and *Nereocystis luetkeana* (ANOVA, $F_{(9,49)} = 5.60$, $p < 0.001$).

For the subcanopy kelp *Costaria costata*, two species of articulated coralline algae, *Bossiella schmittii* (22.3 ± 7.3 individuals·cm^{-2}) and *Calliarthron tuberculosum* (79.2 ± 23.6 individuals·cm^{-2}), observed primarily in kelp forest habitat, had significantly higher sporophyte densities than all other coralline algal species and bare rock (Figure 5a, Appendix S2: Table S6A). All other coralline species significantly differed in sporophyte densities from bare rock (mean ± *SE* density of 1.2 ± 1.1 individuals·cm^{-2}). The two articulated coralline algae had mean densities at 66.8- and 18.8-fold higher than bare rock and 25.5- and 7.2-fold higher than the next highest mean densities on the non-coralline fleshy red alga *Hildenbrandia* spp. (3.1 ± 2.9 individuals·cm^{-2}), respectively. The density of the kelp *C. costata* on *Hildenbrandia* spp. was not significantly different from *B. schmittii*.

Settlement of the canopy kelps *Macrocystis pyrifera* and *Nereocystis luetkeana* was similar across coralline algal species and bare rock, with those coralline algae observed in high relative abundance in kelp forests and urchin barrens, both showing little significant difference among each other (Figure 5b,c, Appendix S2: Table S6B,C).

For *Macrocystis pyrifera* (Figure 5b), none of the coralline algal treatments showed any significant differences in mean kelp density compared with bare rock (312.5 ± 134.3 individuals·cm^{-2}). An exception being the fleshy red alga crust *Hildenbrandia* spp. (2.9 ± 1.1 individuals·cm^{-2}), which had the lowest mean sporophyte density, with a 106.9-fold reduction in mean sporophyte density compared with bare rock, and was significantly lower than all other treatments except *Crusticorallina painei* (85.6 ± 42.1 individuals·cm^{-2}), *Crusticorallina muricata* (119.4 ± 96.8 individuals·cm^{-2}), and dead *Crusticorallina* (highest variability with 369.0 ± 286.5 individuals·cm^{-2}). *Bossiella orbigniana* (39.2 ± 9.2 individuals·cm^{-2}), observed only in kelp forest habitats, had the lowest sporophyte density of all the coralline species and was significantly lower by a 4.9-fold reduction than *Calliarthron tuberculosum* (191.9 ± 34.5 individuals·cm^{-2}) and by an 8.3-fold reduction than *Lithothamnion glaciale* (326.99 ± 122.1 individuals·cm^{-2}), both also observed primarily in kelp forest habitat. There were no other significant differences among coralline algal species (maximum fold change of 3.9 between non-significantly different coralline algae), with several species found in high relative abundance in kelp forests having no significant differences from those in urchin-barren habitat (Appendix S2: Table S6B).

For *Nereocystis luetkeana* (Figure 5c), the highest observed mean density was on bare rock (1156.4 ± 333.3

