Methods for Marks et al 2020 "Niche Complementarity and Resistance to Grazing Promote the Invasion Success of Sargassum horneri in North America"

Methods are documented separately for the data presented in each data table:

Table 1: Seasonal distribution of algal biomass

Description:

Sargassum horneri may decrease the abundance and taxonomic richness of native algae through competition, or it may display a seasonal abundance that is complementary to native algae. To test these hypotheses, we compared the native algal assemblages in experimental plots from which *S. horneri* was continually removed (hereafter referred to as S–) with those in unmanipulated control plots with *S. horneri* left intact (S+) over three years. This experiment was conducted at Isthmus Reef (33.4476° N, 118.4898° W) at 6 m depth, within the range where *S. horneri* is most abundant. Twenty-four 1 m^2 plots separated by a distance of at least 2 m were established on areas of reef comprised of >90% rock and with a high density (i.e., at least 30 individuals) of *S. horneri*. *S. horneri* was removed from 12 randomly assigned plots (S–) beginning in spring 2014 and every 6 to 12 weeks thereafter until summer 2017. S– plots had a 30 cm wide buffer zone around the perimeter where *S. horneri* was removed to minimize potential edge effects such as shading by individuals outside of the plot. Removal entailed divers using knives to pry all *S. horneri* holdfasts off the substrate, minimizing disturbance to the other biota within the plot as much as possible. Since competitive interactions may vary with time and among seasons, we sampled the algal communities in all S+ and S– plots just prior to the initial removal of *S. horneri* in spring 2014 and quarterly thereafter (i.e., summer, autumn, winter and spring) over three consecutive growing seasons (2014–2015, 2015–2016 and 2016–2017).

Algae were identified to the lowest taxonomic level possible, which in most cases was species, and measurements of all understory and subcanopy-forming algae were taken in order to estimate the damp biomass of algae in each plot. The abundance of low-lying understory algae was measured as percent cover using a uniform point contact (UPC) method that involved recording the presence and identity of all algae intersecting 49 points distributed in a grid within each 1 m^2 plot. Percent cover was determined as the fraction of points a taxon intercepted × 100. Although multiple organisms may intersect a single point if they overlay one another, a taxon was only recorded once at a given point even if it intersected that point multiple times. Using this technique, the percent cover of all taxa combined in a plot can exceed 100%, but the percent cover of any individual species or morphological group cannot. This sampling resolution was sufficient to detect species covering at least 2% of the area in a quadrat. If a species was present in the plot and not recorded at one of the 49 points, then it was assigned a percent cover value of 0.5%. Since percent cover does not necessarily scale with biomass for larger subcanopy-forming algae, we recorded the density and the average size of these taxa. Damp biomass was estimated from density and size data of subcanopy algae and percent cover data of understory algae using taxon-specific relationships obtained from the literature or developed specifically for this project.

We specifically examined the relationships between *S. horneri* and the native subcanopy-forming and understory algal assemblages. All but two species of algae recorded in the study plots were native to the

region; the non-native *Sargassum muticum* and *Codium fragile* occurred in low abundance and are not reported here.

Table 2: Percent transmission of photosynthetically active radiation through *S. horneri* canopy

Description:

Sargassum horneri may compete with native algae by reducing the amount of light reaching algae growing beneath its canopy which is formed during the spring. To determine the amount of shading caused by the *S. horneri* canopy we calculated the percent transmission of photosynthetically active radiation (PAR, 400–700 nm) in twelve 1 m^2 permanent plots where *S. horneri* was removed (S–) and twelve unmanipulated control plots (S+) plots (see Description for Table 1 for details). Light was measured using a handheld spherical quantum sensor (LI-COR Model LI-192) oriented vertically in the center of each plot 30 cm above the bottom. Ten readings of Photosynthetic Photon Flux Density (PPFD in μmol m^{-2} s^{-1}) were taken in each plot and averaged. Percent transmission was calculated from the average of 10 PPFD readings taken at the surface before and after the dive as:

$$\%\ \text{transmission PAR} = \left[1 - \frac{\text{PAR}\ \overline{sfc} - \text{PAR}\ \overline{plot})}{\text{PAR}\ \overline{sfc}}\right] * 100$$

Sampling was done in spring 2014, 2015, 2016 and 2017. Measurements in 2014 were taken as a baseline prior to the removal of *S. horneri* from S– plots.

The damp biomass of *S. horneri* was calculated based on life stage-specific counts of all *S. horneri* within plots. Densities were converted to damp biomass using life stage-specific size-to-biomass formulas.

