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Biomass loss reduces growth and resource translocation in giant kelp *Macrocystis pyrifera*

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ABSTRACT: The biomass dynamics of primary producers have important implications for the structure and function of ecosystems. Along the wave-swept coastline of central California, USA, biomass removal by wave action is a key driver in the primary productivity of giant kelp forests, yet the mechanisms of regrowth within giant kelp *Macrocystis pyrifera* are not well understood. To examine the physiological consequences of biomass loss on *Macrocystis*, a manipulative experiment was used to simulate biomass removal by wave action. Growth rates were measured as the number of new fronds produced through time, and the $\delta^{13}C$ and $\delta^{15}N$ values of juvenile fronds were used as a proxy for carbon and nitrogen translocation in support of growth. The experimental removal of biomass significantly constrained the growth of new fronds and, under extreme levels, led to mortality. The growth rate and isotopic composition of juvenile fronds on sporophytes with a portion of canopy biomass intact recovered to pre-disturbance values within 4 mo. In contrast, a reduction in growth rates as well as a permanent depletion in $\delta^{13}C$ and $\delta^{15}N$ values was observed when the canopy was completely removed and the magnitude scaled with biomass loss. These results suggest that the translocation of carbon and nitrogen to juvenile fronds from near-surface biomass is a critical process affecting growth in giant kelp. The spatial variability and physiological consequences of biomass loss among individuals may therefore play an important role in the biomass dynamics of giant kelp forests across multiple temporal and spatial scales.

KEY WORDS: Stable isotopes · Carbon · Nitrogen · Resource allocation · Disturbance · Recovery

INTRODUCTION

Kelps (Laminariales, Phaeophyceae) function as foundation species in many temperate marine ecosystems, due largely to their complex morphology and rapid growth rates (Dayton 1985, Graham 2004, Bolton 2010). Kelp forests are among the most productive ecosystems on earth (Reed & Brzezinski 2009), and their high rates of primary productivity are driven by the collective biomass and rapid growth of the kelps themselves (Mann 1973, Reed et al. 2008). However, the extent of habitat and energy provided by kelps can be strongly influenced by a suite of environmental factors including wave disturbance (Seymour et al. 1989, Graham et al. 1997), light and nutrient availability (Chapman & Craigie 1977, Gerard 1982, Dunton 1985), and grazing pressure (Dayton et al. 1984, Konar & Estes 2003). These biotic and abiotic drivers can greatly influence the key biological processes that ultimately regulate kelp population dynamics.

Much debate surrounds the relative importance of top-down (grazing and trophic interactions) and bottom-up (resource availability) forces in regulating community structure and primary productivity in kelp-dominated systems (Foster et al. 2006, Halpern et al. 2006, Foster & Schiel 2010). Importantly, the relative influence of these drivers is not consistent across systems. For example, some mid- to high-latitude kelp systems such as the Aleutian Islands

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and the northwest Atlantic exhibit strong top-down control through trophic cascades that alter grazing pressure (Estes & Palmisano 1974, Steneck et al. 2002). Alternatively, the productivity of giant kelp *Macrocystis pyrifera* forests in California, USA, is strongly regulated by bottom-up oceanographic processes, most notably nutrient limitation (Jackson 1977, Zimmerman & Kremer 1986, Dayton et al. 1999). While *Macrocystis* growth is often nutrient limited in southern California, growth is more strongly regulated by wave disturbance in central California (Bell et al. 2015).

In central California, intermittent upwelling occurs from early spring to early summer and reduces nutrient-limited growth of kelp populations (Graham et al. 2008). Despite greater nutrient availability, the annual net primary productivity (NPP) of *Macrocystis* forests in central California is approximately half the NPP of those in southern California. The lower rates of NPP in central California are largely due to the loss of biomass from wave disturbance (Reed et al. 2011). Thus, large-scale patterns of biomass dynamics in *Macrocystis* populations can be fundamentally altered by physical disturbance. Kelps have unique physiology and resource translocation strategies, which facilitate their large size and high growth rates (Schmitz & Lobban 1976). Although the mechanisms of regrowth following disturbance remain unclear, they are likely linked to the ability of kelps to efficiently translocate resources to areas of new growth.