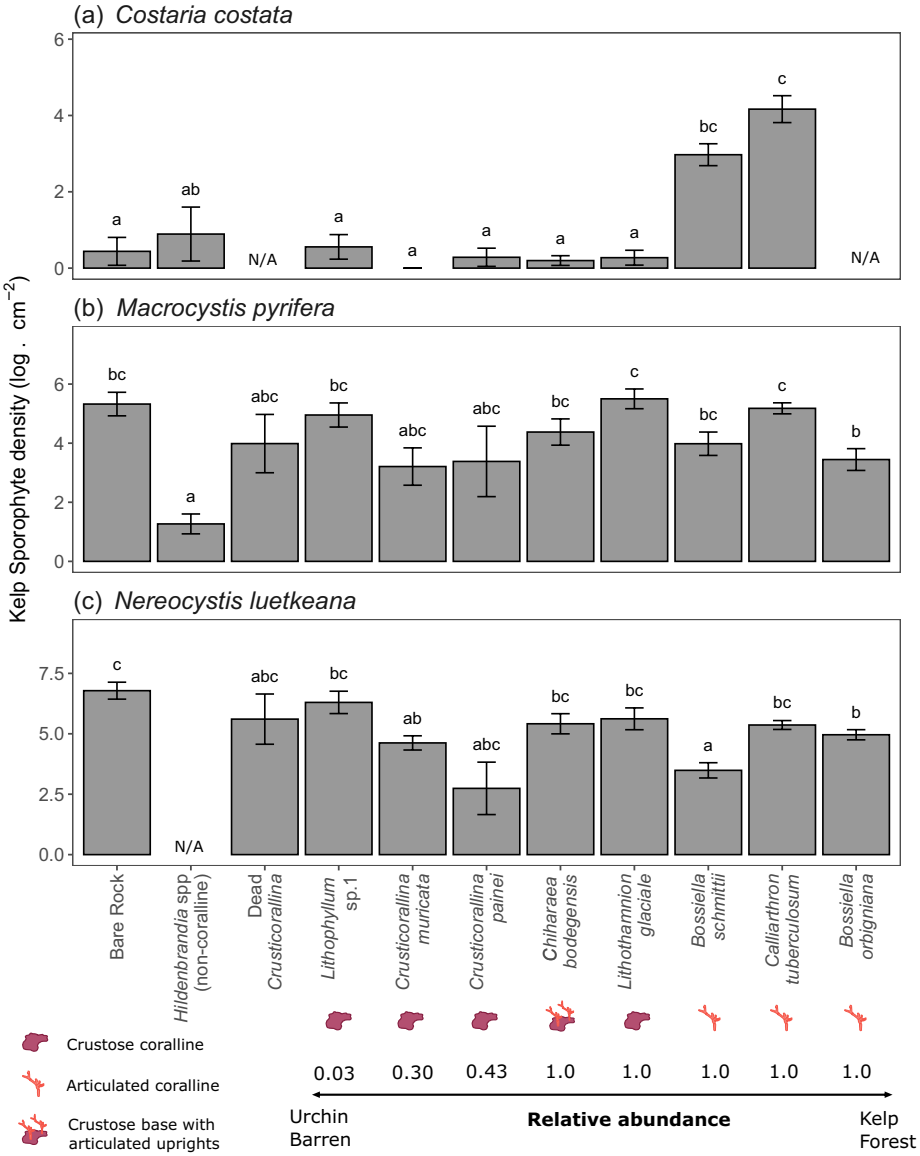

FIGURE 5 Mean juvenile sporophyte (±*SE*) density of (a) *Costaria costata*, (b) *Macrocystis pyrifera*, and (c) *Nereocystis luetkeana* juvenile sporophytes on different coralline algal species. Shown on a log scale to better visualize differences. Different letters above bars indicate significant differences (Tukey's test, $p < 0.05$). The relative abundance of each coralline algal species in kelp forest compared with urchin barren habitat is shown and is based off data collected by Hind et al. (2019). The relative abundance of the fleshy red *Hildenbrandia* spp. was 0.83 in kelp forests compared with urchin barrens. See Table 1b for sample sizes.

individuals·cm^{-2}), which was significantly higher than *Bossiella schmittii* (39.4 ± 9.2 individuals cm^{-2}) with a 29.3-fold difference, *B. orbigniana* (158.2 ± 33.2 individuals·cm^{-2}) with a 7.3-fold difference, and *Crusticorallina muricata* (130.9 ± 32.0 individuals·cm^{-2}) with an 8.9-fold difference. *Bossiella schmittii* had the lowest sporophyte density of all the coralline species and was significantly lower than all other corallines, with differences ranging from 4.0- to 23.3-fold, except *Crusticorallina painei* (48.7 ± 32.0 individuals·cm^{-2}, with only a 1.2-fold difference) and *Crusticorallina muricata* (3.3-fold difference). Unlike *Macrocystis pyrifera* and *Costaria costata*, the density of kelp sporophytes on *Hildenbrandia* spp. was not recorded in the *N. luetkeana* treatment due to several *Hildenbrandia* spp. individuals dying. However, another fleshy red algal crust, *Peyssonnelia* spp., showed a low sporophyte density of 15.2 ± 3.0 ($n = 2$) individuals·cm^{-2} (not shown on Figure 5 as no statistical test could be run with $n = 2$). The fleshy red alga *Peyssonnelia* spp. had similarly low values compared with *Hildenbrandia* spp. for both *M. pyrifera* (5.9 individuals·cm^{-2}, $n = 1$) and *C. costata* (3.0 ± 3.0 individuals·cm^{-2}, $n = 2$), respectively. There were no further significant differences among coralline algal species, with similar densities observed on coralline algal species in high relative abundance in kelp forest and urchin-barren habitat. Similar to *M. pyrifera*, the *N. luetkeana* sporophyte density on dead

Crusticorallina (1373.2 ± 962.7 individuals·cm^{-2}) had high variability and was not significantly different from any other treatment (alga or bare rock).

DISCUSSION

We found that red sea urchin larval metamorphosis was induced by coralline algal species as well as the fleshy red algal crusts (*Hildenbrandia* spp.). However, contrary to our hypothesis, percent urchin metamorphosis was not significantly higher in response to coralline algal species that are more abundant in urchin barrens compared with those in kelp forests. Instead, urchin larvae metamorphosed at similar percentages across all algal species examined. These results build upon those of Rowley (1989) who observed similar densities of recently settled juvenile red (*Mesocentrotus franciscanus*) and purple (*Strongylocentrotus purpuratus*) urchins on fleshy red algal crusts dominant in kelp forests and coralline algae dominant in urchin barrens. Similarly, Pearce and Scheibling (1990) observed that percent metamorphosis of green urchins (*Strongylocentrotus droebachiensis*), abundant in temperate waters in the northern Pacific and Atlantic oceans, did not differ across one articulated coralline and two crustose coralline species. These results are in contrast, however, to reported coralline species-specific larval settlement/metamorphosis of some species of abalone (e.g., O'Leary et al., 2017; Roberts et al., 2010), corals (e.g., Deinhart et al., 2022), sea stars (e.g., Doll et al., 2023), and some other urchin species (e.g., Nielsen et al., 2015; Taniguchi et al., 1994). For example, differences in percent metamorphosis were recorded for the Japanese sea urchin (*Mesocentrotus nudus*) across extracts from three morphologically identified coralline algae (Taniguchi et al., 1994), and two Australian urchin species (*Heliocidaris erythrogramma* and *Holopneustes purpurascens*) differed in percent metamorphosis between the morphologically-identified articulated coralline species *Corallina* "*officinalis*" and *Amphiroa* "*anceps*" (Huggett et al., 2006; Nielsen et al., 2015; Swanson et al., 2004). Together, these results suggest that invertebrate preference (or lack of preference) for different coralline species may not be generalizable and will need to be investigated on a species-by-species basis.