Table 3: Vertical distribution of algal biomass

Description:

Sargassum horneri may display a spatial distribution that differs from native algae and contributes to its invasion success. We examined the degree of spatial complementarity between *S. horneri* and native algae by comparing their biomass across the depth range within which most species of brown algae at Santa Catalina Island occur (0 – 30 m). Scuba divers counted the number of recruit (defined as < 5 cm tall) and adult (defined as > 5 cm tall) *S. horneri* and native species of subcanopy-forming macroalgae within 1 m^2 quadrats placed every 5 m along transects at four sites that ran perpendicular to shore from the intertidal to 30 m depth or where the reef transitioned to sand, whichever came first. Density data were converted to units of damp biomass using the method described in Table 1. Since these algae grow only on hard bottom substrate, we visually estimated the percent cover of rock within each quadrat and standardized density estimates to m^{-2} hard bottom. We performed these surveys in April of 2016, the time of year when the biomass of *S. horneri* reaches its peak. Although smaller native understory species may also compete with *S. horneri,* limits on bottom time prevented us from sampling them.

Measured depths were adjusted relative to the Mean Lower Low Water (MLLW). The total biomass of each species within a quadrat was calculated as the sum of the biomass of its juvenile and adult stages.

Table 4: Herbivore preference assessed by grazing assays

Description:

The primary grazers at Santa Catalina Island include sea urchins and herbivorous snails. *Centrostephanus coronatus*, the most abundant species of urchin, takes refuge in crevices and forages within <1 m from its shelter during the night before returning to the same location before sunrise. This behavior leads to the formation of urchin "halos" where they commonly graze down algae within small home ranges.

We performed grazing assays and surveys of benthic algae within and adjacent to urchin halos to assess whether the palatability of *S. horneri* differed from that of other algae. In September 2016, replicate arrays consisting of *Sargassum horneri*, its native and introduced congeners *S. palmeri* and *S. muticum* and the native kelps *Macrocystis pyrifera* and *Eisenia arborea* were deployed at Isthmus Reef for periods of 48 h. Arrays were either exposed to grazing by urchins and snails or placed inside cages nearby that were designed to exclude these grazers. Cages were constructed from 1 cm-gauge plastic mesh and were cylindrical in shape (1 m in height and 0.5 m in diameter) with mesh covering the top. Cages were open at the bottom and a 1 m-wide weighted skirt secured them to the reef and prevented grazers > 1 cm from entering through the bottom of the cage. All urchins and snails were removed from the cages at the beginning of each assay.

During each of the four deployments, 15 arrays containing one sample of each of the five target species of algae were placed in urchin halos while another 15 were placed inside cages designed to exclude grazers. Urchin halos were defined as sections of the reef adjacent to a small ledge where >10 urchins were found and grazing activity was apparent from a lack of algae growing within a 30 cm radius. Some herbivorous snails were also present in the halos, including *Tegula eiseni*, *Tegula aureotincta*, *Megastrea undosa* and *Norrisia norrisii*. Cages were left in the same location for the duration of the experiment, but we selected unique halos for each deployment so that herbivores would be naïve to the arrays. In the day preceding each deployment, we collected and weighed similarly sized blades or thalli of the five target species. Damp weights were quantified prior to deployment and immediately after collection by spin-drying samples for 10 s before weighing them. Three repeat measurements of each sample were taken by re-hydrating the sample and repeating the drying and weighing process. The average of three replicate measurements for each sample was used to optimize our ability to detect small changes in tissue loss.

Herbivore preference was assessed by comparing algal weights measured before and after deployment in the exposed versus caged arrays. We calculated the percent of biomass lost as:

$$\% \Delta = \left[\frac{G_{final} - G_{initial}}{G_{initial}} \right] * 100$$

where $G_{initial}$ and G_{final} represent the mean of the three replicate weights measured for each sample before and after deployment respectively.

Table 5: Herbivore preference assessed by algal percent cover

Description:

To provide a more time-integrated assessment of the feeding preferences of grazers, we tested whether the relative abundance of *S. horneri* differed from that of native algae in heavily grazed areas during the final deployment of the grazing experiment (see Description for Table 4 for details). We did this by measuring the percent cover of all subcanopy and understory algae in 1 m^2 quadrats placed adjacent to the 15 urchin halos and at 15 nearby reference locations (i.e., non-halo) with high algal cover. Percent cover was assessed using the uniform point contact sampling method (see Description for Table 1 for details). We ignored encrusting algae and unoccupied space in order to focus on the differences between the foliose algal species that are likely to be consumed by the grazers. Algae were identified to the lowest taxonomic level possible.

For further details, see:

Marks LM, Reed DC, Holbrook SJ (2020) Niche Complementarity and Resistance to Grazing Promote the Invasion Success of *Sargassum horneri* in North America. Diversity, 12(2).