Protocol

Chlorophyll to carbon ratio estimated from aerial hyperspectral imagery

Tom W. Bell and David A. Siegel

Estimates of Kelp Canopy Biomass/Age

To track the progression of giant kelp canopy development, we estimated canopy biomass and age using Landsat 5, 7 and 8 satellite imagery. The combination of these sensors delivers an image every 8 - 16 days, with 1 - 2 cloud-free images per month. Landsat multispectral imagery has been used to estimate the canopy biomass of giant kelp across the NE Pacific (Cavanaugh et al. 2011; Bell et al. 2020). Briefly, each 30 m pixel is modeled as a linear combination of one temporally stable giant kelp canopy spectral endmember and one of 30 temporally varying seawater spectral endmembers, which are unique to each image date, using Multiple Endmember Spectral Mixing Analysis (MESMA; Roberts et al. 1998). The estimated fractional cover of kelp canopy in each pixel was compared to diver-estimated kelp canopy biomass at two sites in the SBC measured by the Santa Barbara Coastal Long Term Ecological Research Project (SBC LTER) from 2003 – 2017 ($r^2 = 0.64$, $p < 0.001$; Bell et al. 2020; 2021a). We determined canopy age by setting the first date where canopy biomass was observed in a pixel as age = 1 day. Long-term, annual kelp persistence was determined using a seasonal 34-year spatial time series of kelp canopy biomass from 1984 – 2017. Pixels were classified as occupied for a given year if kelp canopy was present at least once during that year. Persistence of an individual pixel was defined as the proportion of years that the pixel was occupied by kelp. Regional scale persistence was the mean annual occupancy of all pixels within the 1 km coastline segment.

Laboratory Analysis of Kelp Canopy Physiological Condition

Field samples of giant kelp canopy chlorophyll *a* pigment concentration and carbon content were taken at three sites in the SBC: Arroyo Quemado (34.4677 N, 120.1191 W), Arroyo Burro (34.4003 N, 119.7446 W), and Mohawk (34.3941 N, 119.7296 W) kelp forests (Bell et al. 2018). All sites were sampled monthly from August 2012 – August 2015. Fifteen mature blades were collected haphazardly from different plants inside a permanent 40 x 40m plot at each site. The blades were standardized for age by selecting blades approximately two meters from the tip of an actively growing frond (representing a blade age of ~14 days). Blades were placed in a sealed plastic bag which was immediately placed on ice in an opaque cooler. The blades were then transported to the lab where they were stored at 4°C until being processed within 24 hours of collection. In order to examine changes in pigment concentration with canopy age, blades from frond cohorts were collected every 3 weeks from March – September 2019 at Mohawk reef. One hundred fronds were tagged two meters from the growing tip and one blade from each of 10 different individuals were collected at the tag site. At each sampling period, 1 blade was sampled from 10 previously tagged fronds at the tag site and an additional 100 fronds were tagged two meters from the growing tip.

A 5 cm square was cut from the center of each blade approximately 5 cm above the pneumatocyst and any epibionts were removed (blades usually had no epibionts). The reflectance of the square was then measured between 350 – 800 nm, at 1 nm intervals, using a Shimadzu UV 2401PC spectrophotometer with an integrating sphere attachment (Bell et al. 2015b; 2018). Chlorophyll *a* concentration was determined from a 0.8 cm^2 disc excised from the center of each square. Each disc was weighed and placed in 4 mL of dimethyl sulfoxide for 45 minutes at room temperature in the dark. The disc was then removed and washed with 1 mL water before being placed in 5 mL of a 3:1:1 acetone, methanol, and water solution for 2 hours at 4°C in the dark (Seely et al. 1972). The extracts were placed in individual quartz cuvettes and absorbance was measured between 350 – 800 nm using the spectrophotometer. Chlorophyll *a* concentration was determined using known absorbance-based equations (Seely et al. 1972). A separate 5 cm^2 disc was excised from each blade near the pneumatocyst

and rinsed in a 10% HCl solution to remove any residual calcium carbonate from epibionts. These discs were weighed and combined for each site and date before being placed in a drying oven at 60°C for several days, after which, dry mass was recorded. The dried discs were ground to a fine powder and analyzed for carbon and nitrogen content using an elemental analyzer (Carlo-Erba Flash EA 1112 series, Thermo-Finnigan Italia, Milano, Italy). Chl:C was calculated by dividing the mass of chlorophyll *a* by the dry mass of carbon for each disc.

Hyperspectral Estimates of Kelp Canopy Physiological Condition

Giant kelp blade Chl:C was estimated using an algorithm developed from laboratory reflectance spectra of recently matured kelp blades (Bell et al. 2015b). The 1 nm resolution laboratory reflectance spectra were degraded to ~10 nm bands consistent with the Airborne Visible/Infrared Imaging Spectrometer (AVIRIS). The difference in pseudoabsorbance (ln 1/Reflectance) between bands centered at 658 nm and 677 nm were related to the Chl:C of the kelp blades by equation 1,

Eq. 1 $$\text{Chl:C} = 0.0353e^{-7.53x}$$

where x is equal to the difference in pseudoabsorbance between the two bands. Cross validation analysis found that this relationship explained 76% of the observed variance in laboratory assessed Chl:C (Bell et al. 2015b).

The AVIRIS sensor provided hyperspectral image swaths (11 km width) of the SBC approximately three times per year (April, June, August) from 2013 – 2015 as part of the HyspIRI Preparatory Airborne Campaign (hyspiri.jpl.nasa.gov/airborne). The AVIRIS sensor provides imagery of upwelling spectral radiance in 224 contiguous, 10 nm bands (400 – 2500 nm) at a pixel resolution of 18 m. For this study, orthocorrected level-2 reflectance products were used. All imagery is freely available (aviris.jpl.nasa.gov/).

The laboratory derived giant kelp Chl:C algorithm was then applied to the AVIRIS hyperspectral imagery of the SBC. One issue with scaling from kelp blade measurements in the laboratory to measurements of kelp canopy in the imagery is that each image pixel is a mixture of giant kelp canopy and seawater. To account for differences in fractional cover between pixels, MESMA was used to calculate the proportional kelp cover for each pixel. Only pixels with greater than 10% kelp cover were used in the analysis. For each image date, each kelp pixel was normalized for proportional kelp cover using the empirical linear relationship between the difference in reflectance for the bands centered at 658 nm – 667 nm and the fractional kelp cover in that pixel. Equation 1 was then applied to all imagery to estimate the Chl:C of each kelp pixel in the SBC for each image date. To validate the algorithm for floating giant kelp canopy, we compared field sampled Chl:C from each site to the mean Chl:C of the four AVIRIS pixels that overlaid each site for the sampling date closest to each image acquisition. If an image date fell between two field sample dates (>5 days) the later sampling date was used to account for changing environmental conditions. Field and image estimated Chl:C were compared using a reduced major axis least squared regression.

Relationship of Kelp Physiological Condition to Environmental Variables and Canopy Age

To determine the relationship between SST and Chl:C estimated over regional scales, the coastline of the SBC was divided into 1 km segments. All kelp pixels were binned into their closest coastline segment. If the coastline segment contained >15 classified kelp pixels, the mean of those pixels was calculated and assigned to that segment, for each image date. Each coastline segment was assigned a mean SST for each image date by taking the mean of all MODIS SST pixels within a 5 km radius for images between 2 and 20 days previous to the AVIRIS image date. Each segment's Chl:C and SST were compared using a linear regression across each image date. Since the overall relationship across all dates may not

be linear, a generalized additive model was used to elucidate the potentially non-linear fit using the mgcv package in R (Wood 2006).

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