Despite differences in the surface microbiomes of *Lithophyllum* spp., Hapalidiales sp.1, and all other coralline algal crusts and the differences in surface microbiomes known to occur between articulated and crustose coralline algal morphologies (Lemay et al., 2021), there was no significant difference in red urchin larval metamorphosis across coralline species. Furthermore, a reduction in the microbiome load induced by antibiotics did not significantly influence urchin percent metamorphosis. These results suggest that the chemical cue used by red urchin larvae for metamorphosis comes primarily from coralline algae and not from the associated surface microbiome. This is consistent with Pearce and Scheibling (1990), who observed that a reduction of the microbiome of one coralline algal crust had no effect on percent metamorphosis rates of the green urchin *Strongylocentrotus droebachiensis*. There have been conflicting results, however, on the role of microbiomes in settlement/metamorphosis of invertebrate larvae, with some studies demonstrating that the cue comes from the coralline itself (Pearce & Scheibling, 1990; Roberts et al., 2010) and others showing it comes from the associated microbiome (Huggett et al., 2006; Johnson & Sutton, 1994; Nielsen et al., 2015). While this may be taxon-specific, one recent study on coral larvae suggests there may be redundant cues in both the microbiome and the coralline alga that promote metamorphosis, with cues from coralline algae being stronger than those from the microbiome (Gómez-Lemos et al., 2018).

Our results indicate that red urchins have generalist metamorphosis patterns across coralline algal species. Lack of settlement differences across coralline species suggests that this component of early recruitment could be similar across kelp forest and urchin-barren habitats, which counters the assumption of higher recruitment facilitation in urchin barrens (Baskett & Salomon, 2010; Breitburg, 1984; Filbee-Dexter & Scheibling, 2014). Despite potential similarities in coralline-mediated metamorphosis patterns across these two habitats, other pre- and post-metamorphosis processes such as larval supply, hydrodynamic conditions, predation, and physical abrasion also play important roles in structuring adult urchin densities within the habitats (Cameron & Schroeter, 1980; Konar & Estes, 2003; Rowley, 1989; Schroeter et al., 1996). Further studies combining measurement of larval delivery rates, settlement/metamorphosis, and survival at both early and late recruitment stages are required to elucidate potential differences in establishment of viable adult populations between the two habitats.

Spores from the canopy kelps, *Macrocystis pyrifera* and *Nereocystis luetkeana*, were able to settle and germinate into juvenile sporophytes on several crustose and articulated coralline algal species observed in both urchin barrens and kelp forests. This stands in contrast to our initial hypothesis, and several studies that have reported inhibition by a range of different coralline algal species (Johnson & Mann, 1986; Suzuki et al., 1998; Vermeij et al., 2011). It has been demonstrated, however, that chemical inhibition can vary among both kelp and coralline species (Kim et al., 2004, Villas Bôas & Figueiredo, 2004) and that it is the sloughing (loss of unstable surface epithallial cells) of some coralline algal species that causes wide-scale inhibition of epiphytic algal species (Johnson & Mann, 1986). The canopy kelps sporophyte densities on most of these corallines were not significantly different than that occurring on

bare rock collected from the same locations. These results suggest that the settlement of juvenile canopy kelps would be similar on coralline algal species and bare rock in the marine environment. For these canopy kelp species, the lowest densities were seen on fleshy red algal crusts (*Hildenbrandia* spp. and *Peyssonnelia* spp.), suggesting that these fleshy crusts, which can have high abundances in both kelp forests and urchin barrens, may inhibit development of sporophytes. These results are similar to what Okamoto et al. (2013) observed for the kelp *Saccharina bongardiana* with sporophyte density significantly reduced by greater than 97% on fleshy red algal crusts (*Hildenbrandia* spp. and *Ralfsia* spp.) compared with bare rock, but with kelp sporophyte densities similar on bare rock and coralline crusts (unidentified spp.).

