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Phenology and the Response to Disturbance of the Fucoid, Stephanocystis osmundacea

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California State University, Monterey Bay

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PHENOLOGY AND THE RESPONSE TO DISTURBANCE

OF THE FUCOID, *STEPHANOCYSTIS OSMUNDACEA*

A thesis presented to

the faculty of Moss Landing Marine Laboratories

and Department of Natural Sciences

California State University, Monterey Bay

In partial fulfillment

of the requirements for the degree

Master of Science

in Marine Science

by Cody Dawson

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CALIFORNIA STATE UNIVERSITY MONTEREY BAY

The Undersigned Faculty Committee Approves the
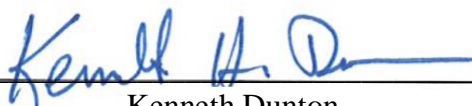
Thesis of Cody Dawson:

PHENOLOGY AND THE RESPONSE TO DISTURBANCE
OF THE FUCOID, *STEPHANOCYSTIS OSMUNDACEA*

_____
Michael Graham, Advisor
Moss Landing Marine Labs

_____
Scott Hamilton
Moss Landing Marine Labs

_____
Kenneth Dunton
University of Texas at Austin

_____
Kris Roney, Dean
Associate VP for Academic Programs and Dean of Undergraduate and Graduate Studies

_____
Approval Date

Phenology and the Response to Disturbance of the Fucoid,
Stephanocystis osmundacea
by
Cody Dawson
Master of Science in Marine Science
Moss Landing Marine Laboratories, 2018

ABSTRACT

Nearshore rocky ecosystems along exposed shorelines experience frequent disturbances due to turbulent swells and wave action. These disturbances directly affect subtidal algal communities that provide biogenic habitat along the coast. This habitat shapes faunal communities by providing refuge through structural complexity. In central California, kelps are the most notable providers of biogenic habitat, but, seasonally, a prolific fucoid, *Stephanocystis osmundacea*, adds a considerable amount of habitat into the environment. While diminutive and bushy during the winter, this alga produces canopy-forming reproductive fronds during the spring and summer months that add to the biogenic refuge. The purpose behind this study was to understand how the frequency and timing of disturbances affect the physiology of *Stephanocystis*. This was accomplished by performing manipulations on the reproductive and vegetative tissues of the alga, including: full reproductive removal (-R), haphazard vegetative blade damage (-V), no removal (C), and damage of both reproductive and vegetative structures (-All). By using measurements of changes in total length (cm) as a proxy for biomass we provided an *in situ* assessment of the response to disturbance by the alga. This external growth response was coupled with stable isotope analysis of changes in carbon and nitrogen isotopes as a bioindication of fitness. Removal of reproductive fronds during spring elicited a dormancy response, while damage to the vegetative tissue reduced growth, possibly by limiting overall photosynthetic capacity. These results suggest that spring frond growth is important to reproductive fitness and removal can stimulate a life history trade-off between reproduction and survival. Winter manipulations elicited no response due to the dormancy period of this species. Enrichment values for $\partial^{13}C$ and $\partial^{15}N$ were consistent with reported values for growth in other brown algal species but, because of the timing of extraction, the internal chemistry of the individuals rebounded and the ability to detect a response was lost. Both the natural and manipulated populations had similar $\partial^{13}C$ and $\partial^{15}N$ values when separated by tissue and time of year, which indicates that while the alga may be impacted from an external perspective, it will recover internally and stay as a viable part of the reproductive population. Understanding how these seaweeds respond to biomass loss provides a better perspective of disturbance effects on this species and the ecosystem it helps support.

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INTRODUCTION

Structural or habitat complexity is a cornerstone of a productive and biologically diverse ecosystem (Anderson 1994, Steneck et al. 2002, Willis & Anderson 2003, Matias et al. 2010). This complexity is determined by habitat heterogeneity, defined as vertical and horizontal vegetation and landscape structure, and "keystone" structures, which are the physical components of the vegetation and landscape. Meta-analyses indicate that habitat heterogeneity and structure positively influence biodiversity in terrestrial and marine ecosystems (Tews et al. 2004), and these factors are often used to inform conservation and management decisions, such as the placement of marine reserves (Roberts et al. 2003). The types of structures that are known to improve biodiversity marine ecosystems can be biogenic (e.g., coral, mussels, macroalgae) or abiogenic (e.g., substrate, pier pilings, oil rigs) (Kovalenko et al. 2012). Each type of structural habitat produces a spatially heterogeneous environment that helps sustain higher species richness (Torres-Moye et al. 2013).

In coastal marine ecosystems along the California coast, prolific seaweeds such as the giant kelp, *Macrocystis pyrifera*, provide biogenic structure that creates habitat and refuge; these species are often labeled as foundation species (reviewed in Graham et al. 2007). The term "foundation species" refers to an organism that is disproportionately important to the community structure (Dayton 1972) and helps ameliorate environmental stress for space and refuge (Stachowicz 2001) and act as a provision for resources. The vertical structure produced by a *Macrocystis* canopy provides physical orientation and adds to the complexity of the habitat, essentially serving as an extension of the substratum into the water column (Quast 1971, Wheeler 1980). The presence of *Macrocystis* has been shown in past studies to dramatically enhance abundances and richness of fishes (Ebeling et al. 1980, Carr 1989, Holbrook et al. 1990,

Anderson 1994, Graham 2004) and invertebrates (Coyer 1984) in kelp beds, while its role on understory algal communities is more dependent on its absence due to light competition (Reed & Foster 1984, Santelices & Ojeda 1984, Holbrook et al. 1990, Clark et al. 2004). It is generally considered that any disturbances affecting foundation species (e.g., *Macrocystis*) are likely to have cascading effects throughout their associated ecosystems (Reed et al. 2011).

Along the central California coastline, storm-driven swells are a frequent and major source of disturbance on wave-exposed shores (Graham et al. 1997). These swells bring kinetic wave energy that is absorbed by dense kelp forests that are formed during summer months (Seymour et al. 1989). This constant barrage of wave energy is the primary cause of kelp biomass removals (Dayton et al. 1992, Graham 1997, Reed et al. 2011), due in part to the drag of the high surface area provided by its biogenic structures (Seymour et al. 1989). Storm disturbances fragment this habitat on a variety of spatial-scales, depending on the severity of the event, thus creating a network of patches (Dayton et al. 1984). Ultimately, storm disturbances are one of the most important factors influencing kelp population dynamics and the productivity of this foundation species up and down the coast (Reed et al. 2011), while also providing a window for understory algae to recruit to the bare space that is freed up (Reed & Foster 1984, Clark et al. 2004).

Sub-canopy algal assemblages tend to be comprised of opportunistic settlers and can provide similar structural amenities seen in canopy-forming species. Low-lying stipitate kelp beds of *Pterygophora californica* are often used as foraging grounds for surfperch (Ebeling & Laur 1985), while similarly sized *Ecklonia* spp. beds have been shown to harbor higher abundances of fishes (Tuya et al. 2009) and invertebrates (Goodsell et al. 2004). In addition to kelp species, there are some fucoids that create sub-surface and surface canopies in the subtidal

(Coleman et al. 2008, Marzinelli et al. 2013). Fucoids have been known to act as "foundation species" in the Baltic Sea (Råberg & Kautsky 2007), the Mediterranean Sea (Benedetti-Cecchi & Cinelli 1992, Bulleri 2002, Chiminée et al. 2013), Australia (Marzinelli et al. 2013), New Zealand (Schiel 2006), and southern California (Gunnill 1982, 1986). Ecologically, members of the Fucales tend to be perennial "shrubs" that will persist in intertidal and subtidal environments for years (Schiel 1985, Gunnill 1986, Chapman 1995) and provide varying amount of shelter and protection from environmental stressors (Råberg & Kautsky 2007, Wernberg et al. 2011, Marzinelli et al. 2013). One fucoid proposed to have similar ecological relevance in central California subtidal ecosystems is the canopy-forming *Stephanocystis osmundacea* (Schiel 1985).

Stephanocystis osmundacea (formerly *Cystoseira osmundacea* [Draisma et al. 2010]), ranges from Oregon to Baja California, Mexico and is found intertidally and subtidally down to 30 m (Spalding 2003). *Stephanocystis* has a basal, vegetative thallus that is somewhat diminutive (<1m), which apically produces large, annual, pneumatocyst-bearing fronds during early spring into mid-fall that contain reproductive material (i.e., conceptacles housing oogonia and antheridia). Reproductive fronds can sometimes be seen forming canopies that intermingle with *Macrocystis* at depths between 6-9 m (Schiel & Foster 2015). These fronds can encompass up to 80% of the biomass for an individual, but are subjected to large-scale removals during winter storms (Schiel 1985).

Biomass loss in fucoids through tissue removal is often mitigated in the alga by translocating energy and nutrients for regrowth, reproduction, and chemical defenses in wounded/nearby tissues (Chapman 1995). These responses utilize transport machinery within the thallus of an individual (Diouris & Floc'h 1984). Carbon- and nitrogen-based compounds are transported via translocation through sieve elements contained in the medullary matrix of the

stipe and blades (Moss 1983), though the process is much slower in fucoids (2-4 cm/hr; Diouris & Floc'h 1984) than kelps (10s-100s cm/hr; Schmitz 1981). Thallus healing and the investment in reproductive structures benefit from the ability of fucoids to translocate compounds through the thallus (Lehvo et al. 2001, Hurd et al. 2014). After a disturbance event removes some amount of tissue, the cellular response of the sieve elements facilitates transport of polysaccharides to plug the wound site within 6-24 hours (Hurd et al. 2014). The medullary cells underneath the plugged wound push through and become new, highly pigmented, lateral filaments, which increase the density of surface thylakoids, indicating a shift towards increased respiratory function and higher rates of photosynthesis for compensation (Fagerberg and Dawes 1977). Van Alstyne (1989) and Honkanen and Jormalainen (2002) described the ability to adventitiously branch from wound sites and exhibit compensatory growth after small-scale disturbances of their fucoid study species', alluding to the idea that alternative processes can be utilized by seaweeds to respond to tissue loss. In the case of Honkanen and Jormalainen (2002), *Fucus vesiculosus* also compensated for tissue loss by increasing its reproductive output, thus leading to a shrinking of the vegetative thallus. A variety of methods have been used to understand how tissue chemistry is affected by biomass loss in fucoids (e.g., chemical defenses: Van Alstyne 1988, Connan et al. 2004, Hemmi et al. 2004; metabolite production: Lehvo et al. 2001), but the investigation into isotope ratios (e.g., $\partial^{13}C$ & $\partial^{15}N$) for these chemical compounds has been underutilized in most fucoid species.

Stable isotopes have been used extensively in ecosystem studies as tracers of nutrients or organic material through systems (Petersen & Fry 1987, reviewed in Michener & Lajha 2007). Much of the work done has used autotroph-based carbon and nitrogen values to better understand how food is dispersed or utilized by primary and secondary consumers in a given system

(Stephenson et al. 1984, Dunton et al. 2012). For terrestrial and marine autotrophs, the ratio of heavy to light isotopes of carbon ($\partial^{13}C$) and nitrogen ($\partial^{15}N$) are distinct indicators of photosynthetic pathways (Marshall et al. 2007) and environmental conditions (Handley & Raven 1992) respectively, while both elements can be attributed to growth (Brenchley et al. 1997, 1998, Dayton et al. 1999).

Research utilizing $\partial^{13}C$ analysis has become a powerful tool because of the distinct values shown in photosynthetic machinery (Farquhar et al. 1989) and carbon-based storage compounds (Fox 2013). A well-studied enzyme involved in photosynthesis, ribulose bisphosphate carboxylase/oxygenase (RuBisCO), actively discriminates against the heavier carbon (^{13}C) during passive diffusion of CO_2, which is often reflected in more negative $\partial^{13}C$ values (Farquhar et al. 1989, Marshall et al. 2007). Because RuBisCO is a key component in the transformation of inorganic carbon into useable organic compounds, that discriminatory behavior has been transitioned to marine applications given the ability of micro- and macroalgae to uptake and use inorganic carbon sources in the form of CO_2 and bicarbonate (HCO_3^-) (Raven et al. 2002a, b). Increases in macroalgal biomass have been shown to be correlated with $\partial^{13}C$ values (Carvalho et al. 2009), but the determination of inorganic carbon sources in aquatic environments is slightly more difficult due to water motion and the presence of a diffusive boundary layer surrounding the alga (O' Leary 1988, Hurd 2000). The correlative relationship between $\partial^{13}C$ and growth was used effectively by Fox (2013, 2016) as a response variable for biomass loss in *Macrocystis*. Kelps can mobilize stored carbon reserves (e.g., laminarin; Chapman & Craigie 1978) by converting the storage compound into the more manageable sugar

alcohol, mannitol, and translocating it to areas of active growth (Kremer 1981). Fox (2013)

noticed high ∂^{13}C enrichment values for areas of *Macrocystis* frond initiation likely due to that

mobilization of ∂^{13}C enriched in stored carbohydrates in response to the loss of biomass, which

was a similar result seen in studies by Carvalho et al. (2007, 2009) using ∂^{13}C as an indicator for

rapid growth in another kelp, *Undaria pinnatifida*.

Nitrogen is often used in conjunction with carbon to help describe patterns of growth or

recovery in marine autotrophs, especially when referencing seasonal patterns and the response to

biomass loss (Chapman & Craigie 1977, Gagne et al. 1982, Gerard 1982, Brenchley et al. 1997,

1998). Most of these studies use inorganic nitrogen species (i.e., NO_3^- & NH_4^+) to trace

environmental changes through their roles in growth (Hanisak 1979), usually during times of

increased photoperiod (Fujita et al. 1989). Nitrogen isotope ratios (∂^{15}N) have been implemented

in determining the outside sources of nutrients with relatively small differences between nitrogen

species (Cohen & Fong 2005). Within some central California upwelling systems, ∂^{15}N values

have been shown to reflect oceanographic conditions in canopy-forming macroalgal species with

isotope values from upwelled seawater being observed in canopy blade tissue not long after

(Foley & Koch 2010, Fox 2016). In addition to being an indicator of the productivity of the

system, nitrogen enrichment values have been correlated to essential autotrophic compounds

needed for growth and photosynthesis (e.g., proteins and amino acids: Macko et al. 1987; lipids

and chlorophylls: Bidigare et al. 1991, Chikaraishi et al. 2005) giving further validity into the use

of nitrogen isotopes for other marine autotrophs, especially biomass dependent canopy formers.

