

Supplementary Information

Extended Materials and Methods

Field surveys and species identification

We conducted subtidal surveys (July 11-14, 2014) at four rocky reef sites; two kelp forests and two urchin barren sites on the central coast of British Columbia, Canada, where sea otter populations have been recovering (since around 1980) from extirpation due to the fur trade (1)(Fig. S4). The sites encompassed relatively consistent physical parameters (depth range, aspect, substrate) but were selected to provide a range of different sea urchin densities, facilitated in part by the length of time each site has been occupied by sea otters (34, 18, or 0 years at the time of sampling). At each site, sea urchin density and adult stipe density of kelps (Laminariales) and Desmarestialean algae (≥ 15 cm) were quantified in 18 stratified random 1 m^2 quadrats (depths 4-15 m below mean low water) that spanned six 30 m horizontal transects laid between two depth contours (see 2 for details). We also measured sea urchin test diameters, enabling us to calculate mean urchin biomass for each site (3).

To quantify total coralline cover and the specific cover of genetically identified species, we estimated percent species cover and took samples from six 0.25 m^2 quadrats placed randomly along the first transect (10-12 m depth below MLW) at each site.

Individuals were grouped into morpho-species based on differences in colour, texture, thickness or margin structure and were given temporary field identification. Specimens were collected by SCUBA using a chisel and hammer and each placed separately in a Ziploc bag. Samples were transferred to vials containing silica gel in order to desiccate the material and preserve DNA quality. DNA extraction, PCR amplification and

sequencing followed Hind *et al.* (4). The *psbA* barcode region (863bp) was amplified for most specimens, although COI-5P (664bp) or *rbcL* (1400bp) regions were amplified for some specimens (Dataset S1). All three of these gene regions have been tested and established as species level genetic markers for coralline algae (4, 5). Sequences were then searched against the Barcode of Life Database (BOLD), GenBank and a private reference library of DNA sequences, using blast (6) for accurate molecular species identification. Sequences that failed to match any known sequence or that matched a sequence from an undescribed species were assigned to a genus and given a consecutive number (e.g., *Lithophyllum sp.1*). In order to calculate site-level abundances of coralline species, abundances from each quadrat were averaged.

Crust thickness measurements were mostly made on samples removed from the plots, when specimen size permitted. To increase sample size, additional measurements were made on herbarium samples from the British Columbia coast that had previously been identified using molecular sequence data. There was no clear difference between measurements taken from within the plots and those added from outside collections. For two species, crust thickness measurements were only taken from outside collections due to a lack of large enough specimens within the quadrats. No crust thickness measurements were taken for *Lithothamnion sp.1* since only very small fragments were obtained and no herbarium vouchers were available for measuring. Thus, *Lithothamnion sp.1* and all articulated species were excluded from the analysis of crust thickness. Community-weighted mean thickness was calculated for each quadrat using the sum of the proportion of total coralline cover occupied by each species multiplied by its thickness.

Statistical analysis

In order to determine whether urchin biomass, kelp density or total coralline cover differed between sites, we performed ANOVAs (one factor, four levels) followed by Tukey post-hoc tests. Urchin biomass and kelp density data were log-transformed and coralline cover data were arcsin-squareroot transformed prior to analysis to address non-normality. To examine community level diversity, we generated rarefied species accumulation curves for each site using 999 resampling permutations. We also calculated three diversity indices that were intended to incorporate species abundance to varying degrees: species richness, Shannon diversity and Simpson's index. These indices were calculated for each quadrat and mean values were compared using nested ANOVAs on habitat type, given site as a random nested factor. To examine coralline assemblage composition and beta diversity, we conducted principle coordinate analyses and PERMANOVA using Bray-Curtis dissimilarity and assigned quadrats as replicates. Post-hoc pairwise comparisons were then conducted to determine which sites were significantly different. To test for potential shifts in crust thickness across communities, we compared community weighted mean thickness of sites with an ANOVA. We also tested for a correlation between community weighted mean thickness and total coralline cover using a linear model.