In contrast, the densities of juvenile sporophytes of the subcanopy kelp *Costaria costata* were significantly higher on articulated coralline algal species, particularly *Calliarthron tuberculosum*, than on all other species. Settlement on these articulated corallines was also significantly higher than on bare rock, which may be due to the fact that the kelp was outcompeted by filamentous algae found on the rocks at the end of the experiment. We suspect that a similar phenomenon could also occur in the marine environment where kelp spores settling on bare rock are outcompeted by other faster growing algal species. A similar trend was observed by Parada et al. (2017) for the intertidal kelp *Lessonia spicata* where crustose coralline algae inhibited kelp spores that readily settled on articulate coralline algae in the laboratory, a pattern that also translated to the relative number of recruited juvenile sporophytes observed on those corallines in the field. Although it is unclear at which stage of the settlement process *C. costata* was inhibited by crustose coralline algae (initial spore settlement, germination, or gametophyte development), Kim et al. (2004) has suggested that there can be chemical inhibition preventing both spore settlement and germination. Further work is needed to determine at what stage inhibition occurs. Unfortunately, attempts to visualize kelp spore settlement and germination on the surface of coralline algal crusts using fluorescent stains were unsuccessful due to autofluorescence of the coralline itself (e.g., Okamoto et al., 2013, CellTracker™ green, Invitrogen; present study, CellTracker™ blue, Invitrogen). Future studies should investigate different colors or types of fluorescent stains (e.g., antibody staining of brown algal cell walls) to minimize coralline autofluorescence.

We did not test the antibiotic treatments in the kelp settlement experiments. However, for canopy kelps, no significant differences were observed in settlement for those species identified as having different microbiome communities. In contrast, the subcanopy kelp *Costaria costaria* settled on articulated coralline over crustose species, which have previously been demonstrated by Lemay et al. (2021) to have different microbiome communities. The fact that these spores readily settled on control glass cover slips with no associated microbiome and a similar sporophyte density suggests no positive facilitation role is provided by the microbiome. However, a negative interactive effect of the microbiome on kelp spore settlement and development cannot be ruled out and requires further investigation as studies have shown that for red and green macroalgae, certain bacteria species can inhibit algal spore germination (Egan et al., 2001).

Results from the present study demonstrate that the settlement and development of kelp across different coralline algal species is variable and not generalizable, which could have major implications for kelp forest recovery. That canopy kelps can still settle onto coralline algal species abundant in urchin barrens suggests that coralline algae do not have a strong negative impact on kelp forest recovery as previously thought, and removal of these species prior to kelp restoration efforts may not be necessary (e.g., Bulleri et al., 2002; Eger et al., 2022; Jung et al., 2022). Nevertheless, although canopy kelps are able to settle into both kelp forest and urchin-barren systems, survivability and spore supply to these sites are still important factors when considering recovery and maintenance of these ecosystem states. For example, studies from the northwestern Atlantic Ocean have shown that re-establishment of kelp spores and recovery of kelp beds following reductions of urchins at several sites ranged from 18 months to 4 years depending on the proximity to the nearest reproductive kelp beds (Johnson & Mann, 1988; Keats et al., 1990). However, results presented here of greater kelp spore settlement on articulated coralline algae over bare rock, suggest that coralline algal communities may need to transition to a community resembling that observed in kelp forests with abundant articulated coralline species in order for subcanopy kelps to recruit back into the system. A similar result was demonstrated by Barner et al. (2016) who showed that the recruitment of the intertidal kelp *Hedophyllum sessile* (as *Saccharina sessilis*) depended on the presence of articulated coralline algae. We know that differences in benthic coralline algal communities exist in well-established kelp forest compared with urchin-barren habitats (Hind et al., 2019); however, it is less clear over what time scales coralline communities diverge. Coralline algae are considered to be slow growing with growth rates varying among species (Dethier & Steneck, 2001; Fisher & Martone, 2014), and therefore, the timescale for recovery of diverse coralline algal assemblages, with both crustose and articulated species, from those observed in urchin-barren habitats is unclear but could take several years. Coralline community dynamics require further investigation, particularly in relation to kelp forest recovery.

CONCLUSIONS

Kelp forests offer a wide range of ecosystem functions and significant economic value. The present study enhances our understanding of the bottom-up roles that coralline algae play in maintaining or restoring these crucial coastal ecosystems. Contrary to our hypotheses, we found that canopy kelps *Macrocystis pyrifera* and *Nereocystis luetkeana* and red sea urchins (*Mesocentrotus franciscanus*) can settle or metamorphose on many coralline algal species located in both kelp forest and urchin-barren habitat types (and at the same level that they settle on bare rocks), indicating generalist patterns of settlement and metamorphosis. The subcanopy kelp *Costaria costata*, however, showed a species-specific settlement pattern, preferentially settling on two species of articulated coralline algae (and at a higher level than on bare rocks), indicating that these particular coralline species actively enhance the settlement of this subcanopy kelp. Surprisingly, and despite significant differences in microbiome structure across coralline species, our data suggest that urchin larval metamorphosis cues likely originated from coralline thalli and not from the microbiomes. The application of molecular-assisted identification methods is critical for understanding the important, and often species-specific, roles played by coralline algae in the ecology of nearshore ecosystems.