The purpose of this study was to use isotope analyses and couple them with physical

measurements to better understand the ability of *Stephanocystis* to rapidly attain high amounts of

vertically structured biomass and assess how the species would respond to that biomass being lost during two critical points, the summer growth period (Schiel 1985) and the winter dormancy period (Gunnill 1980). The following questions were addressed: (1) How significant is the overall biomass produced by *Stephanocystis* (e.g., greater or less than the reported 80% by Schiel [1985]) to the fitness of the alga? (2) Will values of $\partial^{13}C$ and $\partial^{15}N$ vary positively or negatively with temporal changes in biomass (e.g., through discrimination or fractionation within the tissue vs. diffusive incorporation)? (3) If reproductive biomass is removed during frond initiation, will *Stephanocystis* continue growth in other parts of the thallus or will growth be halted? And, will that be reflected in $\partial^{13}C$ and $\partial^{15}N$ values? (4) If biomass is altered before the overwintering period, will *Stephanocystis* respond through mortality or be unaffected? And, will $\partial^{13}C$ and $\partial^{15}N$ values reflect that response or be more comparable to the natural thallus values?

I hypothesized that biomass production during the 2016 growth season would be a large portion of the overall biomass throughout the year (on par with Schiel 1985). In conjunction with this, I predicted carbon isotopes would correlate with tissue production through high enrichment (an overall internal increase in heavy inorganic carbons for use in photosynthesis) values during the spring reproductive frond initiation with a possible crash in the fall senescence period. I also anticipated that nitrogen isotope values would show a similar pattern of enrichment due to upwelling pulses (Foley & Koch 2010) and growth during the spring and summer months. For the experimental portion of the study, I hypothesized that damage to reproductive fronds during the summer growth period would cause those branches to become dormant while possibly shunting resources for biomass production to other areas of the thallus, similar to the results seen by Van Alstyne (1989). Damage to vegetative tissue would likely exhibit a similar reaction, but

due to it being a lower portion of the overall biomass the disturbance would likely be less detrimental to the individual's ability to rebound. Because the manipulation happened during a growth period, I predicted that the ratio for $\partial^{13}C$ in damaged individuals would be more enriched in areas where new frond initiation could occur (i.e., terminal edges of blades) and less enriched in the holdfast, which is most likely a source for carbon-heavy storage compounds (Fox 2016). Values for $\partial^{15}N$ were expected to mimic $\partial^{13}C$ due to similar needs within the thallus for nitrogen-rich compounds (e.g., lipids and proteins: Macko et al. 1987, Bidigare et al. 1991) to assist in growth of new fronds. Lastly, I hypothesized that any manipulation carried out before a winter dormancy period (Gunnill 1980) in *Stephanocystis* would elicit little to no physical response, but the internal $\partial^{13}C$ and $\partial^{15}N$ values would reflect an individual's ability to store enriched carbon compounds in the holdfast and any tissues damaged would have slightly enriched values for both ratios due to the natural fucoid healing response (Hurd et al. 2014).

METHODS & MATERIALS

Study Site

Stillwater Cove in Pebble Beach, CA was chosen for this study because of available biomass records from a baseline *Stephanocystis* study done by Schiel (1985) within the Cove. Stillwater Cove is located at the northern end of the Carmel Bay in central California (36.34°N, 121.56°W). The cove opens to the south and is mostly protected from large storm-related northwest swells during the winter (Reed & Foster 1984) and allows canopy formers, like *Macrocystis*, to form full summer canopies while also reaching a winter minimum due to the occurrence of less frequent southwest swells (Clark et al. 2004). The reduced frequency in direct

swells allowed for this study to be carried out through the summer and winter months from March 2016 to March 2017 without reducing too much of the annual swell-driven disturbances seen along the California coast.

Natural Variability

Collections to assess the natural variability in *Stephanocystis* biomass took place within Stillwater Cove once a month from March 2016 through March 2017. These collections were meant to compare the biomass production of *Stephanocystis* in the work done by Schiel (1985), while adding isotope analyses to better understand the internal chemistry involved in this annual production of biomass.

Removal of 5 entire *Stephanocystis* individuals occurred mid-month for 13 months with the use of SCUBA. Collections were done at a depth of ~7m within Stillwater Cove for the purpose of replicating the optimal depth for canopy growth (Schiel 1985). The size class addressed by this study was determined from a combination of data from two separate pilot studies that indicated a mature (i.e., an individual that is able to produce a surface canopy) size range for the perennial portion of *Stephanocystis* to be on average 50.39 cm ± 15.27 SD (n=15) in vegetative length (shown in Fig. 1 as VL). The individuals chosen for collection were no less than 40cm to ensure the inclusion of plants on the lower limit of the "mature" size range. Removals were performed using a dive knife to pry between the holdfast of the individual and its associated substrate with care not to damage any of the vegetative or reproductive tissues. Each individual was bagged in a separate 30 x 60 cm U-line bag (25µm thickness) and kept in a dark cooler in seawater for transport to Moss Landing Marine Laboratories (MLML).

At MLML, the *Stephanocystis'* thalli were relieved of epiphytes (floral and faunal) and subsequently washed with de-ionized (DI) water then spun and pat dry. Measurements to estimate productivity were taken from cleaned individuals. Three main measurements were used to estimate productivity in *Stephanocystis*: total length (cm), biomass (g), and reproductive frond count. Total length was measured from the top of the holdfast to the longest point of the thallus (reproductive or vegetative). Biomass was recorded as dry weight (g), which involved weighing the entire thallus of an individual and drying it using an oven/drying rack at ~60°C for at least 48 hours and then weighing it once more.

After measurements, 1-2g subsamples from each individual were taken from the reproductive fronds, blades, and holdfast for use in isotope analysis. For this study, reproductive fronds were characterized by the presence pneumatocysts, but were not necessarily fecund (i.e., bearing conceptacles with antheridia and oogonia) throughout the entire sampling period. Blades were any leaf-like structure protruding from the hard, woody stipe and holdfast was any tissue below a stipe and connected to the substrate. All dried 1-2g subsamples were transferred into polyethylene milling vials and pulverized in a ball mill for 10-20 minutes depending on density of tissue. The powder produced by this milling process was transported to the Center for Stable Isotope Biogeochemistry (CSIB) at the University of California, Berkeley. At UCB, 6 mg portions of each sample were weighed out into aluminum bullets for subsequent combustion in an Elementar analyzer with an Isoprime 100 unit for carbon and nitrogen isotope ratio determination. The standards used to calibrate the analyzer and anchor the ∂-ratios were Pee Dee Belemnite for carbon and atmospheric nitrogen. These standards were variable at a value of 0.01 $\pm$ 0.02 ‰. The $\partial^{13}C$ and $\partial^{15}N$ values were coupled with the physical measurements for productivity to determine how the temporal shifts in biomass can be correlated to internal

chemistry and if that biomass production was on a similar scale as described by Schiel (1985). The productivity measurements and isotope values for natural variability were used as baselines for comparison in the second portion of the study.

Experimental Manipulations to Test Biomass Removal Response

To address the question of how *Stephanocystis* would respond to biomass removals, two experimental manipulations were set up at different points in the annual reproductive biomass life cycle (i.e., growth and senescence). These experiments were located in Stillwater Cove at ~7m depth within a 10m diameter kelp clearing; growth and senescent periods were addressed by performing one manipulation in March 2016 and one in October 2016, letting both run through a 6-month period. Each manipulation site had a total of 20 tagged *Stephanocystis* individuals that were split into four separate treatments (n=5/treatment). Tagged individuals all reached at least 40cm in vegetative length to replicate the constraints for a "mature" *Stephanocystis*.

The four treatments were developed to address how the removal of various tissue biomass would affect *Stephanocystis* through its physical and chemical responses. The treatments (Fig. 2) consisted of (1) an unmanipulated control (C) for comparison against the manipulation treatments, (2) a reproductive biomass removal (-R) in which all reproductive fronds were excised to gain an understanding of how loss of the highly productive tissue will impact the individual, (3) a vegetative biomass removal (-V) in which blade tissue was haphazardly trimmed while leaving all stipe and holdfast tissue intact; this treatment was to better understand the physiological effect that removing the most photosynthetically active tissue would have on reproductive frond initiation, (4) and the fourth treatment was a combination of

both types of biomass removal (-All) to determine the impact that a less tissue discriminant type of disturbance might have on the recovery of *Stephanocystis*.

Response assessment was done on a monthly basis with the use of SCUBA. New reproductive frond growth and total length were recorded to evaluate how each treatment was affecting *Stephanocystis*. Total length was used as an indicator of productivity in a similar manner as shown by previous fucoid biomass studies (Schiel 1985, Mathieson & Guo 1992). At the conclusion of the 6-month periods, all individuals were extracted and transported to MLML for processing. Once at MLML, epiphytes were removed and individuals were washed with DI water then spun and pat dry. Final biomass assessments were taken using total length and dry weight. Subsamples from the individuals were taken for reproductive (if applicable), blade, and holdfast tissues to be combusted and analyzed for carbon and nitrogen isotope determination in an identical manner as described in the above *Natural Variability* subsection. Isotope analysis was used as the proxy for the internal response to biomass loss because of similar applications by other macroalgal studies as indicators of new growth or resource movement (Carvalho et al. 2007, 2009, Fox 2016).

Statistical Analyses

Analysis of the natural variability was addressed using one-way fixed-factor Analysis of Variances (ANOVAs) to determine the variation in biomass and length due to time. Linear regressions were used to determine the thallus region accounting for the variability in total biomass and total length throughout the 13-month collection period and to assess the viability of using total length as an in-field proxy for biomass. Two-way fixed-factor ANOVAs were used to understand the temporal chemical variability associated with biomass production and loss within

the thallus through comparisons between $\partial^{13}C$ (‰), $\partial^{15}N$ (‰), and bulk carbon and nitrogen (C:N ratio) with month, tissue type, and their interaction.

 Experimental manipulation data were analyzed using one-way ANOVAs with fixed-factors. Because the data were inherently non-independent due to the same individuals being measured throughout each experiment, an overall growth rate was calculated by using the start date total length value and comparing it to the month with highest amounts of growth seen in the experimental period (e.g., $\frac{Final-Initial}{Time}$). This gave average values for each treatment's growth rate (cm • d^{-1}), which were then used in the ANOVA. This test was constructed to understand the physical response to disturbance through the growth rate in total length by comparing it with the independent 'treatment' variable. As a follow-up, a comparative two-way ANOVA was used to assess how different each experimental period was from the other using the fixed factors of 'period' and 'treatment'. Post-hoc testing was done using a Tukey's HSD analysis to determine relative differences in treatments for all ANOVAs described.

 In order to address the response to biomass loss in tissue chemistry, tissue type was incorporated into a two-way fixed factor ANOVA along with treatment for $\partial^{13}C$ (‰), $\partial^{15}N$ (‰), and bulk carbon and nitrogen (C:N ratio) for each experiment. Simplification for tissue chemistry values to address any non-independence was unnecessary because all values were obtained through analysis done at the completion of each experiment. This test attempted to connect the chemical signatures of biomass recovery to each treatment based on when the experiments were performed. All analyses were subsequently analyzed with a post-hoc Tukey HSD test to verify which treatment or time point accounted for the most variance in both the natural and experimental populations.

RESULTS

Natural Variability in Stephanocystis *Biomass*

Overall thallus biomass in *Stephanocystis* varied throughout the sampling period (Fig. 3, Table 1), which was attributed to reproductive frond initiation in spring and large amounts of reproductive biomass in the summer months. This relationship is tightly correlated with temporal changes in total biomass being highly influenced by the production of reproductive biomass (Fig. 4; Regression: $F_{1,63}=649.3$, $p<0.001$, $r^2=0.91$). Although this increase in biomass was due to reproductive frond initiation, these fronds were not always fecund (i.e., containing receptacles bearing antheridia and oogonia) at the times in the sampling period where they were most numerous (Fig. 5). This result was only apparent in a small sample size (*n*=5) of the larger population in Stillwater Cove and thus the short period of fecundity in 2016 could be a misrepresentation of the true population or a reaction to the strong El-Niño event that happened that year, which might have curtailed the normal reproductive cycle with lower nutrient availability and warmer waters. The percentage of total biomass that was accounted for by reproductive tissue reached close to 80% during the study (Fig. 6), which is consistent with the findings by Schiel (1985). This temporal variability was seen with regard to the total length as well, mainly due to high amounts of growth in the summer months, (Fig. 7, Table 1b) and was accounted for by the reproductive frond length (Fig. 8; Regression: $F_{1,63}=18321.4$, $p<0.0001$, $r^2=0.99$). The percentage of total length represented by reproductive fronds during the peak growth period was about 90% (Fig. 9). The relationship between biomass and length was used as the in-field proxy for productivity for the experimental portion of the study because of the positive correlative relationship between factors (Fig. 10; Regression: $F_{1,63}=154.9$, $p<0.001$,

$r^2=0.706$). Overall, the variability in total biomass was directly correlated with reproductive growth, which could be an indicator of changes in the environment.