Macrocystis exhibits the highest rates of resource translocation among the kelps and is comprised of a complex network of meristems (Schiel & Foster 2015). These meristems rely on resources exported from mature canopy blades near the surface (Parker 1963, Schmitz & Lobban 1976, Schmitz & Srivastava 1979). New blades on actively growing *Macrocystis* fronds are produced from the apical meristem. Frond initials are new apical meristems that are produced at the base of adult fronds (Schiel & Foster 2015). Actively growing frond initials are referred to as juvenile fronds when they become greater than 1 m in length (Lobban 1978a). The apical meristems on adult and juvenile fronds, along with newly produced blades, are the primary regions of growth in *Macrocystis* and depend on carbohydrates and amino acids exported from mature blades to meet metabolic demands (Parker 1966, Schmitz & Srivastava 1979). When the apical meristem of a frond is removed or damaged, the impacted frond directs the export of all resources towards the juvenile fronds and frond initials at its base (Schmitz & Lobban 1976, Lobban 1978b). Thus, the location and magnitude of biomass loss of kelp fronds (i.e. just surface canopy biomass vs. a full frond) will likely drive variability in resource translocation to juvenile fronds among *Macrocystis* sporophytes.

Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of juvenile fronds may provide unique insights on patterns of carbon and nitrogen translocation in *Macrocystis.* The translocation of carbohydrates has been shown to increase the $\delta^{13}C$ of tissues in vascular plants (Gessler et al. 2009, Werner & Gessler 2011), which suggests that stable isotope ratios reliably track physiological processes associated with the movement of internal resources. Furthermore, the $\delta^{13}C$ of *Macrocystis* frond initials has been shown to be biomass dependent and may reflect translocation of carbohydrates from the surface canopy (Fox 2013). Nitrogen translocation has only been observed in *Macrocystis* once (Hepburn et al. 2012), and the rates and directionality of transport remain poorly understood. However, the $\delta^{15}N$ ratios of macroalge provide detailed information about the primary sources of nitrogen being assimilated (Cohen & Fong 2005) and may follow a pattern of enrichment during translocation similar to that observed with $\delta^{13}C$. If so, a decrease in the $\delta^{13}C$ and $\delta^{15}N$ of juvenile fronds following biomass loss may provide evidence that carbon and nitrogen translocation to juvenile fronds has been reduced.

The primary objectives of this study were to (1) quantify the effects of the magnitude and location of biomass loss on the growth of new fronds in *Macrocystis*, and (2) determine if biomass loss can reduce the translocation of carbon and nitrogen to juvenile fronds. The variable and context-dependent nature of regrowth in *Macrocystis* is driven by its complex morphology and integrated strategy of resource translocation. By quantifying the effects of biomass loss on growth and the translocation of key resources within individual sporophytes, we can refine our understanding of the physiological mechanisms affecting biomass dynamics and recovery from disturbance in *Macrocystis* populations.

MATERIALS AND METHODS

Study site and environmental parameters

Stillwater Cove is located within the northern corner of Carmel Bay in central California (36.59073° N, 121.94590° W). The cove is oriented to the southwest and is relatively protected from large winter swells. The *Macrocystis* beds in Stillwater Cove are repre-