Coralline algae have long been thought to contribute negatively to alternate ecosystem states devoid of any fleshy macroalgae species. To the contrary, our results indicate that for systems dominated by canopy kelp species, coralline algal species found in this habitat likely do not negatively impact initial kelp settlement, highlighting their important bottom-up role in these ecosystems. Instead, non-calcified fleshy red crusts (e.g. *Hildenbrandia* spp. and *Peyssonnelia* spp.) likely have the most negative impact, as they promoted the highest mean urchin percent metamorphosis, although not significant, and supported some of the lowest juvenile canopy kelp densities. Ongoing efforts to conserve and restore kelp forests would be well advised to pay close attention to the bottom-up roles played by coralline algae and fleshy crusts in the assembly of kelp forest ecosystems.

AUTHOR CONTRIBUTIONS

Brenton A. Twist: Conceptualization (lead); formal analysis (lead); investigation (lead); writing – original draft (lead). **Florent Mazel:** Conceptualization (equal); formal analysis (equal); investigation (equal); writing – review and editing (equal). **Stefanie Zaklan Duff:** Conceptualization (equal); investigation (supporting); writing – review and editing (equal). **Matthew A. Lemay:** Conceptualization (equal); writing – review and editing (equal). **Christopher M. Pearce:** Conceptualization (equal); funding acquisition (supporting); writing – review and editing (equal). **Patrick T. Martone:** Conceptualization (lead); funding acquisition (lead); supervision (lead); writing – review and editing (equal).

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DATA AVAILABILITY STATEMENT

All data and code are available in Zenodo at https://doi.org/10.5281/zenodo.7799500. In addition, DNA sequences for coralline algae species and associated microbiome are found on NCBI's GenBank and European Nucleotide Archive (ENA), respectively.

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REFERENCES

Andersen, R. A. (2005). *Algal culturing techniques*. Elsevier Academic Press.

Barner, A. K., Hacker, S. D., Menge, B. A., & Nielsen, K. J. (2016). The complex net effect of reciprocal interactions and recruitment facilitation maintains an intertidal kelp community. *Journal of Ecology*, *104*, 33–43.

Baskett, M. L., & Salomon, A. K. (2010). Recruitment facilitation can drive alternative states on temperate reefs. *Ecology*, *91*, 1763–1773.

Bertness, M. D., & Callaway, R. (1994). Positive interactions in communities. *Trends in Ecology & Evolution*, *9*, 191–193.

Bosence, D. W. J. (1983). Coralline algal reef frameworks. *Journal of the Geological Society*, *140*, 365–376.

Breitburg, D. L. (1984). Residual effects of grazing: Inhibition of competitor recruitment by encrusting coralline algae. *Ecology*, *65*, 1136–1143.

Bulleri, F., Bertocci, I., & Micheli, F. (2002). Interplay of encrusting coralline algae and sea urchins in maintaining alternative habitats. *Marine Ecology Progress Series*, *243*, 101–109.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*, 581–583.

Cameron, R., & Schroeter, S. (1980). Sea urchin recruitment: Effect of substrate selection on juvenile distribution. *Marine Ecology Progress Series*, *2*, 243–247.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., & Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 4516–4522.

Cornwall, C. E., Diaz-Pulido, G., & Comeau, S. (2019). Impacts of ocean warming on coralline algal calcification: Meta-analysis, knowledge gaps, and key recommendations for future research. *Frontiers in Marine Science*, *6*, 186.

Deinhart, M., Mills, M. S., & Schils, T. (2022). Community assessment of crustose calcifying red algae as coral recruitment substrates. *PLoS ONE*, *17*, e0271438.

Dethier, M. N., & Steneck, R. (2001). Growth and persistence of diverse intertidal crusts: Survival of the slow in a fast-paced world. *Marine Ecology Progress Series*, *223*, 89–100.

Doll, P. C., Uthicke, S., Caballes, C. F., Diaz-Pulido, G., Abdul Wahab, M. A., Lang, B. J., Jeong, S. Y., & Pratchett, M. S. (2023). Settlement cue selectivity by larvae of the destructive crown-of-thorns starfish. *Biology Letters*, *19*, 20220399.

Egan, S., James, S., Holmström, C., & Kjelleberg, S. (2001). Inhibition of algal spore germination by the marine bacterium *Pseudoalteromonas tunicata*. *FEMS Microbiology Ecology*, *35*, 67–73.

Eger, A. M., Marzinelli, E. M., Christie, H., Fagerli, C. W., Fujita, D., Gonzalez, A. P., Hong, S. W., Kim, J. H., Lee, L. C., McHugh, T. A., Nishihara, G. N., Tatsumi, M., Steinberg, P. D., & Vergés, A. (2022). Global kelp forest restoration: Past lessons, present status, and future directions. *Biological Reviews*, *97*, 1449–1475.

Filbee-Dexter, K., & Scheibling, R. E. (2014). Sea urchin barrens as alternative stable states of collapsed kelp ecosystems. *Marine Ecology Progress Series*, *495*, 1–25.

Fisher, K., & Martone, P. T. (2014). Field study of growth and calcification rates of three species of articulated coralline algae in British Columbia, Canada. *Biological Bulletin*, *226*, 121–130.