While the physical response to temporal abiotic factors was pronounced throughout the sampling period/year, tissue chemistry showed a slightly more muddled response when referring to environmental abiotic shifts (e.g., light, temperature, etc.). A link between the external or physical change in thalli and internal or chemical change was indeterminate based on initial findings. Both $\partial^{13}C$ and $\partial^{15}N$ content varied monthly and by tissue type as well as the interaction between the two factors, while bulk carbon and nitrogen represented as their ratio (C:N) provided no significance as an interaction term in the model (Fig. 11, Table 3). Significant differences were due to holdfast chemistry being isotopically more enriched in $\partial^{15}N$, while reproductive tissue was more enriched in $\partial^{13}C$ (Fig. 12, Table 3). Blade tissue skewed more towards being chemically similar to reproductive tissue, but did bridge the boundary between the two thallus regions (Fig. 12). A majority of the variability observed in C:N was due to holdfast and blade tissue compared to reproductive tissue at various months (Fig. 11). The patterns seen in C:N were consistent with isotope data, but provided less detail due to bulk carbon and nitrogen having less of a physiological relevance and being more important for overall productivity of the individuals.

Experimental Manipulations to Test Biomass Removal Response

The variability in growth rate for the summer manipulations was reflective of the type of treatment each individual received (Fig. 13, Table 4). The –Repro and –All treatments were the main cause of the variance between treatments because of their extreme negative response to

disturbance compared to the Control and -Veg treatments. A visual representation of this disparity is seen in Figure 14, which shows the lack in ability of the –Repro and –All treatments to recover from the initial manipulation. Because there was no recovery by individuals that received these two treatments, their growth rate never reached the same levels seen by the Control and –Veg treatments (Fig. 14). This suggests that reproductive frond initiation in spring is important to sustain any new growth through the summer months.

The manipulations performed for the winter experimental period showed minor fluctuations in growth rate throughout the period and varied only slightly due to treatment type (Fig. 11). All variability during this period can be accounted for by the negative growth rate seen in the -Veg treatment ($-1.22 \text{ cm} \cdot \text{d}^{-1} \pm 0.82$ SD) compared to the relatively minute fluctuations in growth rate of the other three treatments (Table 5). The natural population's variability seen in the winter months (Fig. 7) mimics the experimental population regardless of a manipulation type. It is likely that during the dormancy period in *Stephanocystis*, any biomass removed has little to no effect in comparison to the critical growing period in the spring and summer months.

Comparing the growth rate for each experimental period by treatment gave further justification to the physiological concept that tissue proliferation and time of the year are important to *Stephanocystis* growth and survival (Table 6). Post-hoc testing determined that the treatment driving the variation between both periods was the Control during the summer. All other treatments were closely related in terms of variance, which can connect the ideas that removal of photosynthetically active tissues like reproductive fronds have an overtly detrimental effect on growth and when those tissues are gone, *Stephanocystis* may go into a dormancy or overwintering phase no matter the time of year.

Tissue type was the main driver of inter-thallus tissue chemistry variance in the summer (Table 7) and winter (Table 8) experimental periods. This variability can be attributed to the observation that reproductive and blade tissues were enriched in ∂^{13}C compared to holdfast tissue (see Fig. 12). Reproductive and blade tissues are, usually, more enriched in ∂^{13}C because of their active role in photosynthesis and diffusion of various dissolved inorganic carbons. This was apparent in the values for reproductive (-19.95 ± 0.69 SE and blade (-19.82 ± 0.62 SE) tissues regardless of time of year. Holdfast tissue is consistently more enriched in ∂^{15}N for each experimental period (Summer: 12.42 ± 0.21 SE, Winter: 12.03 ± 0.36 SE) possibly due to nitrogen-rich structural molecules used to reinforce the holdfast. Combining both experiment's tissue isotopes showed a noticeable difference between tissue types for each of the experiments (Fig. 16). These differences were seen in the three tissue types for both experiments, but also between both sampling periods (Table 9). The pattern seen in these discrepancies is solely based on the ∂^{13}C enrichment in the reproductive and blade tissues because of the two separately weighted carbon isotopes at different points of the year. The measurable statistical differences in ∂^{15}N were credited to holdfast tissue compared to the other tissues regardless of experimental period, but might be affected by timing of sample extraction (e.g. end of summer vs. end of winter) due to seawater chemistry or other abiotic factors.

DISCUSSION

Biomass Variability

Seasonal fluctuations in biomass, whether due to reproduction, disturbances, or annual senescence, are a regular occurrence for many terrestrial (e.g., deciduous trees) and aquatic macro-autotrophs (fucoid: Gagné et al. 1982; seagrass: Erftemeijer & Herman 1994; kelp: Reed et al. 2011, Rodriguez et al. 2013). These alterations in biomass cause a range of physiological responses in the organism depending on life history traits and abiotic environmental factors. In terrestrial plants, producing reproductive tissues or structures can have a dramatic impact on the individual's fitness depending on species (reviewed in Harper 1987). This variation in biomass due to reproductive effort is seen extensively in fucoids (McCourt 1985, Schiel 1985, Mathieson & Guo 1992, Brenchley et al. 1998, Wernberg et al. 2001), where most species experience at least one reproductive growth period which accounts for a large portion of their total biomass. This is followed by a period of overwintering in a smaller life history stage. For *Stephanocystis,* this pattern was consistent with the literature where the increase in total biomass was indicative of whether or not an individual was in the reproductive growth season (Figs. 3 & 9). This investment into reproduction suggests a trade-off for propagation over vegetative growth as proposed by McCourt (1985) for fucoids. Reproductive biomass is so energetically important and costly that the "overwintering" or dormancy seen during the winter months is indicative of an individual's need to recover through photosynthesis and nutrient uptake. It can be noted that although production of reproductive fronds did create considerable amounts of structure, that tissue was only fecund for a short period in the growing season (Fig. 9). This could be due to the study taking place during an El Niño year, which has been shown to affect the health of large seaweeds (Tegner & Dayton 1987), or possibly a delayed onset of fecundity due to its positive

correlation with plant size as mentioned by De Wreede and Klinger (1990). Variability in fecundity has not been well documented and the results from this study were inconsistent with Schiel (1985) who documented fecund individuals in March, which is a similar result seen for the March 2017 natural population samples.

The disturbance of important thallus tissues, like reproductive fronds, in fucoids uncovers an understanding of how dramatically important this biomass can be to the fitness of an individual. In the summer portion of the study, thallus damage elicited negative responses in all treatments with reproductive tissue removal showing an inability in the individuals to recover by the end of the experiment (Fig. 14). While the structural complexity provided by the fronds of *Stephanocystsis* and *Macrocystis* might be similar, their response to disturbance is vastly different. *Macrocystis* may respond with a reduction or slowing of growth in new fronds (Fox 2016), but the fertility of the sporophylls may only take a short time (~83 d) to rebound completely from direct disturbance (Geange 2014). The lack of a capacity for *Stephanocystis* to rebound from the disturbance performed here is consistent with previous studies noting the fucoid's slow growth strategy and preference to defend rather than regrow (McCourt 1985, Mathieson & Guo 1992, Van Alstyne 1988, 1989), which is supported by the growth vs. reproduction hypothesis as evidenced by limited vegetative growth as a side effect of reproduction (reviewed in Obeso 2002). This can be compared to the lag in reproductive frond initiation seen in the vegetative tissue manipulations (-Veg). The short delay in growth (Fig. 14) suggests that there might be a need for the wound site to be healed (Hemmi et al. 2004) before resuming normal reproductive growth. Individuals in the –Veg treatment never reached the peak growth seen in either the natural population or controls, which could mean that the energy used

to repair the damaged tissue reduced the individual's overall capacity for growth, a response similarly found in *Fucus* sp. (Honkanen & Jormalainen 2002).

Energetic needs are vastly different for *Stephanocystis* during the winter months. After the annual removal of reproductive fronds by storms and connective tissue senescence, the surviving vegetetative thallus stays small throughout the winter (Schiel 1985). Chapman (1995) reviewed several instances for other fucoids that noted that the strain of reproductive frond growth leads to depletion of nutrient stores, which get replenished during the overwintering period. This diminutive form was seen throughout this study in the natural population (Fig. 3) and the controls in the winter manipulation (Fig. 15). During the manipulation period, the consequences of damaging tissues were apparently reduced possibly due to individuals having already started their dormancy phase, as seen in other fucoids (Le Lann et al. 2012). Although most treatments varied similarly in growth rate during the winter period, the vegetative manipulation seemed to experience the largest negative growth rate. The apparent senescence could be from high energy swells adding damage to the thallus or it could be an artifact of the haphazard sampling design due to individuals of that treatment being over manipulated. Because of the turbulent nature of the water during the winter months, the physiological response of *Stephanocystis* to halt growth could be aided by swell energy potentially pruning any fresh tissue. An overwintering period in *Stephanocystis* might have evolved over time to deal with the unique physical factors of living in a subtidal ecosystem.

Physio-chemistry

Algal tissues are formed using their internal resources, whether that be stored carbohydrates or freshly produced photosynthates (Hatcher et al. 1977, Gagne et al. 1982). This

production is mediated by microscopic machinery like carbon concentrating mechanisms (CCMs), most notably the enzymes carbonic anhydrase (CA) and RuBisCO. This enzymatic process allows many macroalgae incorporate diffusive CO_2 as well as bicarbonate (HCO_3^-) into the thallus to build tissues (Roleda & Hurd 2012). Some added ways macroalgae incorporate these two inorganic carbon sources is through light-independent carbon fixation with the help of two less carbon selective enzymes, phosphoenolpyruvate carboxylase (PEPC) and phosphoenolypyruvate carboxykinase (PEPCK) (reviewed in Gomez & Huovinen 2012). This enzyme can utilize bicarbonate as its substrate for carboxylation in times of low light (e.g., winter) and shown to be an active part of non-photosynthetic carbon acquisition in *Stephanocystis* (Cabello-Pasini & Alberte 1997), but, as a caveat, often does so without a net carbon gain. Because bicarbonate is abundant in seawater and enriched in $\partial^{13}C$, researchers have demonstrated the ability to link $\partial^{13}C$ enrichment values to periods of high photosynthetic activity due to the internal conversion of HCO_3^- to CO_2 (Raven et al. 2002b), which can elicit high $\partial^{13}C$ values. This type of enrichment can be seen in the natural population during the months right before the spring reproductive frond initiation (Fig. 17) followed by a drop during the summer months. The elevated enrichment values during the winter and spring months might be attributed to low light conditions causing *Stephanocystis* to be utilizing light-independent carbon fixation with PEPC/PEPCK, but more realistically can be due to the increased respiration in low light conditions causing low weight CO_2 to leave the thallus leaving high weight carbon in the tissues. The subsequent summer drop in $\partial^{13}C$ might be related to the carboxylation of bicarbonate or CO_2 into the compounds necessary for this reproductive growth, although

bicarbonate is often thought to be a more energetically costly molecule to convert (Ken Dunton, Pers. Comm.).

These values can be used in conjunction with enrichment values for $\partial^{15}N$, which have been shown to be correlated with proteins and amino acids when around 12‰ internally (Macko et al. 1987), which play a role in the rigid structures (e.g., holdfasts) of seaweeds (Schmid & Stengel 2015). These two isotopic ranges were used to compare temperature, which should vary inverseley with inorganic carbon and nitrogen, and seasonal variability in tissue chemistry. Temperatures that raised up above the yearly average (~12.4°C) are shown to be loosely correlated with drops in isotopic enrichment in both elements for all tissue types. This suggests that the cold, nutrient rich waters combined with seasonal light availability are aiding *Stephanocystis* in photosynthesis and tissue development during the spring months as shown by Fox (2016). The low amount of seasonal variability in the tissues compared to $\partial^{13}C$ is likely attributed to lower amounts of nitrogen fractionation throughout the thallus, which is a product of the small N mass per sample (~1-2% of total) and some interference by atmospheric nitrogen (Handley & Raven 1992).

Isotope values for blade and reproductive tissues tended to cluster by experiment, but this result may be more indicitave of environmental factors and time of the year due to an inability to separate out disturbance effects because all treaments were allowed to rebound. The winter experiment was extracted in March, which is the point when reproductive frond initiation starts. $\partial^{13}C$ enrchiment values for that experiment are reflectant of active photosynthesis as described by Raven et al. (2002) for marine macrophytes and Ishihi et al. (2001) in *Sargassum* spp. This suggests that although the individuals in the winter experiment were manipulated, it had no

effect on their potential for photosynthesis and growth. Conversely, the values for the blade and reproductive tissues extracted in August for the summer manipulations indicated a depletion of carbon enriched photosynthates that mimics the pattern seen naturally (Fig. 16). Holdfast tissue was depleted in ∂^{13}C for both experiments, which could be attributed to it being less photosynthetic overall due to its position in relation to light source (Gao & Umezaki 1988, 1989) and/or it might act as a storage tissue for lighter carbon compounds in a similar way to *Macrocystis* holdfasts (Sargent & Lantrip 1952, Loban 1978).

Assessment of the role of nitrogen isotopes on autotroph physiology and ecology has been messy (Handley & Raven 1992). This can become especially difficult when assessing the impact that biomass removal might have on inter-thallus response. Bidgaire et al. (1991) tried to approach this problem in flowering land plants by looking at the ∂^{15}N of lipids and chlorophylls *a* and *b*. They found that lipids/proteins had higher ∂^{15}N values while the chlorophylls had lower. Applying their results to this study gives a reasonable conclusion to the values seen from each experiment. Holdfasts are naturally more encriched in ∂^{15}N, which might be due to the tissues rigid structure and need for more support molecules (i.e. lipids/proteins). This enrichment is a byproduct of the physiological necessity for the holdfast tissue to be a structural component (Schmid & Stengel 2015) of the alga. Blades and reproductive structures are more depleted in ∂^{15}N, but also more photosynthetically active as reflected by the analysis of ∂^{13}C values. This inverse relationship in the blades and fronds can reasonably is representative of autotrophs due, in part, to the low values of ∂^{15}N in the photosynthetic pigments, chlorophyll *a* and *b* (Bidgaire et al. 1991).

