Methods excerpted from

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*Study Sites.* Macroalgal biomass and irradiance data were collected at two locations at each of four reefs along the mainland coast of the Santa Barbara Channel, California, USA: Arroyo Quemado ($34^0$ 28.048'N, $120^0$ 07.031'W), Carpinteria ($34^0$ 23.474'N, $119^0$ 32.510'W), Mohawk ($34^0$ 23.649'N, $119^0$ 43.762'W) and Naples (34° 25.342'N, 119° 57.102'W). The eight sampling locations ranged in depth from 5.8 m to 8.9 m (MLLW) and were chosen to represent a range of physical and biological characteristics known to influence subtidal macroalgal assemblages in the region. Sea urchin grazing intensity differed dramatically among locations and contributed to the wide array of macroalgal assemblages studied, ranging from low diversity, low biomass assemblages dominated by coralline crusts to high diversity, high biomass foliose assemblages (Arkema et al. 2009). A common (but not always persistent) feature was the presence of the giant kelp, *Macrocystis pyrifera* Linnaeus, which forms a dense canopy at the sea surface that suppresses growth of understory algae below it. We took advantage of the variable light conditions produced by an ongoing *M. pyrifera* removal experiment (see Byrnes et al. 2011) to further increase the range of macroalgal assemblages evaluated. At each reef, *M. pyrifera* was removed from a 2000 $m^2$ plot once per year in early winter and an adjacent un-manipulated 2000 $m^2$ plot served as a control. Areas within these control and removal plots served as sampling locations at each of the four reefs.

*Macroalgal Abundance and Standing Biomass.* Divers using SCUBA surveyed the abundance of all understory macroalgae along permanent 40 m x 2 m transects in the center of each sampling location twice per season (approximately every six weeks) from January 2008 through December 2011. Abundance-derived biomass of all understory macroalgae was estimated using relationships generated from field estimates of abundance and laboratory measurements of biomass (see Table 1). These relationships were derived for 19 taxa that accounted for 97% of the standing biomass of understory macroalgae averaged across all sampling locations during the study period.

We used different measures of abundance for macroalgae of different sizes and morphologies. Percent cover was used to measure the abundance of crustose forms, low lying turfs and foliose algae. The following taxa were included in this group: *Bossiella orbigniana* Decaisne*, Callophyllis flabellulata* Harvey*, Chondracanthus corymbiferus* Kützing*, Corallina chilensis* Decaisne*,* large *Cystoseira osmundacea* Turner (defined as individuals of diameter > 10 cm)*, Desmarestia ligulata* Stackhouse*, Laurencia spectabalis* Postels & Ruprecht*, Polyneura latissima* Harvey*, Rhodymenia californica* Kylin*, Dictyota* spp.*,* family Ectocarpaceae*, Polysiphonia* spp.*, Pterosiphonia* spp., *Halymenia* spp., and crustose coralline algae consisting primarily of *Pseudolithophyllum*

*neofarlowii* Setchell & Mason. Taxon-specific relationships between percent cover and biomass were established using data collected from within 20 to 30 replicate 100 $cm^2$ quadrats strategically placed on the bottom over the course of a year at the study sites. Percent cover was estimated by divers as a proportion of 20 uniformly spaced points within the 100 $cm^2$ quadrat that contacted any foliage of the target taxon. Once points were recorded, all tissue of the targeted taxon within the quadrat was carefully collected, placed in a labeled plastic bag and returned to the laboratory for determination of biomass in units of dry mass. In the laboratory, each sample was weighed damp, dried at 60$^oC$ for three days and then re-weighed. *C. chilensis, B. orbigniana* and crustose coralline algae were de-calcified using a 10% HCL bath prior to drying to obtain measurements of de-calcified dry mass.

Density (number of individuals $m^{-2}$) was used as the measure of abundance for the understory kelps *Laminaria farlowii* Setchell and *Pterygophora californica* Ruprecht and small individuals of the fucoid *Cystoseira osmundacea*. Density was coupled with measurements of individual size to estimate biomass of these species. For large individuals of *L. farlowii* (defined as having a blade width > 15cm) and *P. californica* (defined as having a stipe length $\geq$ 20cm and a stipe diameter > 7mm), biomass was estimated from allometric relationships developed using individuals collected in the field and measured and weighed in the laboratory. Dry mass of large *L. farlowii* was related to total blade length, while dry mass of large *P. californica* was related to the total number of blades > 30 cm in length. Small individuals of these species can be extremely abundant and mean size calculated from a subsample of individuals was used to estimate the biomass of individuals of these species that were smaller than the sizes noted above.