In order to determine whether closely related species were distributed similarly with respect to urchin grazing, we tested for the effect of phylogeny on community assembly at urchin barren and kelp forest sites. We first performed phylogenetic inference using an 856 bp alignment of *psbA* sequences aligned and edited in Geneious

(7). A neighbor-joining tree was then inferred using a Tamura-Nei model in Geneious (7). Next, Blomberg et al.'s K (8) and Pagel's λ (9) were calculated to test for phylogenetic signal on species distributions and on crust thickness. To test for phylogenetic signal on species distributions we calculated differences in average abundance at sites with and without otters (hereafter "habitat preference"). Standard effect size (SES) of mean pairwise distance (MPD) and mean nearest taxon distance (MNTD) were also calculated to test for phylogenetic clustering or over-dispersion relative to communities that were randomly generated but maintained species richness at the quadrat level. Quadrats of each site were used as replicates and a t-test was used for each site to determine whether communities had SES(MPD) and SES(MNTD) values that were greater than (over-dispersed) or less than (clustered) zero (random). Two quadrats that only had one coralline species were excluded from the analysis.

To identify which coralline species were most important in distinguishing urchin barrens and kelp forests, we conducted supervised classification using randomForest analysis (10). Supervised classification is a machine learning approach that uses training data (in this case percent cover data of coralline species for each habitat type) in order to estimate the assignment accuracy of an unknown taxon being placed in the correct habitat type (11). All analyses were conducted in R (R Core Team) using the packages "vegan" (12), 'Phytools' (13), "picante"(14) , and "randomForest" (15), except for the multivariate analyses that were conducted in PRIMER (16).

Supplemental Dataset, Table and Figures

Dataset S1. List of specimens identified by DNA sequence, including collection details, UBC Herbarium accession numbers, and Genbank numbers.

** Please note: Dataset S1 is in separate Excel file **

Table S1. Quadrat-level diversity metrics at kelp forests and urchin barrens

| | | Richness | Shannon | Simpsons |
|--------------------------|--------|-----------|-----------|-----------|
| Kelp Forest | Site 1 | 3.7 ± 0.6 | 2.8 ± 0.4 | 2.5 ± 0.4 |
| | Site 2 | 4.3 ± 0.6 | 3.2 ± 0.4 | 2.8 ± 0.4 |
| Urchin Barren | Site 3 | 3.2 ± 0.5 | 2.1 ± 0.3 | 1.9 ± 0.3 |
| | Site 4 | 2.8 ± 0.5 | 2.0 ± 0.3 | 1.8 ± 0.3 |
| Effect of Habitat(Site): | | P = 0.12 | P < 0.05 | P < 0.05 |

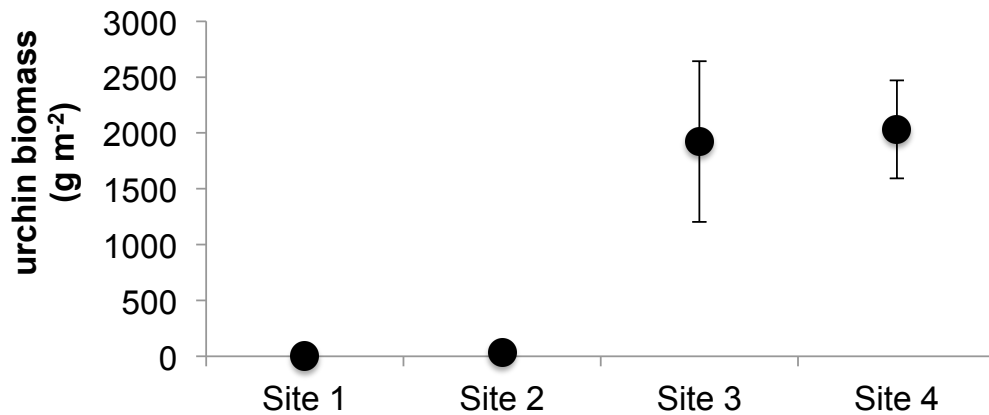


Figure S1. Urchin biomass (untransformed mean ± SE) at kelp forest sites occupied by sea otters (Site 1 = 34 yrs, Site 2 = 18 yrs) and urchin barren sites not occupied by otters.