CONCLUSIONS

Removal of canopy-forming algal biomass has been shown to have negative impacts to the physiology of the individual (Reed 1987, Pfister 1992, Fox 2016) and the associated species within that community (Dayton et al. 1984, Santelices & Ojeda 1984, Bulleri et al. 2002, Edgar et al. 2004). This study has shown that *Stephanocystis osmundacea* populations along the central coast of California experience a naturally occurring biomass removal (e.g. storms), but maintain an ability to withstand the loss of reproductive fronds and overwinter as a perenniating vegetative thallus. These responses were replicated through the experiments and suggest that *Stephanocystis* is evolutionarily programmed to overwinter after reproductive tissue loss. In addition, any disturbances felt through this dormancy phase are not fatal for the individual. While internal thallus chemistry did not reflect the physical response by *Stephanocystis* at the conclusion of the experiments, a reduction in sampling time and an increase in sample size might be able to alleviate these issues. That being said, $\partial^{13}C$ and $\partial^{15}N$ analyses proved to be valuable tools in understanding how *Stephanocystis* populations vary naturally throughout the year and helped set a framework for future experiments on canopy removal effects on tissue chemistry.

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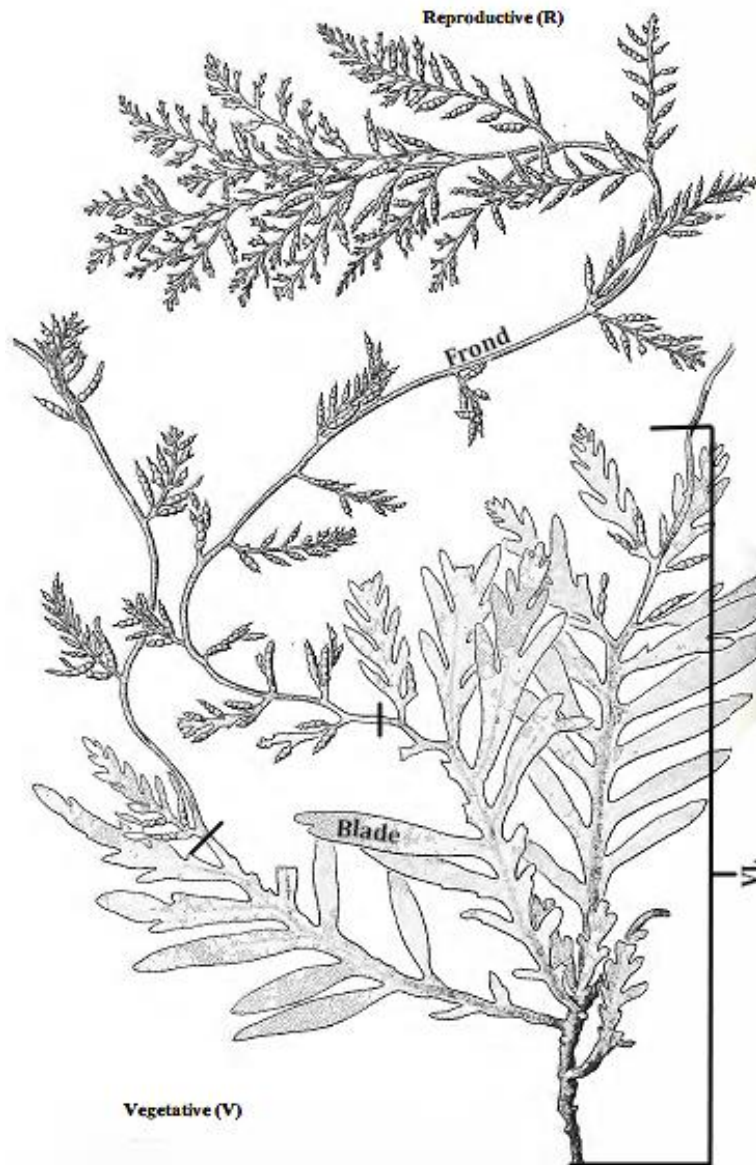
FIGURES

Figure 1. Diagram of *Stephanocystis osmundacea*
(adapted from Abbott & Hollenberg 1976) showing
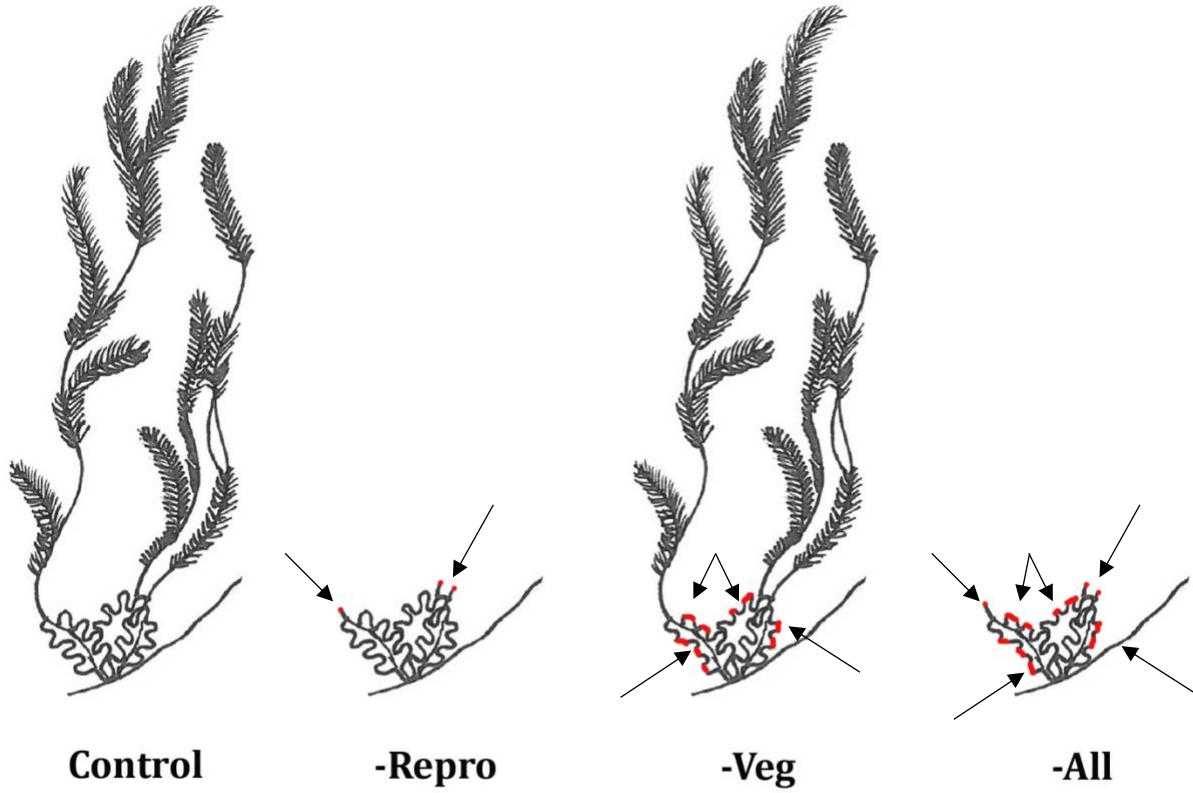dark bars for areas where vegetative thallus terminates

Figure 2. The four treatments performed in the experimental manipulations portion of this study. Each treatments removal area is denoted by red demarcations and arrows.
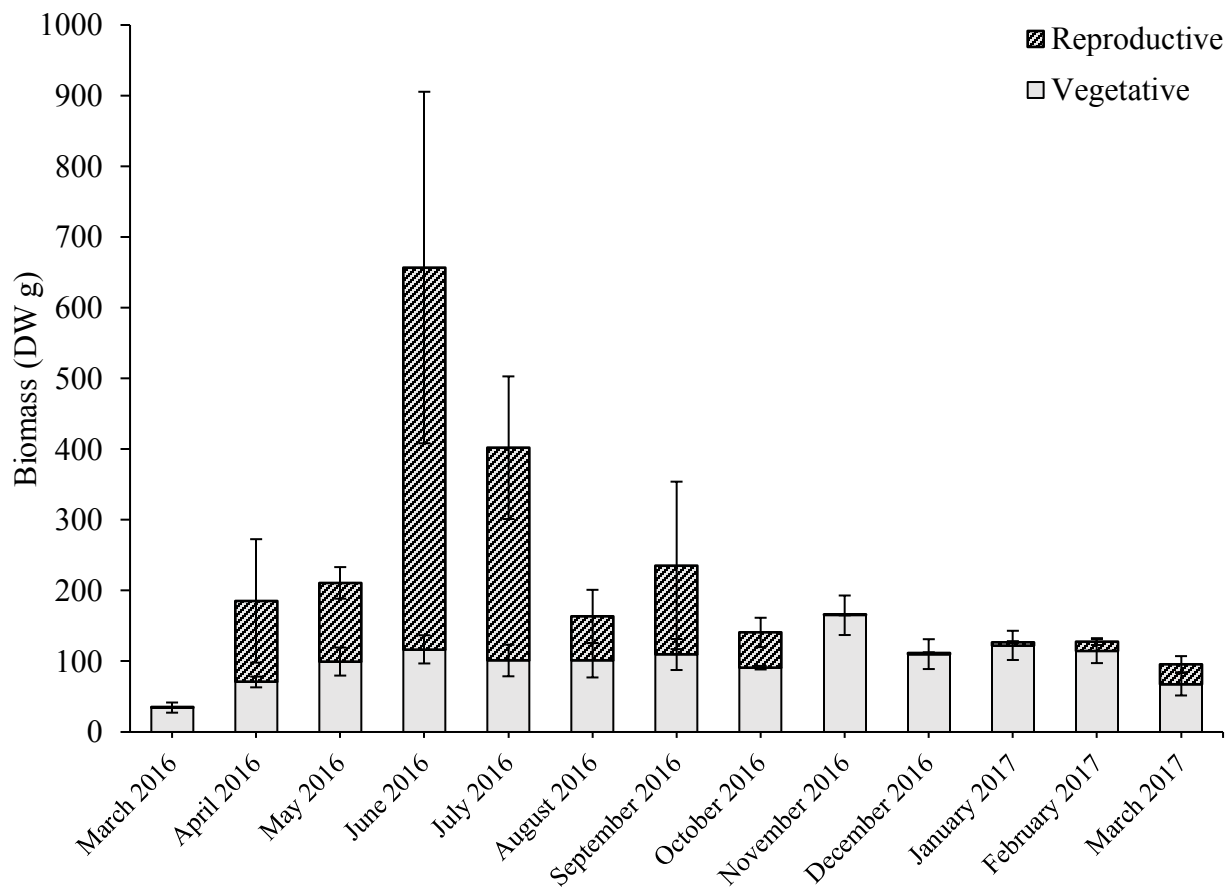
Figure 3. Total biomass (DW g) from the baseline collection (n=5) for each month from March 2016 to March 2017 separated into amount accounted for by reproductive and vegetative biomass. Error bars are ±1 SE.
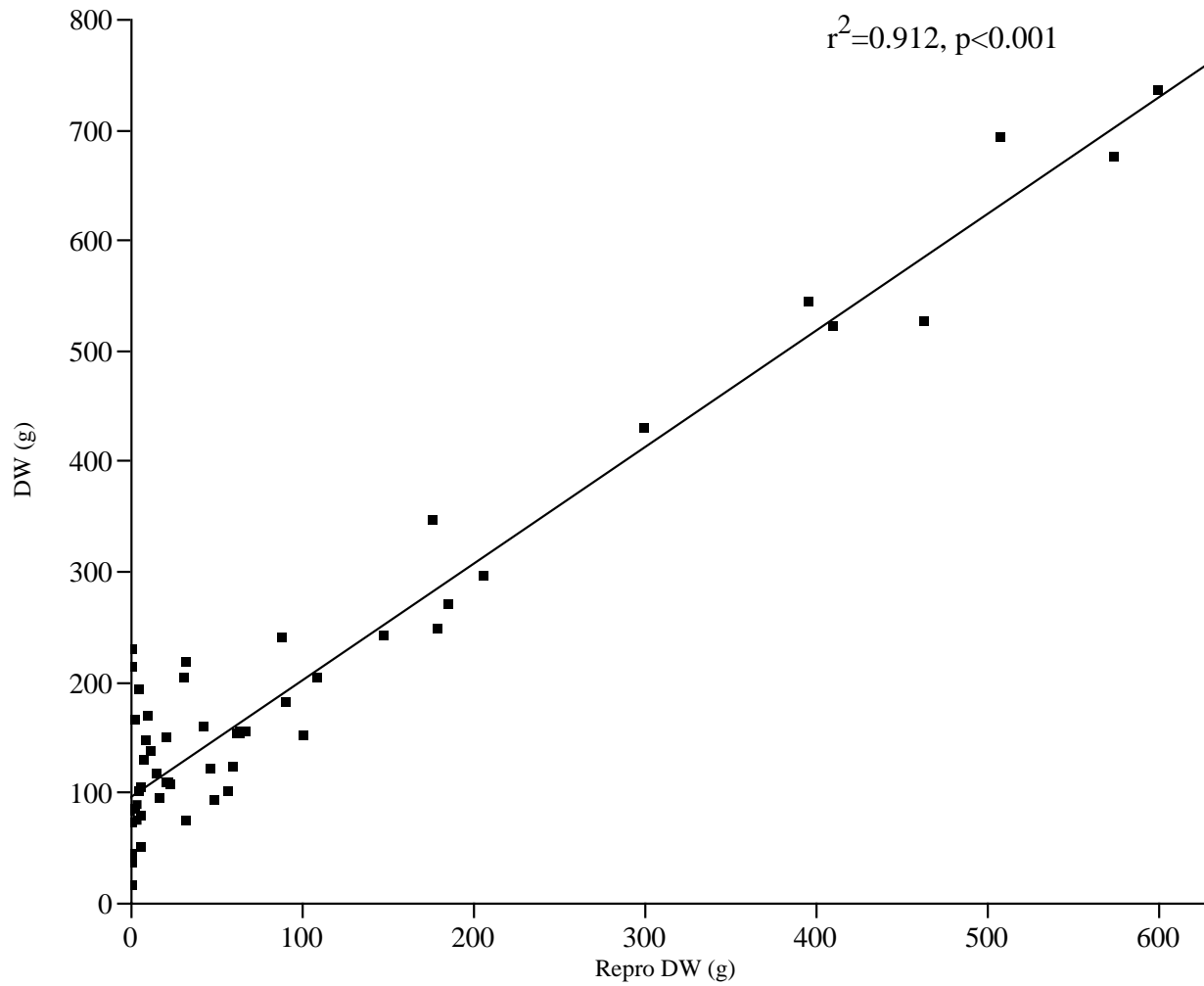
Figure 4. Relationship between total biomass and reproductive frond biomass averaged for each month. Line is best fit (Regression: Total Biomass=95.776+1.054*Repro Biomass; RMSE=48.45).
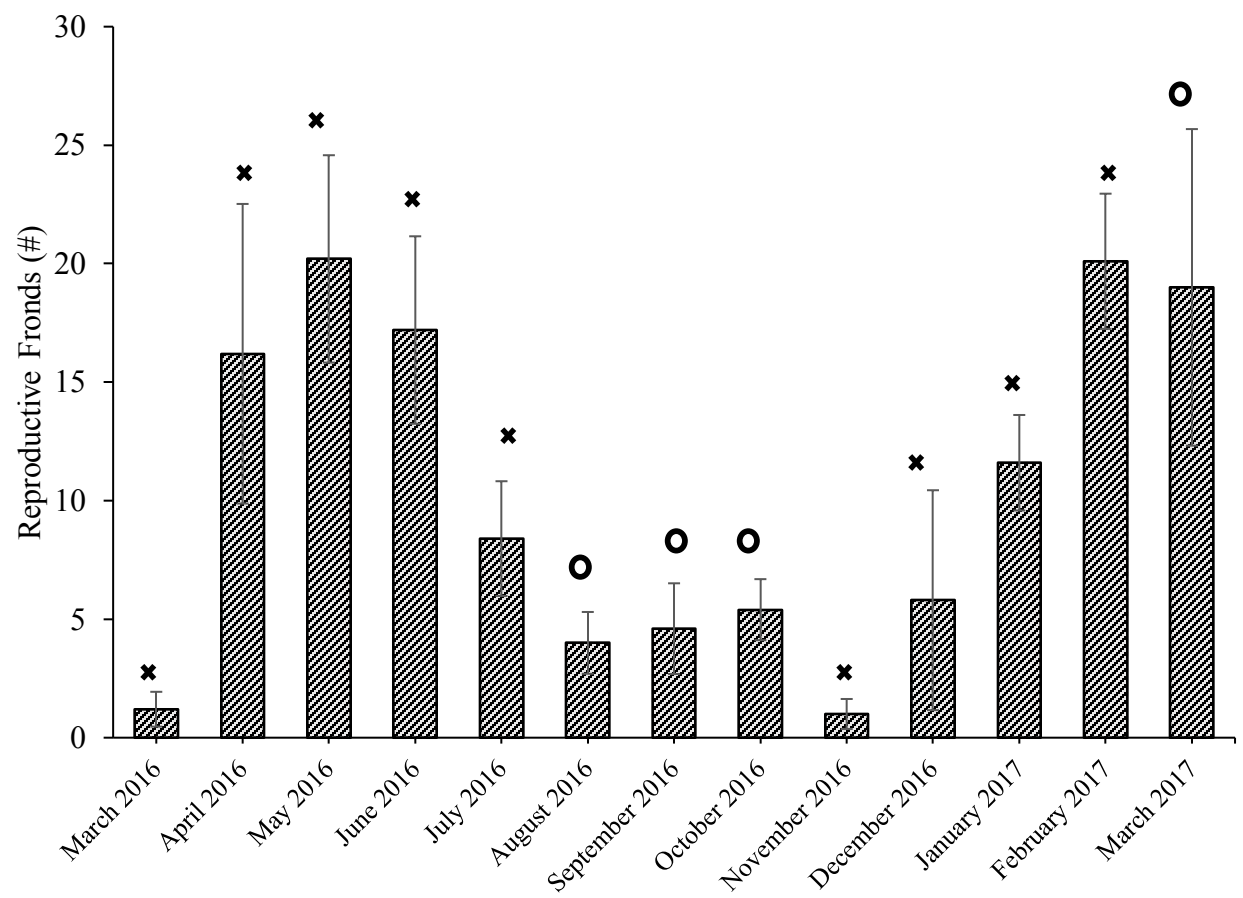
Figure 5. Number of reproductive fronds by count throughout the sampling period. Frond number was highest during the spring and summer growing months with a dip during fall and winter dormancy period. Observations of fecundity are denoted by circles (o), while the absence of reproductive structures within the frond receptacles for the population sampled is denoted by a bold X. Error bars are ±1 SE.