sentative of a typical central California giant kelp forest and exhibit peak canopy biomass during summer (Reed & Foster 1984, Foster & Schiel 1985). All sporophytes used in this experiment were located within the interior of the kelp bed to avoid confounding edge effects (Stewart et al. 2009). The experiment ran from June to November 2011, and environmental conditions were monitored using an array of StowAway Tidbits (0.2°C). The thermistors recorded water temperature at 5 min intervals at depths of 0.5, 4, 8, and 13 m below the surface. Daily water temperature was calculated by averaging values across all depths. Empirical dissolved inorganic nitrogen (DIN: nitrite + nitrate) values were obtained from discrete water samples collected adjacent to each thermistor. Water samples were collected every 2 wk (12 samples per depth across the experiment), transported on ice and frozen until analysis. During the study, NH_4 was only measured in concentrations <1 µmol l^{-1} and therefore not included in the DIN values used to parameterize a predictive linear model between temperature and DIN, DIN = 59.68 ± 2.94 (SE) − 3.75 ± 0.25 × Temp, r^2 = 0.818 (see Fox 2013). Ambient DIN availability during the experiment was used to identify periods where growth rates could have been driven by nutrient limitation rather than treatment effects and to inform temporal patterns in $\delta^{15}N$.

Biomass manipulation

Biomass removals were implemented in the field to elucidate the effects of physical disturbance on the growth of new fronds and the associated patterns of resource translocation in giant kelp. Three replicate blocks of 8 similarly sized (30.2 ± 7.2 [SD] fronds) *Macrocystis* sporophytes were located at 10–12 m depth. Each block was approximately 25 m apart, while the individuals within a block were never more than 10 m apart. In each block, individuals were randomly assigned to 1 of 4 biomass removal treatments (n = 2 per block, 6 per treatment): control (C), cut control (CC), canopy removal (CR), and full removal (FR) (Fig. 1). Two unmanipulated control sporophytes per block were maintained for baseline data on natural rates of frond initiation and chemical composition at the study site. The 6 remaining sporophytes per block were trimmed to 12, non-senescent canopy fronds and given 1 wk to recover prior to the start of the experiment. Fronds greater than 1 m, but not yet in the canopy, were also removed. Frond initials and sporophylls were not removed. This standardization eliminated pre-existing differences in biomass and homogenized the isotopic signatures of juvenile fronds across all experimental sporophytes. The CC treatment was not manipulated beyond this standardization and controlled for effects of frond thinning as well as the presence of a surface canopy. The remaining sporophytes were trimmed once more at the start of the experiment in June 2011. Sporophytes in the CR treatment had all fronds cut to 1 m below the surface of the water column. For the FR treatment, all mature and juvenile fronds were completely removed. The total biomass removed from all experimental sporophytes (CC, CR, and FR) was estimated as the sum of the biomass of each frond removed. Frond biomass was calculated using the quadratic relationship between frond length and biomass determined from 39 *Macrocystis* fronds collected at the study site (biomass = 0.182 − 0.01 × frond length + 0.17 × frond length^2) (see Fox 2013).

The key difference between all treatments was the amount of frond biomass intact on each sporophyte. The FR treatment was designed to replicate extreme physical disturbance from large waves that have been observed to occur at the study site, which can occur regularly during the winter at exposed loca-

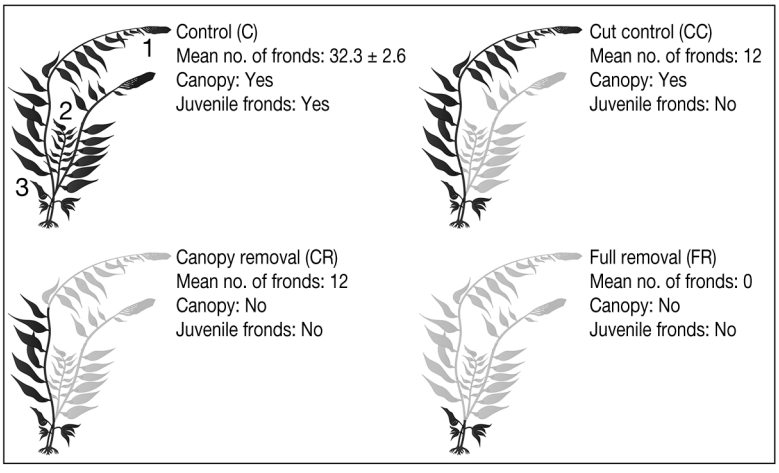

Fig. 1. Visual representation of the experimental treatments. Graphic for each treatment represents the extent and location of biomass removal. For reference, the key morphological features for growth in a mature *Macrocystis* sporophyte are labeled in the Control diagram: (1) mature canopy frond, (2) juvenile frond, and (3) frond initial. See 'Biomass manipulation' section for details. Mean number of fronds corresponds to the average number of fronds (±SD) per sporophyte at the start of the experiment