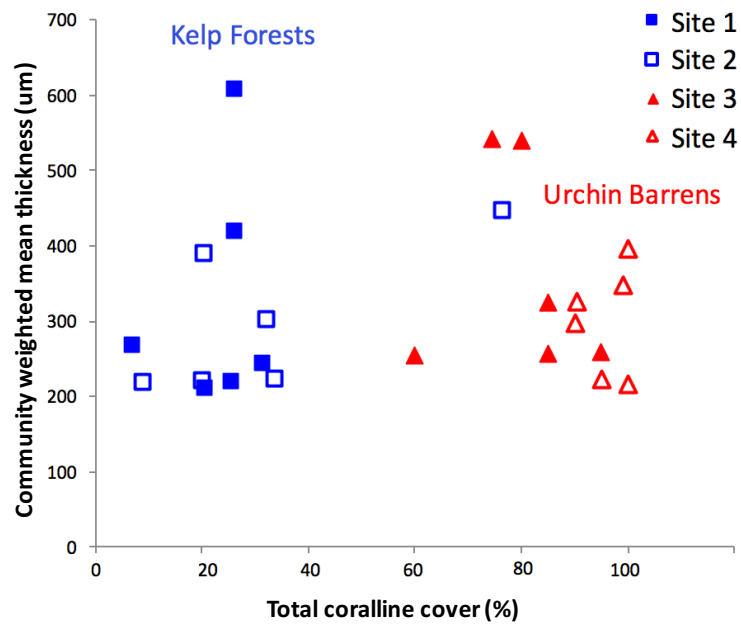


Figure S2. Community weighted means of coralline crust thickness in sites with (blue) and without (red) otters. There was no correlation between community thickness and total percent cover corallines (Linear Regression: $F = 0.2587$, $df = 1 \text{ \& } 22$, $P = 0.6161$).

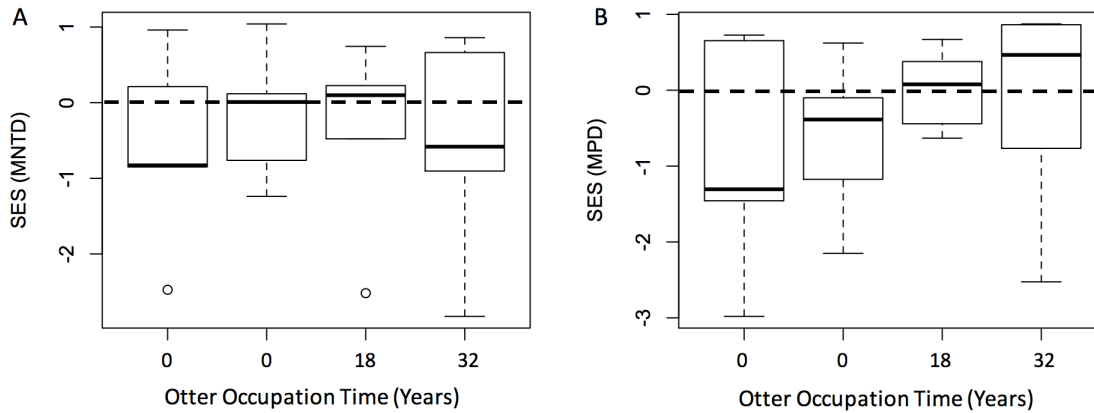


Figure S3. Standard effect size (SES) of mean nearest taxon distance (MPD) (A) and mean paired distance (MPD) of coralline assemblages along a gradient of sea otter occupation. Boxplots represent quadrats ($n = 6$) from all four sites. The dotted line at zero indicates that species are randomly distributed with respect to phylogeny. Positive values indicate phylogenetic over-dispersion. None of the sites were significantly clustered or over-dispersed with respect to phylogeny (t -tests: $P > 0.05$).