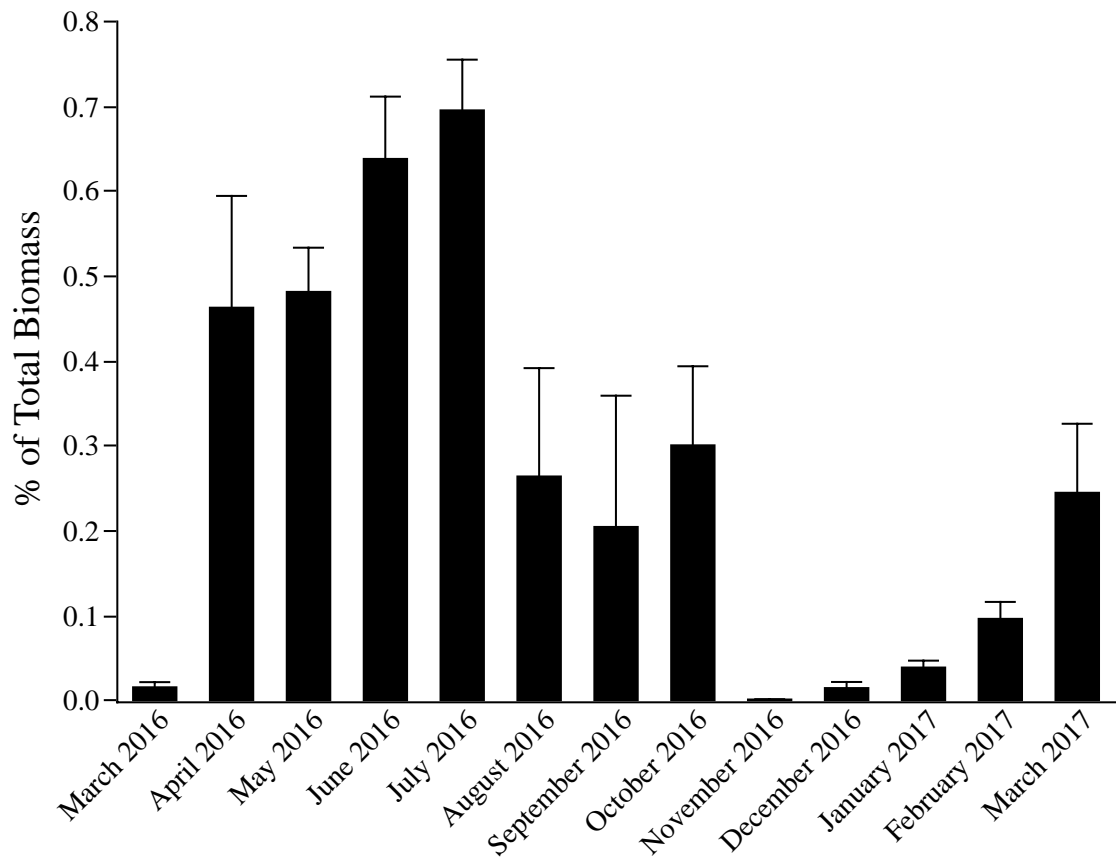Figure 6. The percentage of the total thallus biomass represented by reproductive frond biomass by month. Error bars are ±1 SE.
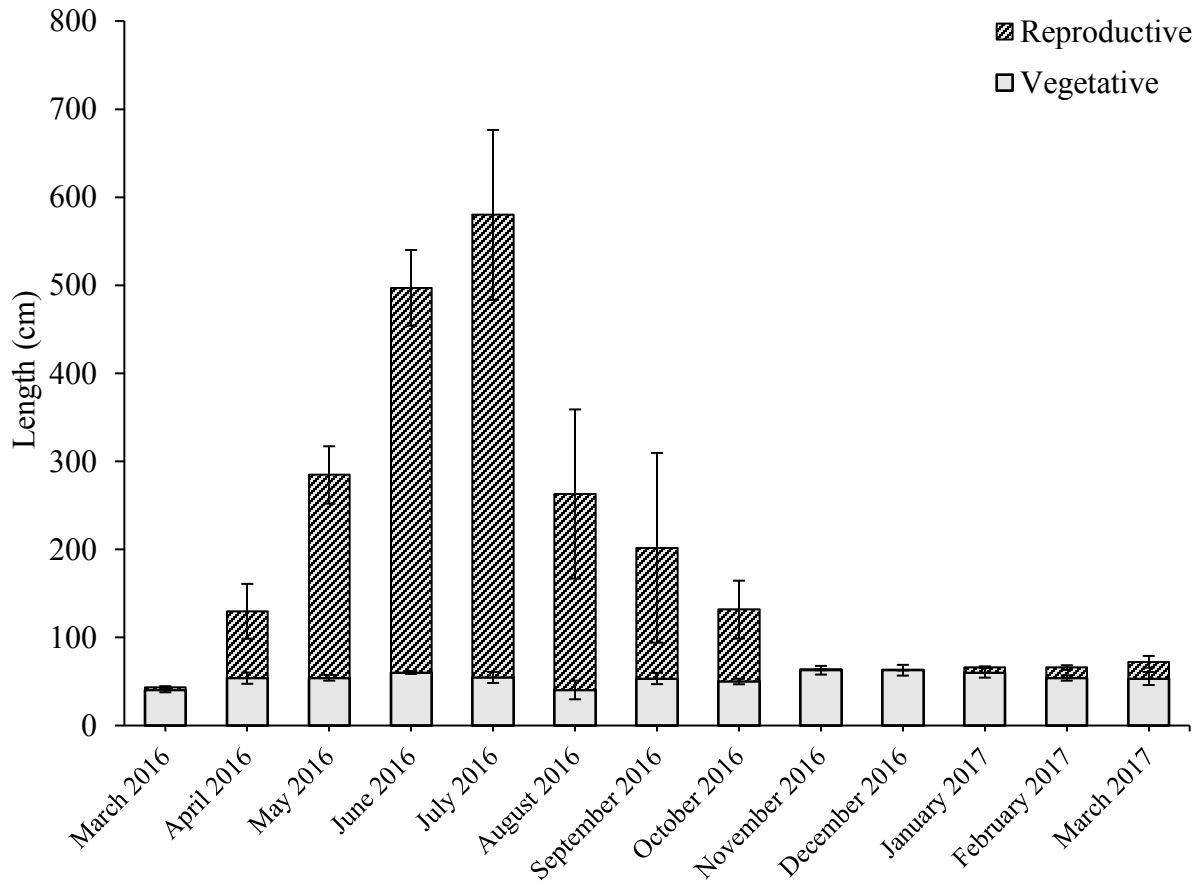
Figure 7. Total length (cm) from the baseline collections (n=5) for each month within the 13 month sampling period. Error bars are ±1 SE.
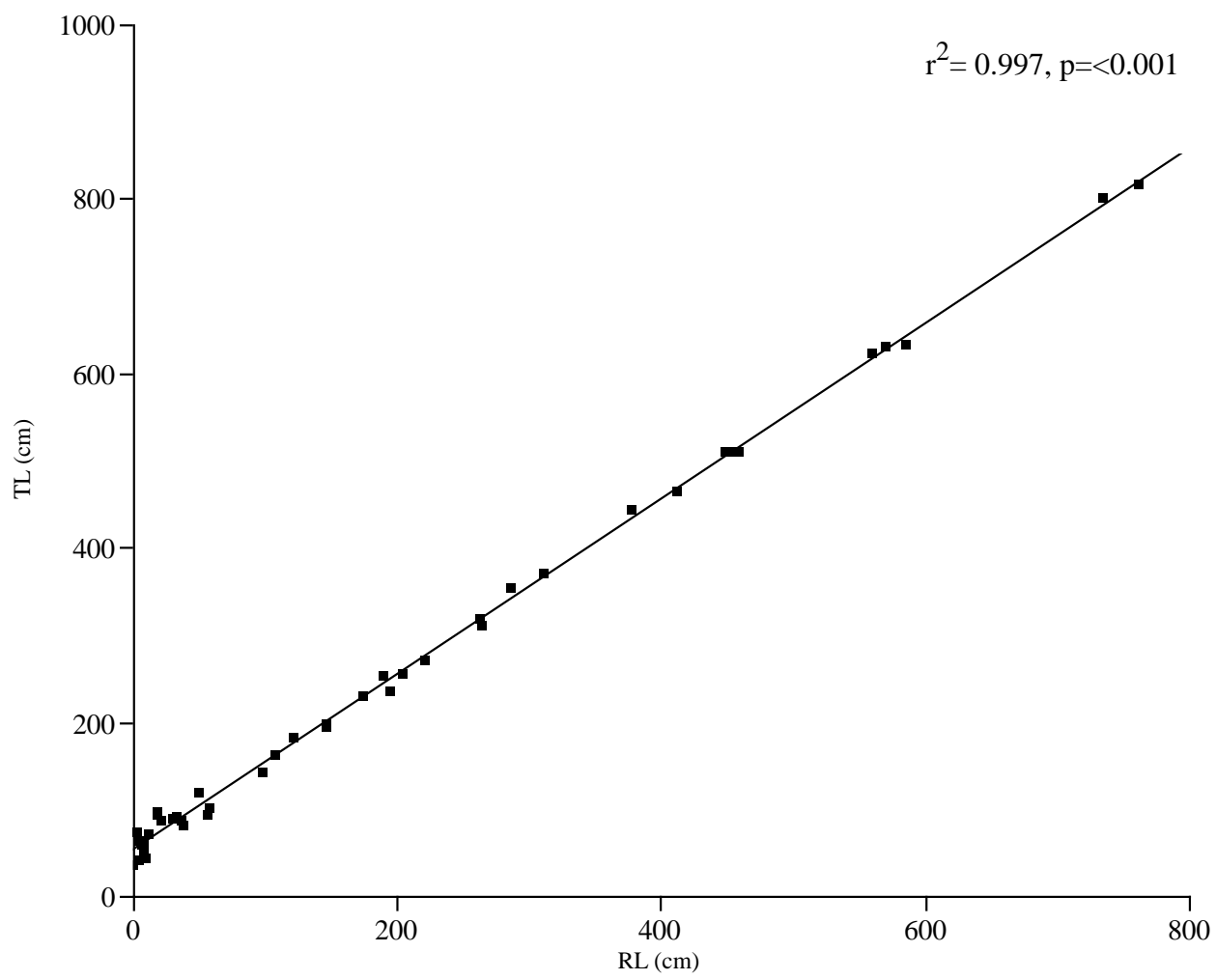
Figure 8. Relationship between total length and reproductive frond length averaged for each month. Line is best fit (Regression: Total Length=53.342+1.004*Reproductive Length; RMSE=11.75).
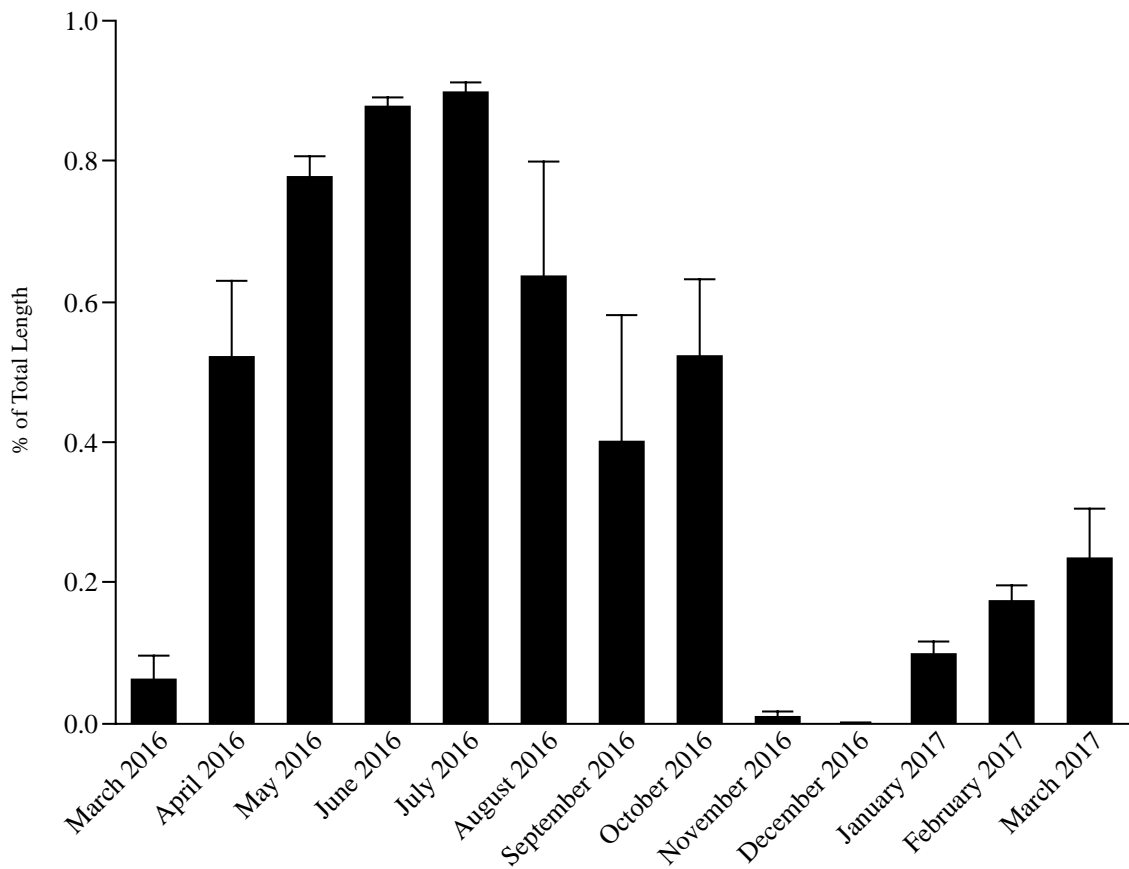
Figure 9. Proportion of total length represented by reproductive frond length by month. Error bars are ±1 SE.
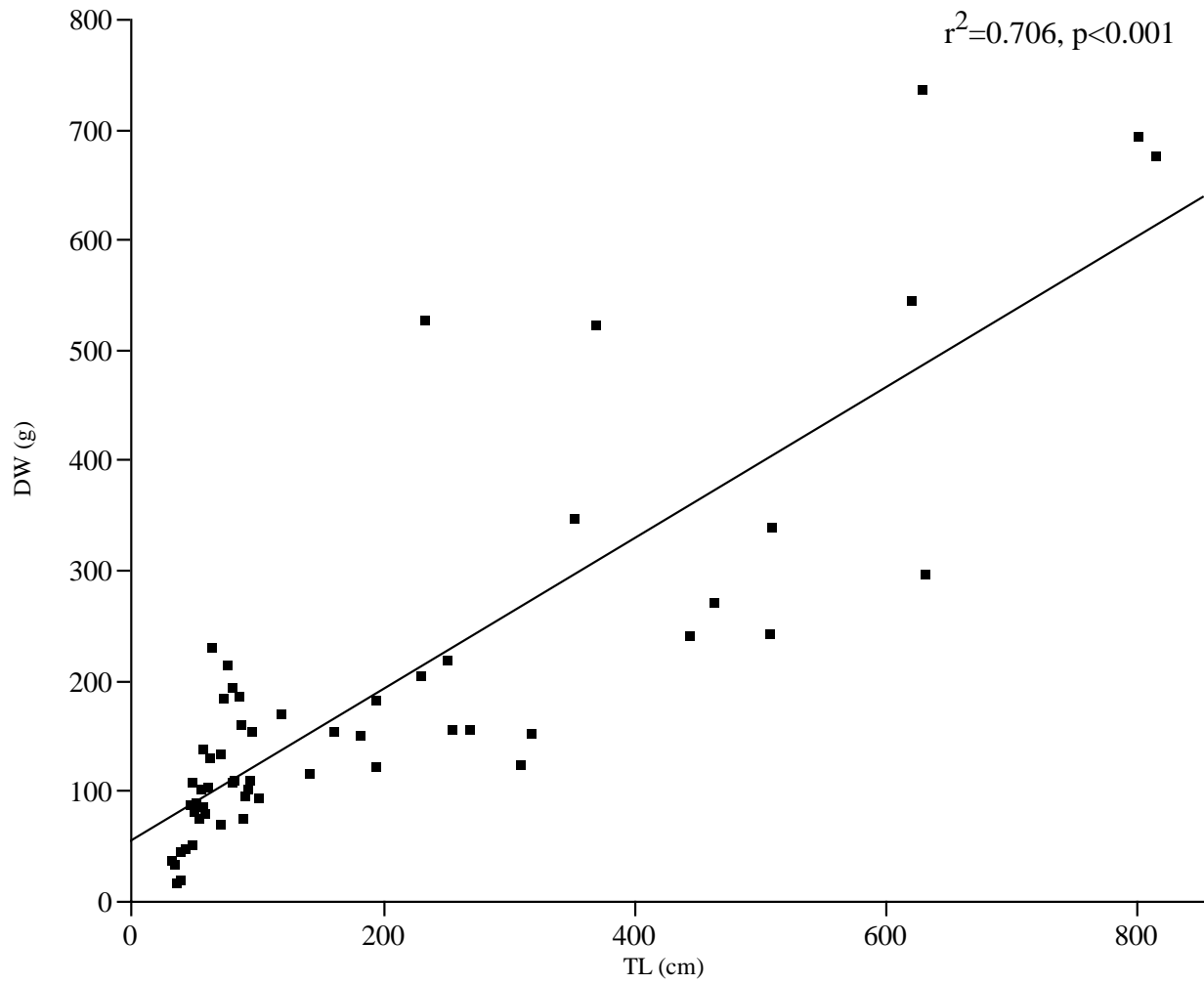
Figure 10. Predictive correlation of total length on total biomass of using monthly mean values. Line is best fit (Regression: Total Biomass=54.801+0.684*Total Length; RMSE=87.58).
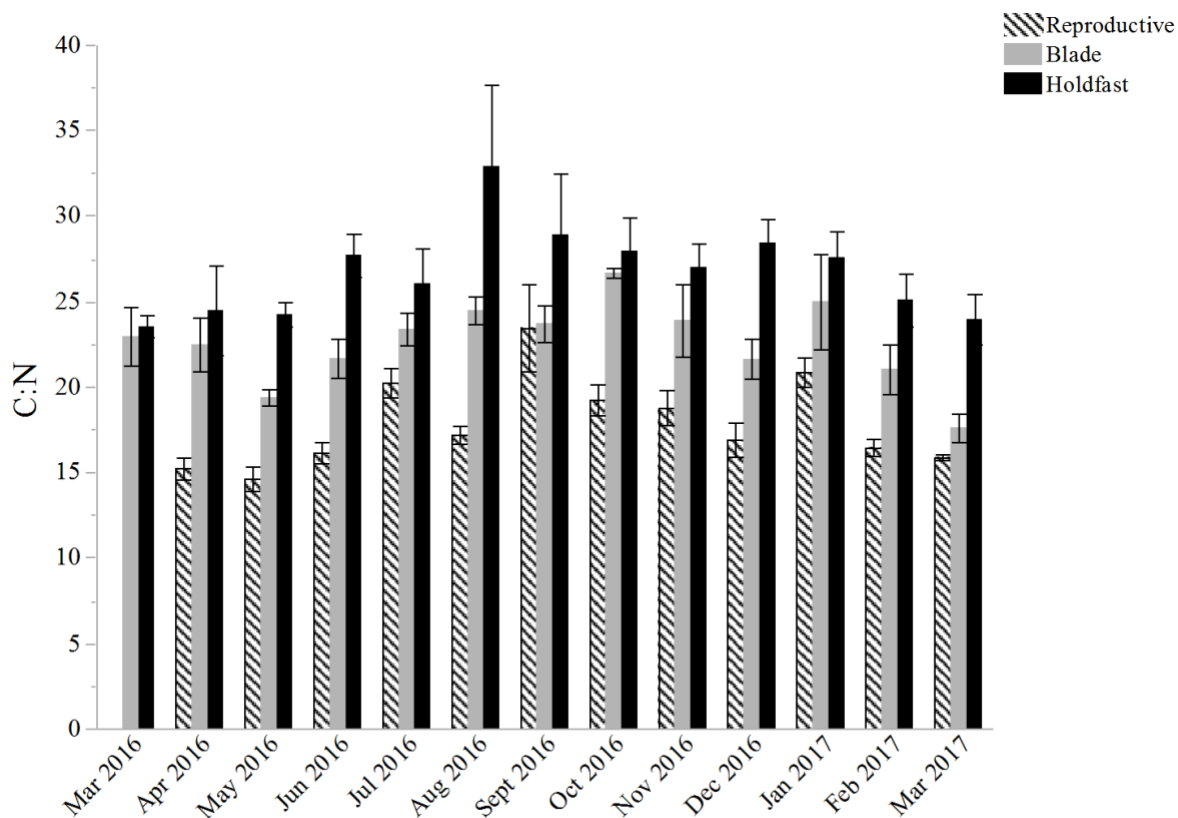
Figure 11. Bulk carbon and nitrogen as a ratio of percent composition of each tissue tracked through time. Hatched bars represent reproductive tissue, solid gray bars represent blade, and the solid black bars represent holdfast tissue. Most of the variability in composition is seen in the summer months and especially between reproductive and holdfast tissues. Errors bars are $1 \pm SE$.
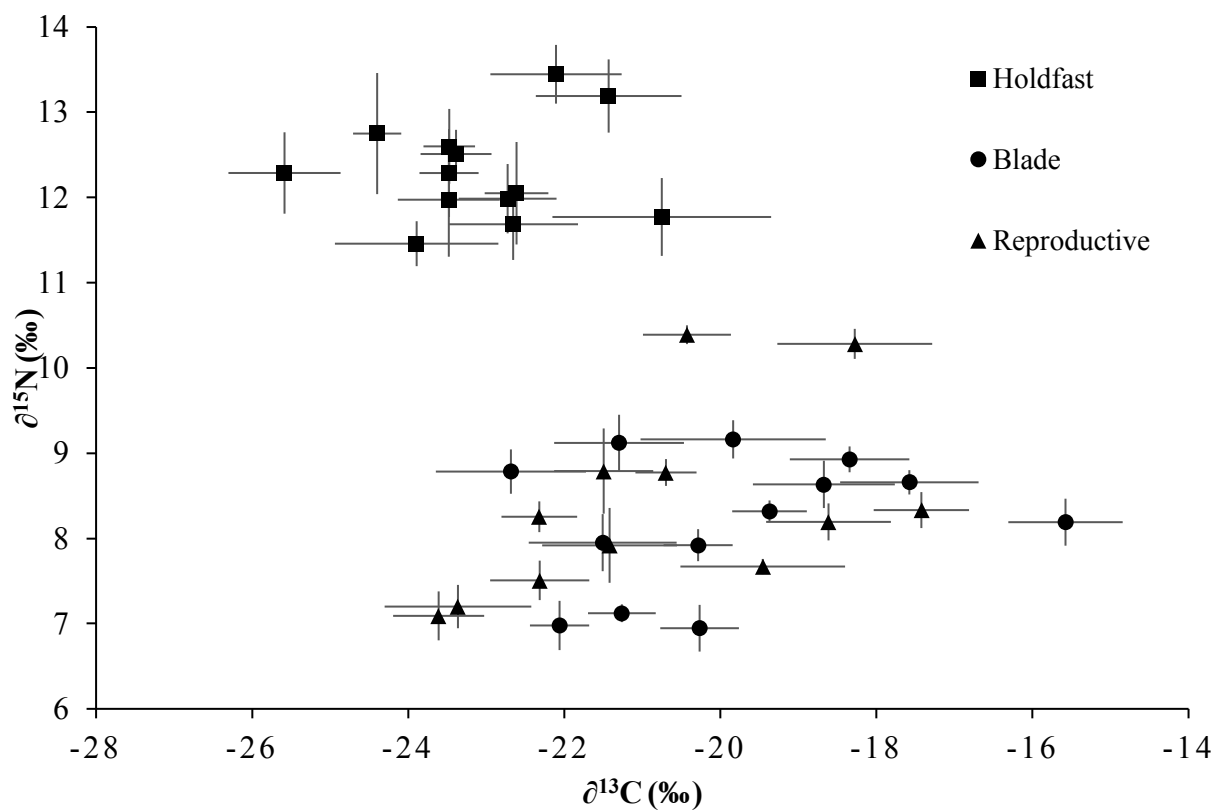