tions along the central California coast. This treatment removed all frond biomass, theoretically eliminating all resource translocation to frond initials. In contrast, the CR treatment simulated moderate physical disturbance by removing the surface canopy and all apical meristems, while leaving 12 fronds that spanned the water column (~10 m from the bottom). Thus, prior to senescence, these fronds would theoretically be able to export carbon and nitrogen to frond initials or juvenile fronds to subsidize new growth.

Sampling design

To determine the effects of biomass removal on growth and recovery potential, sporophytes were sampled monthly from June to November 2011. Growth rate was measured as a function of new fronds produced over time, or frond initiation rate (Zimmerman & Kremer 1986). Extant fronds on each sporophyte were tagged at the start of the experiment and new, untagged fronds greater than 1 m in height (North 1971) were counted and tagged monthly. Frond initials (fronds <0.5 m long) were counted at the base of each sporophyte to represent growth potential. The growth of new fronds was also examined as a function of the available initials in the previous month to identify changes in the efficiency of frond initiation.

Stable isotope techniques were used to assess the effect of biomass loss on resource translocation during recovery from disturbance. Each month, a single blade was collected from ~1 m behind the apical meristem of a randomly selected juvenile frond (2–3 m long) on all sporophytes. Samples were always collected mid-morning due to the diel variability in isotopic signatures observed in vascular plants (Werner & Gessler 2011). Blades were cleaned of epiphytes, and a 2–5 g subsample was removed from the central portion of the blade closest to the pneumatocyst. This tissue represents the youngest portion of the blade and likely provides the best estimate of recent physiological activity. All samples were dried at 60°C for 72 h, homogenized in a ball mill, and analyzed for $\delta^{13}C$, $\delta^{15}N$, %C and %N with a Carlo Erba 1110 elemental analyzer interfaced with a Thermo Finnigan Delta Plus XP mass spectrometer at the University of Wyoming Stable Isotope Facility. Isotopic values are expressed as $\delta^{13}C$ or $\delta^{15}N$, where $\delta = 1000 \times [(R_{sample}/R_{standard}) - 1]$ and R_{sample} or $R_{standard}$ are the ratio of the heavy to light isotope in parts per thousand (‰). The standards used were Vienna-Pee Dee Belemnite (V-PDB) and atmospheric N_2. The within-run run standard deviation of a glutamic standard was <0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$.

Movement of carbohydrate resources from the canopy to juvenile fronds should increase the $\delta^{13}C$ and overall %C of juvenile blade tissue. The $\delta^{15}N$ and %N of juvenile blade tissue should also follow this pattern if the physiological mechanisms associated with the transport nitrogen are similar to those of carbon translocation. A reduction in these tissue chemistry parameters below control values is likely indicative of reduced translocation of carbon and nitrogen from near-surface biomass. Periods of nutrient limitation would likely drive tissue %N values below 1% (Gerard 1982).

Statistical analyses

Pre-existing differences in frond initial number and chemical composition prior to biomass manipulation were tested with a 1-way fixed factor ANOVA. The presence of a stratified water column was tested with 2-way ANOVAs comparing monthly means of temperature and DIN between the surface (0.5 m) and bottom (13 m). The effects of each biomass removal treatment on the growth and chemical composition were tested with 1-way fixed factor ANOVAs on the 5 mo composite means for each sporophyte (n = 6). This approach was used because treatments became unbalanced through time due to the mortality of sporophytes within the FR treatment. Furthermore, low replication and the relatively short period of time in which all treatments could be analyzed similarly precluded my ability to rigorously examine temporal differences in the response variables. Normality and homoscedasticity were verified using the Shapiro-Wilks test and Levene's test, respectively. The effect of block was tested against the 5 mo composite mean of each response variable with a 2-way ANOVA of treatment × block to ensure there was no spatial heterogeneity in the treatment effects. In all cases the effect of block was insignificant ($p > 0.5$) and was removed from the final analyses. An estimate of mean frond length for *Macrocystis* sporophytes at the study site was determined from 85 randomly selected mature fronds. Mean frond length was multiplied by the number of fronds on each sporophyte prior to biomass manipulation to calculate the original sporophyte biomass. The remaining biomass on each sporophyte was calculated by subtracting the removed biomass from the original biomass and was expressed as the percentage of the