We coupled our derivations of biomass with time series data of abundance to estimate taxon-specific biomass through time. The abundance (percent cover or density) (and size in the case of *Laminaria farlowii, Pterygophora californica and Cystoseira. osmundaceae*) of all taxa of understory macroalgae were surveyed at each sampling location approximately every six weeks during the four-year study. Large *P. californica* and *L. farlowii* were counted within the entire 40 m x 2 m area of each sampling location, whereas small *P. californica*, *L. farlowii,* and *C. osmundaceae* were counted in six 1 $m^2$ quadrats uniformly distributed within the sampling area. Percent cover of the remaining species was estimated using a point contact method consisting of a uniform grid of 80 points in a 40 m x 1m area at each sampling location. This method accounted for vegetation layers of multiple taxa, however any single taxon was only recorded once at each point. All estimates of abundance were converted to units of dry mass using the relationships described above. Biomass derivations for the 19 focal species are shown in Table 1. The biomass of rare species not mentioned above (which collectively accounted for 3% of cumulative biomass) was determined using relationships between percent cover and biomass generated for morphologically similar taxa. Estimates of biomass were

summed over all taxa and size classes to yield community estimates of biomass at each sampling location for each time period.

*Bottom Irradiance.* Surface and bottom irradiances were measured using submersible PAR sensors (MKV-L, Alec Electronics, Japan). Sensors were mounted ~30 to 100 cm above the sea surface on a moored vertical spar buoy at each of the four reefs and on a stake 30 cm above the seafloor at each of the eight sampling locations. Irradiance in units of μmol $m^{-2}$ $sec^{-1}$ was recorded once per minute and averaged to obtain hourly estimates over the course of each day. Sensors were retrieved on each survey date and replaced with a clean calibrated sensor. Upon their collection, sensors were placed in plastic bags, returned to the laboratory and evaluated for biofouling by exposing them to 500 watt halogen lamps for 20 minutes while submerged in seawater. Sensors were then cleaned and exposed to the same light field for an additional 20 minutes. We calculated attenuation by the fouling community and corrected light values as an exponentially increasing function of time after deployment. Fouling never changed estimates of bottom irradiance by more than 2%.

*Physiological Measurements.* We used the methods of Miller et al. (2012) to measure photosynthesis versus irradiance and respiration by the 19 most common macroalgal taxa listed in Table 2. We incubated whole thalli (minus the woody stipe and holdfast in the case of the kelp *Pterygophora californica*) in clear acrylic tanks and measured oxygen evolution at nine levels of irradiance (19, 36, 60, 103, 178, 198, 344, 392, and 700 μmol $m^{-2}$ $sec^{-1}$: n = 10 to 20 whole thalli per taxon). This range of instantaneous irradiances encompassed the entire range of values that we observed on the bottom at our sampling locations during the period of study. The initial slope of the relationship between photosynthesis and irradiance at non-saturating irradiance (α) was determined using linear regression of non-saturating irradiance values for each taxon (Jassby and Platt 1976). Photosynthesis at saturating irradiance ($P_{max}$) was estimated for each thallus by fitting the hyperbolic tangent function (Jassby and Platt 1976) using SAS (SAS Institute Inc., North Carolina version 9.1.3). Estimates of $P_{max}$ and α were averaged across replicate thalli to obtain mean estimates for each species or taxonomic group. Units of oxygen were converted to carbon using a photosynthetic quotient of 1.0 (following Rosenberg et al. 1995) and respiration and production rates were standardized to the dry mass of photosynthetic tissue (Table 2).

*Macroalgal Primary Production.* Daily NPP (mg C $m^{-2}$ $d^{-1}$) for each taxon encountered was calculated per Miller et al. (2012), which followed a modified version of the equation of Jassby and Platt (1976):

$$NPP_i = \sum_h P_{\max} * \tanh\left(\alpha_i E_h / P_{\max_i}\right) * b_i - \sum_h R * b_i$$

Where $P_{max}$ is in units of mg C $hr^{-1}$ (g dry mass)$^{-1}$, α is in units of mg C $hr^{-1}$ (g dry mass)$^{-1}$ (μmol $m^{-2}$ $sec^{-1}$)$^{-1}$, E is mean bottom irradiance (μmol $m^{-2}$ $sec^{-1}$) over the course of an hour (h), R is respiration in the dark (mg C $hr^{-1}$ (g dry mass)$^{-1}$) and b is the daily estimate of standing dry biomass (g $m^{-2}$) of an individual taxon (i). Daily standing biomass was estimated using linear interpolation of biomass from one sampling date to the next. NPP was calculated as the sum of gross production and respiration over all daylight hours and respiration over all hours of darkness for each taxon at each sampling location for each day of the year over the four-year study. We used these values to calculate mean NPP for each taxon for early and late portions of each season. Seasons were defined by the solar solstices and equinoxes. Daily NPP for each taxon was summed over all days in a year to obtain annual estimates of NPP at each sampling location in units of kg C $m^{-2}$ $y^{-1}$.