Figure 12. Mean $\partial^{15}N$ and $\partial^{13}C$ for the natural population in each of the months sampled. All months were included and grouped by tissue type due to the variance being mainly explained by the tissues throughout the year. Tissue types are labeled: blade (•), holdfast (■), and reproductive (▲). Error bars for both axes are ±1 SE.
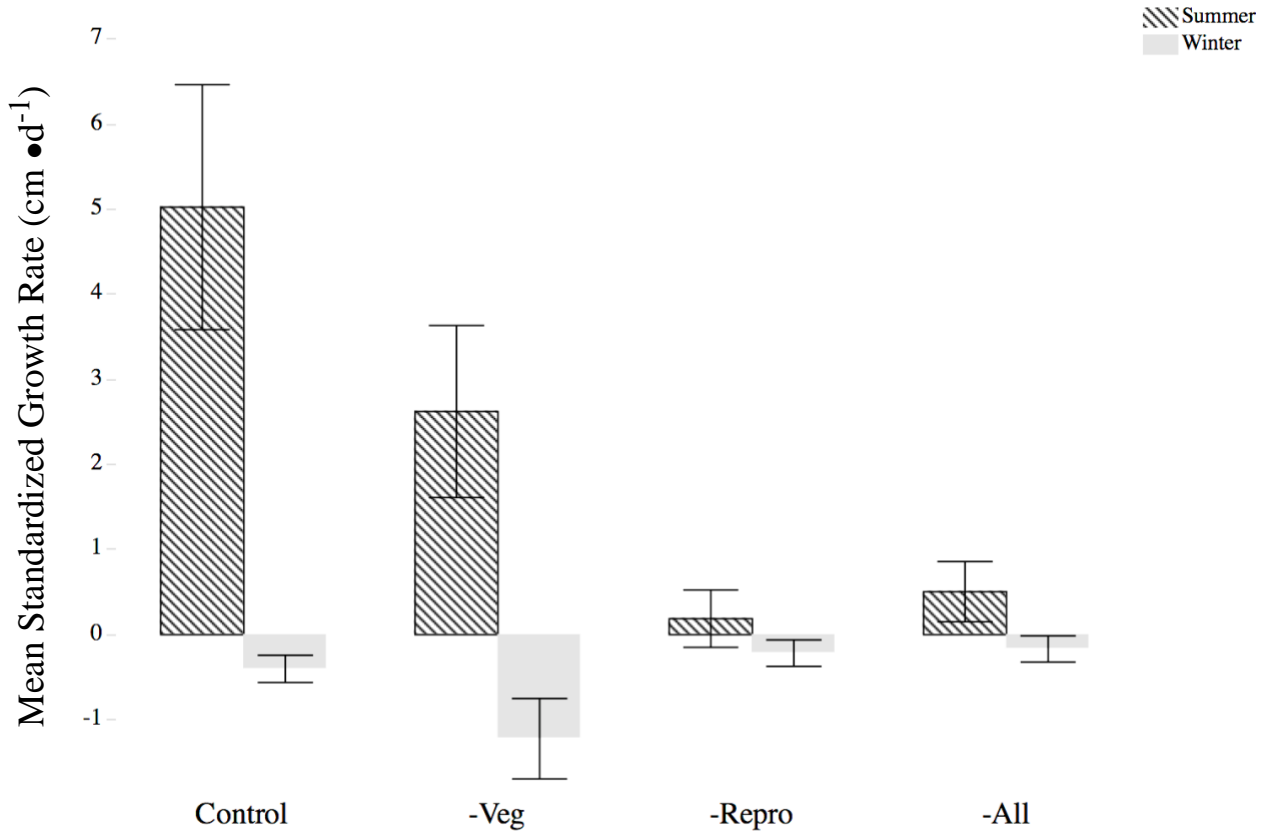
Figure 13. Mean standardized growth rate for all treatments for both experimental periods (summer in hatched bars and winter in gray). Growth was positive at all points during the summer experiments, but diminished with treatments that experienced increasing tissue damage. Winter treatments never saw a period of positive growth. All growth rates were standardized according to maximum growth period. Error bars are $1 \pm SE$.
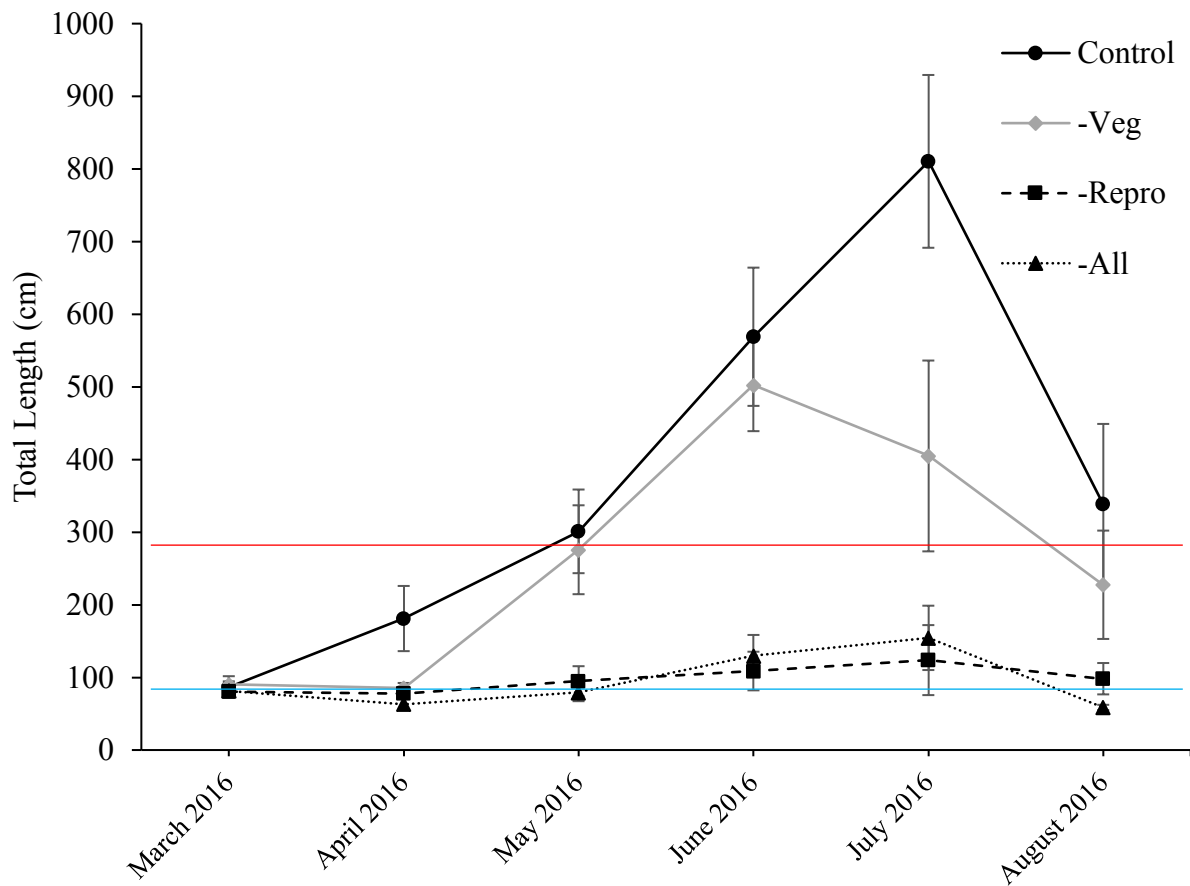
Figure 14. Change in biomass as represented by total length throughout the summer manipulation experiment. Treatments are labeled: Control=solid line (•), -Veg=gray solid line (♦), -Repro=dashed line (▪), and −All=dotted line (▲). These trends are compared to the natural population variability by a blue line that indicates mean length in cm for the dormancy period (98.6 ± 20 cm SE) and a red line that indicates mean length during the growth period (261.55 ± 38 cm SE). Error bars are ±1 SE.
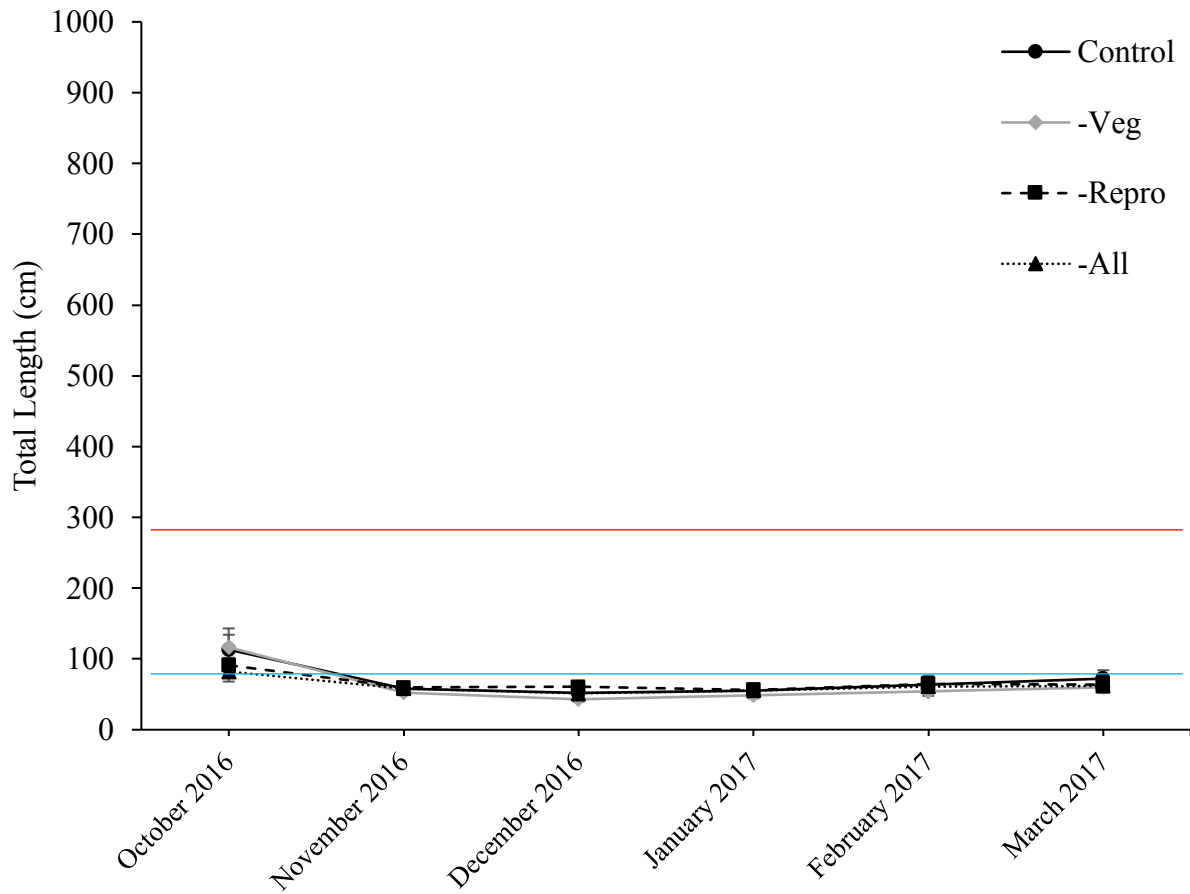