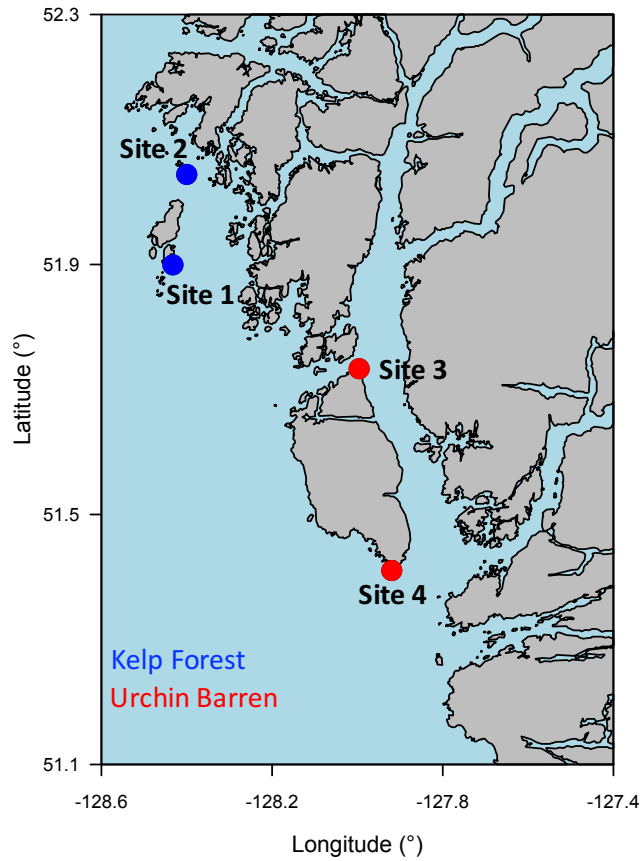


Figure S4. Map of study sites. Sites 1 and 2 are occupied by sea otters and classified as kelp forests. Sites 3 and 4 do not have sea otters and are classified as urchin barrens.

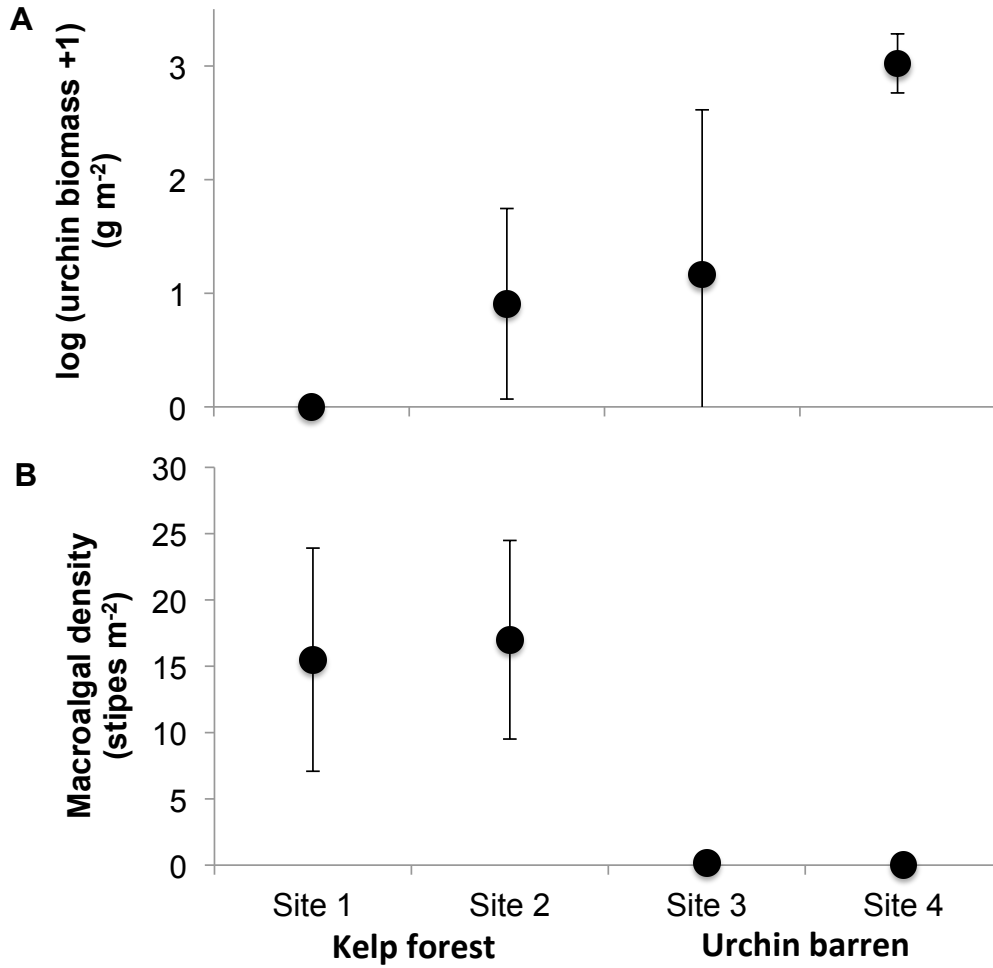


Figure S5: Mean urchin biomass (a), mean adult stipe density of Laminariales and Desmarestiales (b) at kelp forest sites occupied by sea otters (Site 1 = 34 yrs, Site 2 = 18 yrs) and urchin barren sites not occupied by otters. All samples were taken from the same, narrow range of depths (10 – 13m, n = 6).

Supplemental References

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