*Data Analysis.* Daily standing biomass and NPP at each sampling location were summed across all taxa to obtain estimates of each of these variables for the entire understory macroalgal assemblage. NPP and biomass for the entire assemblage were averaged in each season to obtain mean daily estimates for each time period (i.e., early and late portions of each season).

*References*

Arkema K. K., Reed, D. C. & Schroeter, S. C. 2009. Direct and indirect effects of giant kelp determine benthic community structure and dynamics. *Ecology* 90:3126-3137.

Byrnes, J. E., Cardinale, K. C., Cavanaugh, K. C., Holbrook, S. J., Reed, D. C. & Schmitt, R. J. 2011. Climate-driven increases in storm frequency simplify kelp forest food webs. *Glob. Change Biol.* 17:2513–2524.

Jassby, A. D. & Platt, T. 1976. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnol. Oceanogr.* 21:540-547.

Miller, R. J., Harrer, S. L. & Reed, D.C. 2012. Addition of species abundance and performance predicts community primary production of macroalgae. *Oecologia*. 168:797-806.

Rosenberg, G., Littler, D. S., Littler, M. M. & Oliveira, E. C. 1995. Primary production and photosynthetic quotients of seaweeds from Sao Paulo State, Brazil. *Bot. Mar.* 38:369-377.

Table 1. Equations, coefficients of determination ($r^2$) and sample sizes (N) used to estimate biomass from abundance for 19 common macroalgal taxa. Percent of total biomass represents the percentage of the overall mean biomass averaged across all sampling locations and sampling periods contributed by each of the 19 taxa (with standard error) which collectively accounted for 97 % of the assemblage biomass.

| Percent of total biomass | Phylum | Taxon | Equation to estimate biomass (dry g $m^{-2}$) | $r^2$ | N |
|---|---|---|---|---|---|
| 31.5 ( 2.5 ) | Phaeophyta | *Pterygophora californica* | 6.29 ( # blades > 30 cm ) | 0.77 | 145 |
| 17.2 ( 4.2 ) | Phaeophyta | *Desmarestia ligulata* | 2.61 ( percent cover ) | 0.7 | 22 |
| 10.0 ( 0.8 ) | Rhodophyta | *Rhodymenia californica* | 1.22 ( percent cover ) | 0.65 | 33 |
| 9.8 ( 1.1 ) | Rhodophyta | *Chondracanthus corymbiferus* | 1.69 ( percent cover ) | 0.89 | 25 |
| 5.9 ( 0.3 ) | Rhodophyta | *Polyneura latissima* | 3.24 ( percent cover ) | 0.84 | 34 |
| 5.3 ( 1.7 ) | Phaeophyta | *Cystoseira osmundacea* | 3.35 ( percent cover ) | 0.79 | 33 |
| 4.4 ( 0.7 ) | Rhodophyta | *Pseudolithophyllum* neofarlowii | 0.33 ( percent cover ) | 0.78 | 44 |
| 4.3 (1.2 ) | Rhodophyta | *Pterosiphonia dendroidea* | 0.67 ( percent cover ) | 0.77 | 35 |
| 2.6 ( 0.1 ) | Rhodophyta | *Corallina chilensis* | 2.13 ( percent cover ) | 0.79 | 34 |
| 1.1 ( 0.3 ) | Rhodophyta | *Laurencia spectabalis* | 1.96 ( percent cover ) | 0.87 | 26 |
| 1.0 ( 0.1 ) | Rhodophyta | *Gracilaria* spp. | 2.25 ( percent cover ) | 0.64 | 31 |
| 0.9 ( 0.3 ) | Rhodophyta | *Polysiphonia* spp. | 0.8 ( percent cover ) | 0.71 | 28 |
| 0.8 ( 0.1 ) | Rhodophyta | *Halymenia* spp. | 1.24 ( percent cover ) | 0.72 | 25 |
| 0.6 ( 0.1 ) | Rhodophyta | *Bossiella orbigniana* | 2.02 ( percent cover ) | 0.88 | 38 |
| 0.6 ( 0.2 ) | Phaeophyta | *Ectocarpaceae* spp. | 0.33 ( percent cover ) | 0.73 | 19 |
| 0.4 ( 0.1 ) | Rhodophyta | *Cryptopleura ruprechtiana* | 3.88 ( percent cover ) | 0.88 | 26 |
| 0.3 ( 0.1 ) | Phaeophyta | *Laminaria farlowii* | 0.31 ( blade length in cm ) | 0.9 | 52 |
| 0.3 ( 0.1 ) | Rhodophyta | *Callophyllis flabellulata* | 0.7 ( percent cover ) | 0.85 | 45 |
| 0.2 ( 0.1 ) | Phaeophyta | *Dictyota* spp. | 1.08 ( percent cover ) | 0.77 | 24 |