Figure 15. Changes in biomass as represented as total length throughout the winter manipulation experiment. Treatments are labeled: Control=solid line (●), -Veg=gray solid line (◆), -Repro=dashed line (■), and −All=dotted line (▲). These trends are compared to the natural population variability using a blue line that indicates mean length in cm for the dormancy period (98.6 ± 20 cm SE) and a red line that indicates mean length during the growth period (261.55 ± 38 cm SE). Error bars are ±1 SE.
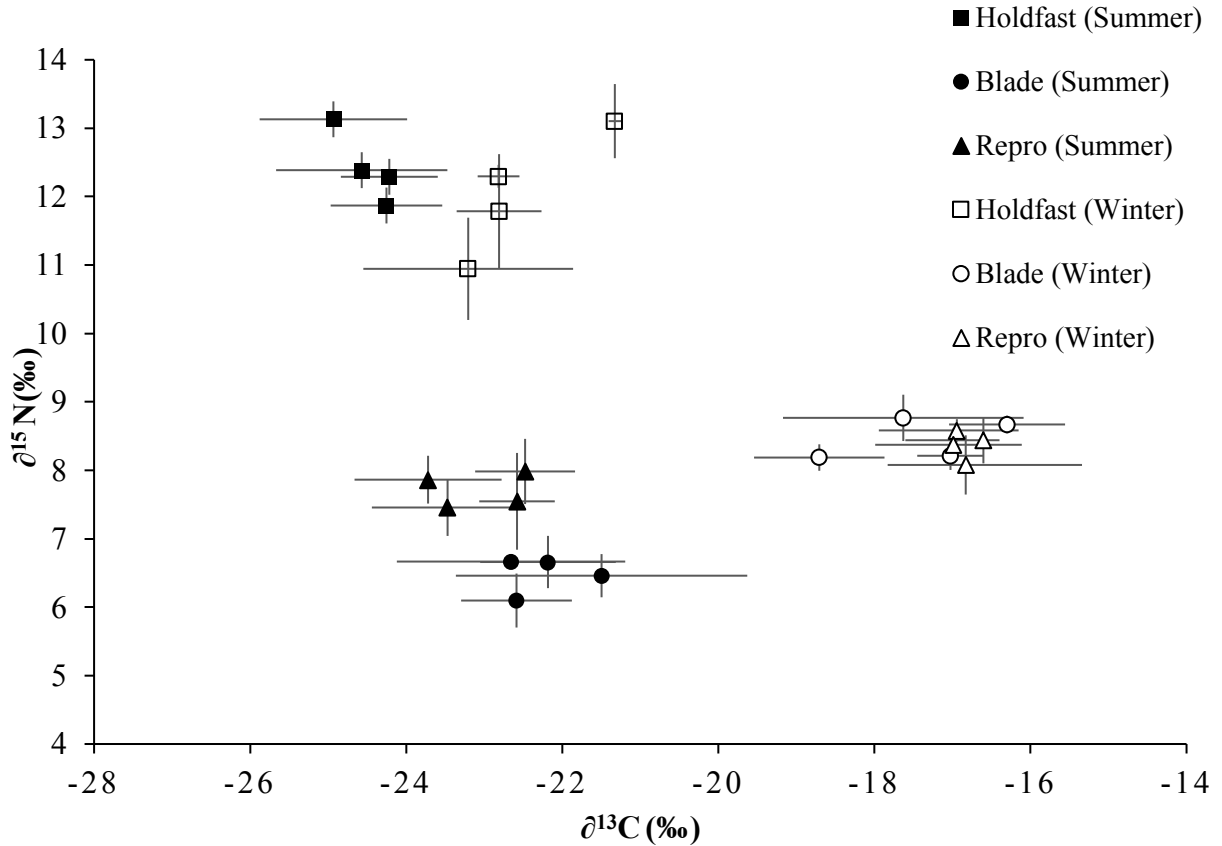
Figure 16. Mean ∂^{15}N and ∂^{13}C for each manipulation experiment (summer represented by solid filled shapes and winter by empty) by tissue types. All treatments are included for both periods because of the inability to detect a significant difference between them (Table 6). Tissue types are labeled: blade (•), holdfast (▪), and reproductive (▲). Samples were collected in August for the summer manipulation and March for the winter manipulation. Error bars for both axes are ±1 SE.
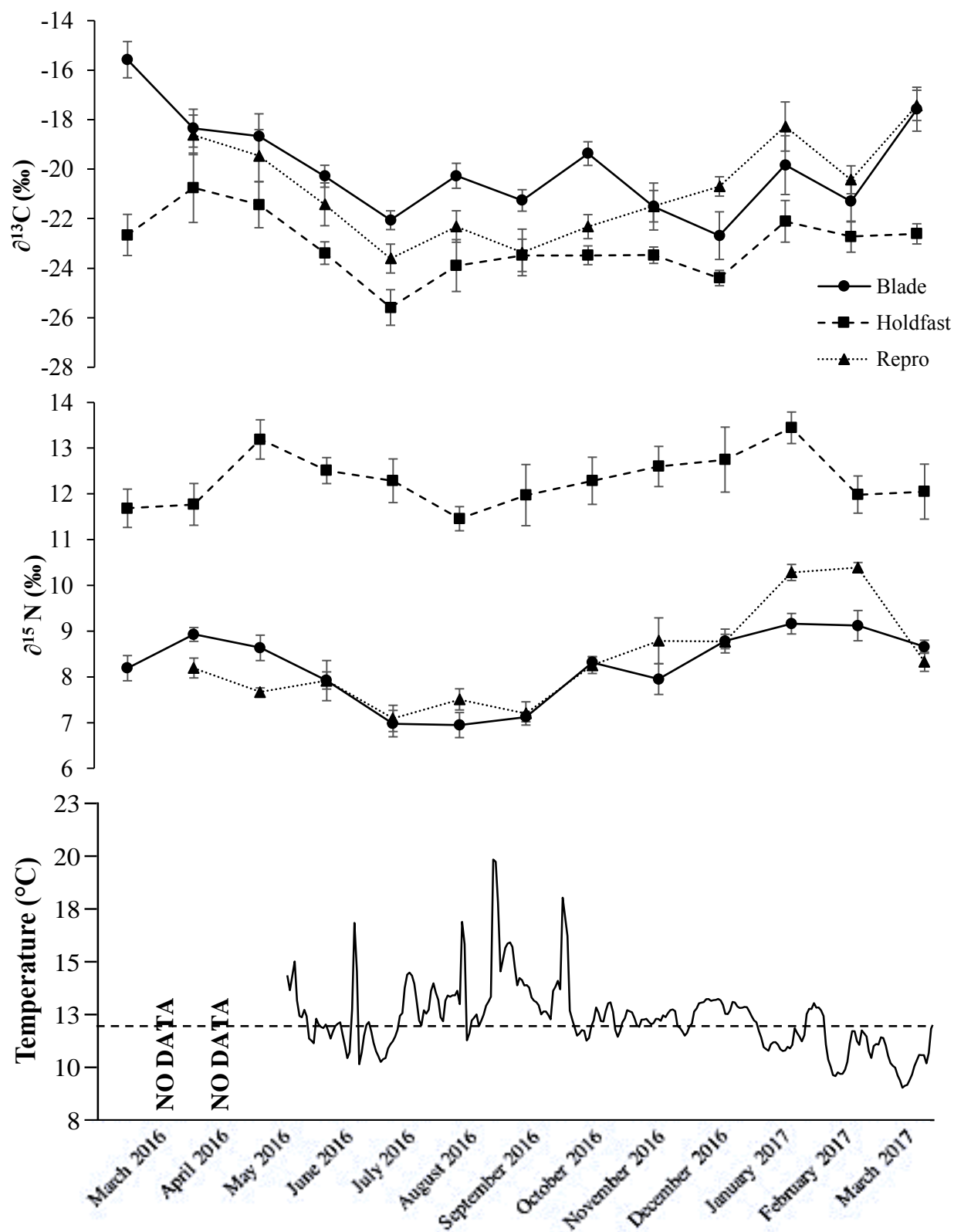
Figure 17. $\partial^{13}C$ and $\partial^{15}N$ values averaged by month per tissue compared to seawater temperature in the bottom graph. Tissue types are labeled: Blade ($\bullet$), holdfast ($\blacksquare$), and reproductive ($\blacktriangle$). Error bars are ± 1 SE. Seawater temperatures were taken from a moored SeapHOx instrument in Stillwater Cove. Dashed line in bottom graph indicates the average temperature in the Cove (~12.4°C).