Fox, J., & Weisberg, S. (2019). *An R companion to applied regression*, 3rd edn. Sage. https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Glöckner, F. O., Yilmaz, P., Quast, C., Gerken, J., Beccati, A., Ciuprina, A., Bruns, G., Yarza, P., Peplies, J., Westram, R., & Ludwig, W. (2017). 25 years of serving the community with ribosomal RNA gene reference databases and tools. *Journal of Biotechnology*, *261*, 169–176.

Gómez-Lemos, L. A., Doropoulos, C., Bayraktarov, E., & Diaz-Pulido, G. (2018). Coralline algal metabolites induce settlement and mediate the inductive effect of epiphytic microbes on coral larvae. *Scientific Reports*, *8*, 17557.

Guenther, R., Porcher, E. M. A., Carrington, E., & Martone, P. T. (2022). Effects of temperature and pH on the growth, calcification, and biomechanics of two species of articulated coralline algae. *Marine Ecology Progress Series*, *700*, 79–93.

Herberich, E., Sikorski, J., & Hothorn, T. (2010). A robust procedure for comparing multiple means under heteroscedasticity in unbalanced designs. *PLoS ONE*, *5*, e9788.

Hind, K. R., Gabrielson, P. W., Lindstrom, S. C., & Martone, P. T. (2014). Misleading morphologies and the importance of sequencing type specimens for resolving coralline taxonomy (Corallinales, Rhodophyta): *Pachyarthron cretaceum* is *Corallina officinalis*. *Journal of Phycology*, *50*, 760–764.

Hind, K. R., & Saunders, G. W. (2013). A molecular phylogenetic study of the tribe Corallineae (Corallinales, Rhodophyta) with an assessment of genus-level taxonomic features and descriptions of novel genera. *Journal of Phycology*, *49*, 103–114.

Hind, K. R., Starko, S., Burt, J., Lemay, M., Salomon, A., & Martone, P. T. (2019). Trophic control of coralline species diversity. *Proceedings of the National Academy of Sciences of the United States of America*, *116*, 15080–15085.

Huggett, M. J., Williamson, J. E., De Nys, R., Kjelleberg, S., & Steinberg, P. D. (2006). Larval settlement of the common Australian sea urchin *Heliocidaris erythrogramma* in response to bacteria from the surface of coralline algae. *Oecologia*, *149*, 604–619.

Johnson, C. R., & Mann, K. H. (1986). The crustose coralline alga, *Phymatolithon* Foslie, inhibits the overgrowth of seaweeds without relying on herbivores. *Journal of Experimental Marine Biology and Ecology*, *96*, 127–146.

Johnson, C. R., & Mann, K. H. (1988). Diversity, patterns of adaptation, and stability of Nova Scotian kelp beds. *Ecological Monographs*, *58*, 129–154.

Johnson, C. R., & Sutton, D. C. (1994). Bacteria on the surface of crustose coralline algae induce metamorphosis of the crown-of-thorns starfish *Acanthaster planci*. *Marine Biology*, *120*, 305–310.

Jung, S., Chau, T. V., Kim, M., & Na, W.-B. (2022). Artificial seaweed reefs that support the establishment of submerged aquatic vegetation beds and Facilitate Ocean macroalgal afforestation: A review. *Journal of Marine Science and Engineering*, *10*, 1184.

Keats, D. W., South, G. R., & Steele, D. H. (1990). Effects of an experimental reduction in grazing by green sea urchins on a benthic macroalgal community in eastern Newfoundland. *Marine Ecology Progress Series*, *68*, 181–193.

Kim, M. J., Choi, J. S., Kang, S. E., Cho, J. Y., Jin, H. J., Chun, B. S., & Hong, Y. K. (2004). Multiple allelopathic activity of the crustose coralline alga *Lithophyllum yessoense* against settlement and germination of seaweed spores. *Journal of Applied Phycology*, *16*, 175–179.

Konar, B., & Estes, J. A. (2003). The stability of boundary regions between kelp beds and deforested areas. *Ecology*, *84*, 174–185.

Krumhansl, K. A., Okamoto, D. K., Rassweiler, A., Novak, M., Bolton, J. J., Cavanaugh, K. C., Connell, S. D., Johnson, C. R., Konar, B., Ling, S. D., Micheli, F., Norderhaug, K. M., Pérez-Matus, A., Sousa-Pinto, I., Reed, D. C., Salomon, A. K., Shears, N. T., Wernberg, T., Anderson, R. J., … Byrnes, J. E. K. (2016). Global patterns of kelp forest change over the past half-century. *Proceedings of the National Academy of Sciences of the United States of America*, *113*, 13785–13790.

Layton, C., Coleman, M. A., Marzinelli, E. M., Steinberg, P. D., Swearer, S. E., Vergés, A., Wernberg, T., & Johnson, C. R.