Table 2. Mean values for laboratory-derived photosynthetic parameters: $P_{max}$ (mg C (g dry mass)$^{-1}$ hr$^{-1}$), α (mg C (g dry mass)$^{-1}$ hr$^{-1}$)(μmol m$^{-2}$ sec$^{-1}$)$^{-1}$ and respiration (mg C (g dry mass)$^{-1}$ hr$^{-1}$) for 19 common taxa. $P_{max}$ is based on gross primary production. Taxa are listed in descending order of their biomass contribution. (see Table 1).

| Taxon | $P_{max}$ (SE) | α (SE) | Resp (SE) | N |
|---|---|---|---|---|
| *Pterygophora californica* | 1.90 ( 0.29 ) | 0.011 ( 0.001 ) | -0.42 ( 0.12 ) | 20 |
| *Desmarestia ligulata* | 3.57 ( 0.39 ) | 0.045 ( 0.006 ) | -1.26 ( 0.24 ) | 16 |
| *Rhodymenia californica* | 3.13 ( 0.94 ) | 0.088 ( 0.023 ) | -1.07 ( 0.38 ) | 14 |
| *Chondracanthus corymbiferus* | 1.89 ( 0.28 ) | 0.013 ( 0.004 ) | -0.36 ( 0.12 ) | 16 |
| *Polyneura latissima* | 6.27 ( 0.62 ) | 0.138 ( 0.031 ) | -1.99 ( 0.48 ) | 23 |
| *Cystoseira osmundacea* | 2.24 ( 0.58 ) | 0.033 ( 0.017 ) | -0.55 ( 0.12 ) | 17 |
| *Pseudolithophyllum* neofarlowii | 6.39 ( 2.04 ) | 0.297 ( 0.035 ) | -2.42 ( 0.38 ) | 24 |
| *Pterosiphonia dendroidea* | 10.19 ( 0.97 ) | 0.232 ( 0.030 ) | -3.65 (0.46 ) | 30 |
| *Corallina chilensis* | 3.55 ( 0.62 ) | 0.209 (0.026 ) | -1.36 ( 0.22 ) | 38 |
| *Laurencia spectabalis* | 5.20 ( 0.77 ) | 0.094 ( 0.018 ) | -1.8 ( 0.54 ) | 12 |
| *Gracilaria* spp. | 3.48 ( 0.53 ) | 0.025 ( 0.003 ) | -1.16 ( 0.22 ) | 27 |
| [1]*Polysiphonia* spp. | 10.19 ( 0.97 ) | 0.232 ( 0.030 ) | -3.65 (0.46 ) | 30 |
| [2]*Halymenia* spp. | 1.89 ( 0.28 ) | 0.013 ( 0.004 ) | -0.36 ( 0.12 ) | 16 |
| *Bossiella orbigniana* | 5.52 ( 0.39 ) | 0.145 ( 0.010 ) | -2.68 ( 0.16 ) | 17 |
| *Ectocarpaceae* spp. | 13.09 ( 1.18 ) | 0.256 ( 0.036 ) | -5.37 ( 1.67 ) | 27 |
| *Cryptopleura ruprechtiana* | 1.49 ( 0.21 ) | 0.029 ( 0.005 ) | -0.41 ( 0.12 ) | 19 |
| *Laminaria farlowii* | 1.36 ( 0.1 ) | 0.005 ( 0.0004 ) | -0.24 ( 0.04 ) | 11 |
| *Callophyllis flabellulata* | 7.26 ( 1.07 ) | 0.24 ( 0.026 ) | -2.37 ( 1.06 ) | 19 |
| *Dictyota* spp. | 5 ( 1.17 ) | 0.08 ( 0.013 ) | -1.23 ( 0.31 ) | 12 |

[1] Values for *Pterosiphonia* spp. were used as a proxy.

[2]Values for *Chondracanthus corymbiferus* were used as a proxy.