TABLES

Table 1. Natural productivity measurement values for each month over the sampling period. Variability seen throughout the year is attributed to the high values in total biomass (TB), reproductive biomass (RB), total length (TL), and reproductive length (RL) during the spring and summer months. Vegetative biomass (VB) and vegetative length (VL) remained consistent month to month.

Mo. Yr.	TB (g)	±SE	RB (g)	±SE	VB (g)	±SE	TL (cm)	±SE	RL (cm)	±SE	VL (cm)	±SE
Mar 16	35.0	7.3	0.5	0.2	34.5	7.2	43.1	2.9	2.8	1.7	40.3	2.5
Apr 16	184.8	85.6	120.8	85.9	64.0	8.7	122.7	27.9	72.7	31.4	50.1	7.3
May 16	185.4	41.0	90.8	23.2	94.7	20.4	259.6	36.1	205.2	34.0	54.4	3.1
Jun 16	395.4	65.8	268.0	64.9	127.4	10.9	490.4	41.4	431.6	41.8	58.8	2.8
Jul 16	402.1	118.1	301.0	100.9	101.1	22.3	579.8	98.9	525.2	96.5	54.6	3.3
Aug 16	163.3	44.9	61.8	37.7	101.5	24.4	262.9	100.7	215.7	99.6	47.2	4.6
Sep 16	235.6	127.4	126.1	118.4	109.6	21.9	201.8	110.1	148.4	107.7	53.4	6.4
Oct 16	140.8	21.3	49.8	20.8	90.9	2.5	131.6	33.5	81.6	32.9	50.0	3.1
Nov 16	165.2	28.0	0.1	0.1	165.1	28.0	63.4	5.3	0.6	0.6	62.8	4.9
Dec 16	111.9	21.7	1.7	0.9	110.1	21.1	62.8	6.1	0.0	0.0	62.8	6.1
Jan 17	127.2	21.2	4.7	1.3	122.5	20.6	66.2	5.5	6.2	1.0	60.0	5.6
Feb 17	127.9	21.4	13.5	4.8	114.4	17.0	65.8	5.5	11.8	2.7	54.0	3.0
Mar 17	95.9	25.4	28.4	11.4	67.5	15.9	72.4	11.7	19.0	6.7	53.4	7.4

Table 2a. Summary statistics for ANOVA of total biomass over time. Time of the year, particularly during the spring and summer months, is driving the variability.

Factor	df	RMS	F-ratio	p-value
Time	12	228.141	2.852	0.004
Error	52	135.091		

Table 2b. Summary statistics for ANOVA of total length over time. Time of the year, particularly during the spring and summer months, is driving the variability.

Factor	df	RMS	F-ratio	p-value
Time	12	386.559	10.437	<0.001
Error	52	119.658		

Table 3. Summary statistics for the ANOVA on inter-thallus chemistry over time using tissue type as an additional factor

Variable	Factor	df	MS	F-ratio	p-value
$\partial^{13}C$	*Time*	12	33.644	11.782	<0.001
	Tissue	2	177.481	62.152	<0.001
	*Time * Tissue*	23	5.644	1.976	0.008
	Error	144	2.856		
$\partial^{15}N$	*Time*	12	6.320	10.555	<0.001
	Tissue	2	336.326	561.750	<0.001
	*Time * Tissue*	23	1.691	2.825	<0.001
	Error	144	0.599		
C:N	*Time*	12	62.604	4.615	<0.001
	Tissue	2	1093.593	80.615	<0.001
	*Time * Tissue*	23	14.666	1.081	0.373
	Error	144	13.566		

Table 4. Summary statistics for ANOVA run for growth rate by treatment for the summer manipulation experiment. Post-hoc testing indicated the variance is primarily between the Control and –Repro and –All treatments.

Period	Factor	df	MS	F-ratio	p-value
Summer	*Treatment*	3	90.231	6.0074	0.0043**
	Error	20	100.132		

Table 5. Summary statistics for ANOVA run for growth rate by treatment for the winter manipulation experiment. Post-hoc testing indicated that most of the variance is attributed to the –Veg treatment's highly negative growth rate.

Period	Factor	df	MS	F-ratio	p-value
Winter	*Treatment*	3	0.8283	4.0537	0.0288*
	Error	14	0.2043		

Table 6. Summary statistics for a two-way ANOVA comparing the growth rates for the summer and winter experimental periods. The model incorporated period (season), treatment, and the interaction term as factors.

Factor	df	MS	F-ratio	p-value
Treatment	3	12.0589	3.9809	0.0156*
Season	1	66.3334	21.8978	<0.001*
Treatment * Season	3	15.8574	5.2348	0.0044
Error	34	3.0292		

Table 7. Summary statistics for the ANOVA comparing the inter-thallus tissue chemistry of the summer manipulation experiment using tissue type and treatment

Variable	Factor	df	MS	F-ratio	p-value
$\partial^{13}C$	Tissue	2	15.718	5.090	0.014
	Treatment	3	1.479	0.479	0.7
	Tissue*Treatment	6	0.439	0.142	0.989
	Error	24	3.088		
$\partial^{15}N$	Tissue	2	118.154	236.185	<0.001
	Treatment	3	0.344	0.688	0.568
	Tissue*Treatment	6	0.442	0.833	0.522
	Error	24	0.500		
C:N	Tissue	2	239.735	11.977	<0.001
	Treatment	3	14.682	0.734	0.542
	Tissue*Treatment	6	10.102	0.505	0.799
	Error	24	20.016		

Table 8. Summary statistics for the ANOVA comparing the inter-thallus tissue chemistry of the winter manipulation experiment using tissue type and treatment

Variable	Factor	df	MS	F-ratio	p-value
$\partial^{13}C$	*Tissue*	2	118.107	62.693	<0.001
	Treatment	3	1.624	0.862	0.474
	*Tissue*Treatment*	6	1.819	0.966	0.469
	Error	24	1.884		
$\partial^{15}N$	*Tissue*	2	51.227	99.564	<0.001
	Treatment	3	1.271	2.471	0.086
	*Tissue*Treatment*	6	0.745	1.447	0.238
	Error	24	0.515		
C:N	*Tissue*	2	365.446	49.235	<0.001
	Treatment	3	10.515	1.417	0.262
	*Tissue*Treatment*	6	14.403	1.940	0.115
	Error	24	7.422		

Table 9. Summary statistics for the ANOVA to compare carbon and nitrogen enrichment values for each experimental season along with tissue type and the interaction between those two factors.

Variable	Factor	df	MS	F-ratio	p-value
$\partial^{13}C$	Season	1	337.394	156.603	<0.001
	Tissue Type	2	105.466	48.953	<0.001
	Season * Tissue	2	28.368	13.167	<0.001
	Error	66	2.154		
$\partial^{15}N$	Season	1	10.936	19.874	<0.001
	Tissue Type	2	160.903	292.424	<0.001
	Season * Tissue	2	8.469	15.392	<0.001
	Error	66	36.316		