(2020). Kelp Forest restoration in Australia. *Frontiers in Marine Science*, *7*, 74.
Lemay, M. A., Chen, M. Y., Mazel, F., Hind, K. R., Starko, S., Keeling, P. J., Martone, P. T., & Parfrey, L. W. (2021). Morphological complexity affects the diversity of marine microbiomes. *The ISME Journal*, *15*, 1372–1386.
Mann, K. H. (1973). Seaweeds: Their productivity and strategy for growth. *Science*, *182*, 975–981.
Mann, K. H., & Breen, P. A. (1972). The relation between lobster abundance, sea urchins, and kelp beds. *Journal of the Fisheries Board of Canada*, *29*, 603–605.
Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet Journal*, *17*, 200.
McCoy, S. J., & Kamenos, N. A. (2015). Coralline algae (Rhodophyta) in a changing world: Integrating ecological, physiological, and geochemical responses to global change. *Journal of Phycology*, *51*, 6–24. https://doi.org/10.14806/ej.17.1.200
Méndez Trejo, M. D. C., Neill, K. F., Twist, B. A., & Nelson, W. A. (2021). Cryptofauna associated with rhodoliths: Diversity is species-specific and influenced by habitat. *Marine Ecology*, *42*, e12647.
Mills, M. S., Deinhart, M. E., Heagy, M. N., & Schils, T. (2022). Small tropical islands as hotspots of crustose calcifying red algal diversity and endemism. *Frontiers in Marine Science*, *9*, 898308.
Nelson, W. A. (2009). Calcified macroalgae–critical to coastal ecosystems and vulnerable to change: A review. *Marine and Freshwater Research*, *60*, 787–801.
Nielsen, S. J., Harder, T., & Steinberg, P. D. (2015). Sea urchin larvae decipher the epiphytic bacterial community composition when selecting sites for attachment and metamorphosis. *FEMS Microbiology Ecology*, *91*, 1–9.
Okamoto, D. K., Stekoll, M. S., & Eckert, G. L. (2013). Coexistence despite recruitment inhibition of kelps by subtidal algal crusts. *Marine Ecology Progress Series*, *493*, 103–112.
Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R. B., Simpson, G. L., Solymos, P., & Stevens, M. H. (2017). vegan: Community Ecology Package. R package version 2.4-3. https://CRAN.R-project.org/package=vegan
O'Leary, J. K., Barry, J. P., Gabrielson, P. W., Rogers-Bennett, L., Potts, D. C., Palumbi, S. R., & Micheli, F. (2017). Calcifying algae maintain settlement cues to larval abalone following algal exposure to extreme ocean acidification. *Scientific Reports*, *7*, 5774.
Paine, R. T. (1984). Ecological determinism in the competition for space: The Robert H. MacArthur award lecture. *Ecology*, *65*, 1339–1348.
Paine, R. T. (1990). Benthic macroalgal competition: Complications and consequences. *Journal of Phycology*, *26*, 12–17.
Parada, G. M., Martínez, E. A., Aguilera, M. A., Oróstica, M. H., & Broitman, B. R. (2017). Interactions between kelp spores and encrusting and articulated corallines: Recruitment challenges for *Lessonia spicata*. *Botanica Marina*, *60*, 619–625.
Pearce, C. M., & Scheibling, R. E. (1990). Induction of metamorphosis of larvae of the green sea urchin, *Strongylocentrotus droebachiensis*, by coralline red algae. *Biological Bulletin*, *179*, 304–311.
Pearce, C. M., & Scheibling, R. E. (1991). Effect of macroalgae, microbial films, and conspecifics on the induction of metamorphosis of the green sea urchin *Strongylocentrotus droebachiensis* (Müller). *Journal of Experimental Marine Biology and Ecology*, *147*, 147–162.
Pessarrodona, A., Assis, J., Filbee-Dexter, K., Burrows, M. T., Gattuso, J.-P., Duarte, C. M., Krause-Jensen, D., Moore, P. J., Smale, D. A., & Wernberg, T. (2022). Global seaweed productivity. *Science Advances*, *8*, eabn2465.
Piazzi, L., Pinna, F., & Ceccherelli, G. (2022). Crustose coralline algae and biodiversity enhancement: The role of *Lithophyllum stictiforme* in structuring Mediterranean coralligenous reefs. *Estuarine, Coastal and Shelf Science*, *278*, 108121.
Quince, C., Lanzen, A., Davenport, R. J., & Turnbaugh, P. J. (2011). Removing noise from pyrosequenced amplicons. *BMC Bioinformatics*, *12*, 38.
Quinlan, Z. A., Ritson-Williams, R., Carroll, B. J., Carlson, C. A., & Nelson, C. E. (2019). Species-specific differences in the microbiomes and organic exudates of crustose coralline algae influence bacterioplankton communities. *Frontiers in Microbiology*, *10*, 2397.
Quinn, T. P., Erb, I., Richardson, M. F., & Crowley, T. M. (2018). Understanding sequencing data as compositions: An outlook and review. *Bioinformatics*, *34*, 2870–2878.
R Core Team. (2022). *R: A language and environment for statistical computing*. Foundation for Statistical Computing https://www.R-project.org/
Roberts, R. D., Barker, M. F., & Mladenov, P. (2010). Is settlement of *Haliotis iris* larvae on coralline algae triggered by the alga or its surface biofilm? *Journal of Shellfish Research*, *29*, 671–678.
Rogers-Bennett, L., & Catton, C. A. (2019). Marine heat wave and multiple stressors tip bull kelp forest to sea urchin barrens. *Scientific Reports*, *9*, 15050.
Rowley, R. J. (1989). Settlement and recruitment of sea urchins (*Strongylocentrotus* spp.) in a sea-urchin barren ground and a kelp bed: Are populations regulated by settlement or post-settlement processes? *Marine Biology*, *100*, 485–494.
Sala, E., Boudouresque, C. F., & Harmelin-Vivien, M. (1998). Fishing, trophic cascades, and the structure of algal assemblages: Evaluation of an old but untested paradigm. *Oikos*, *82*, 425–439.
Scheffer, M., & Carpenter, S. R. (2003). Catastrophic regime shifts in ecosystems: Linking theory to observation. *Trends in Ecology & Evolution*, *18*, 648–656.
Schroeter, S., Dixon, J., Ebert, T., & Rankin, J. (1996). Effects of kelp forests *Macrocystis pyrifera* on the larval distribution and settlement of red and purple sea urchins *Strongylocentrotus franciscanus* and *S. Purpuratus*. *Marine Ecology Progress Series*, *133*, 125–134.
Smale, D. A., Burrows, M. T., Moore, P., O'Connor, N., & Hawkins, S. J. (2013). Threats and knowledge gaps for ecosystem services provided by kelp forests: A Northeast Atlantic perspective. *Ecology and Evolution*, *3*, 4016–4038.
Steneck, R. S., Hacker, S. D., & Dethier, M. N. (1991). Mechanisms of competitive dominance between crustose coralline algae: An herbivore-mediated competitive reversal. *Ecology*, *72*, 938–950.
Suzuki, Y., Takabayashi, T., Kawaguchi, T., & Matsunaga, K. (1998). Isolation of an allelopathic substance from the crustose coralline algae, *Lithophyllum* spp., and its effect on the brown alga, *Laminaria religiosa* Miyabe (Phaeophyta). *Journal of Experimental Marine Biology and Ecology*, *225*, 69–77.
Swanson, R. L., Williamson, J. E., De Nys, R., Kumar, N., Bucknall, M. P., & Steinberg, P. D. (2004). Induction of settlement of larvae of the sea urchin *Holopneustes purpurascens* by histamine from a host alga. *The Biological Bulletin*, *206*, 161–172.
Taniguchi, K., Kurata, K., Maruzoi, T., & Suzuki, M. (1994). Dibromomethane, a chemical inducer of larval settlement and metamorphosis of the sea urchin *Strongylocentroutus nudus*. *Fisheries Science*, *60*, 795–796.
Teagle, H., Hawkins, S. J., Moore, P. J., & Smale, D. A. (2017). The role of kelp species as biogenic habitat formers in coastal marine ecosystems. *Journal of Experimental Marine Biology and Ecology*, *492*, 81–98.
Tebben, J., Motti, C. A., Siboni, N., Tapiolas, D. M., Negri, A. P., Schupp, P. J., Kitamura, M., Hatta, M., Steinberg, P. D., & Harder, T. (2015). Chemical mediation of coral larval settlement by crustose coralline algae. *Scientific Reports*, *5*, 10803.
Twist, B. A., Cornwall, C. E., McCoy, S. J., Gabrielson, P. W., Martone, P. T., & Nelson, W. A. (2020). The need to employ reliable and

reproducible species identifications in coralline algal research. *Marine Ecology Progress Series*, *654*, 225–231.
Twist, B. A., Neill, K. F., Bilewitch, J., Jeong, S. Y., Sutherland, J. E., & Nelson, W. A. (2019). High diversity of coralline algae in New Zealand revealed: Knowledge gaps and implications for future research. *PLoS ONE*, *14*, e0225645.
Vermeij, M. J. A., Dailer, M. L., & Smith, C. M. (2011). Crustose coralline algae can suppress macroalgal growth and recruitment on Hawaiian coral reefs. *Marine Ecology Progress Series*, *422*, 1–7.
Villas Bôas, A. B., & Figueiredo, M. A. (2004). Are anti-fouling effects in coralline algae species specific? *Brazilian Journal of Oceanography*, *52*, 11–18.
Wernberg, T., Smale, D. A., Tuya, F., Thomsen, M. S., Langlois, T. J., de Bettignies, T., Bennett, S., & Rousseaux, C. S. (2013). An extreme climatic event alters marine ecosystem structure in a global biodiversity hotspot. *Nature Climate Change*, *3*, 78–82.
White, H. (1980). A heteroskedasticity-consistent covariance matrix estimator and a direct test for heteroskedasticity. *Econometrica*, *48*, 817–838.
Wright, E. S. (2016). Using DECIPHER v2. 0 to analyze big biological sequence data in R. *R Journal*, *8*, 352–359.
Zeileis, A. (2004). Econometric computing with HC and HAC covariance matrix estimators. *Journal of Statistical Software*, *11*, 1–17.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Supplementary Methods (A–D).

Appendix S2. Supplementary Figures and Tables, containing Figures S1–S4 and Tables S1–S6.

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