original for greater ecological relevance. Linear least squares regression was used to determine the importance of the amount of remaining biomass on frond initiation rates and resource allocation to juvenile fronds.

To examine variation in chemical composition of juvenile blades across time and treatments, a resampling bootstrap approach was used to maximize the possible variation within treatments. Chemical variables were examined in 2-dimensional pairs ($\delta^{13}C$ and $\delta^{15}N$, %C and %N). To test for temporal changes, pre-manipulation samples from all treatments collected in June were pooled and resampled 10 000 times to generate a series of possible global means for all chemical variables. The 95% confidence interval was calculated from these means for each 2-dimensional pair and plotted around their centroid. The monthly mean chemical composition of each treatment was subsequently plotted against the global pre-manipulation mean to examine the trajectories of change. Similarly, to calculate the integrated chemical composition of juvenile blades in each treatment, all post-manipulation data were pooled and resampled to generate a global treatment centroid, 95% confidence interval, and the maximum possible range within each treatment. This approach provided an improved estimate of possible isotopic and bulk elemental compositions that could exist for *Macrocystis* sporophytes outside of this experiment that have experienced comparable levels of biomass loss. All statistical analyses were completed using R (R Core Team 2013).

RESULTS

Environmental conditions

Monthly mean water column temperatures and DIN values ranged from 10.96–13.27°C and 9.75–18.44 µmol l^{-1}, respectively (Fig. S1 in the Supplement; www.int-res.com/articles/m562p065_supp.pdf). Ambient DIN concentration was highest prior to the start of the experiment and lowest in early October. In October interval ambient DIN concentration fell below 5 µmol l^{-1} for 3 and 6 d at 13 and 0.5 m depth, respectively. The water column was stratified during all months except November (temperature — ANOVA: $F_{6,360}$ = 2.40, MS = 1.93, p = 0.03, Tukey's HSD p = 0.7) and (DIN — ANOVA: $F_{6,360}$ = 2.40, MS = 27.3, p = 0.03, Tukey's HSD p = 0.7). By November, the water column was well-mixed and ambient conditions returned to the 6 mo average (12.2°C, 13.77 µmol l^{-1}).

Growth of new fronds

The average percent biomass removed for the standardization to 12 fronds was 44% ± 3.2 (SD) and did not differ between the CC, CR, and FR treatments (ANOVA: $F_{2,15}$ = 0.001, p = 0.71). At the start of the experiment there were an average of 3.8 ± 1.1 (SD) frond initials per sporophyte, and there was no difference among treatments (Table S1 in the Supplement).

Extreme biomass loss, simulated by the FR treatment, resulted in a 78.3% reduction in the average number of frond initials over the 5 mo experiment relative to the control treatment (Fig. 2a, Table 1). Moderate biomass loss did not reduce the average number of frond initials in the CC and CR treatments below that of the controls. All sporophytes in the C, CC, and CR treatments maintained approximately 3–4 initials throughout the experiment (Fig. 2a). Frond initiation rates were reduced by 64.4 and 89% relative to controls in the CR and FR treatments, respectively (Fig. 2b, Table 1). With the exception of one individual, sporophytes in the FR treatment were unable to re-grow fronds and experienced complete mortality by September. Initiation rates of the CC treatment exhibited high inter-sporophyte variability

Table 1. Analysis of variance (ANOVA) outputs testing for variations in different growth metrics across treatments. The 5 mo composite mean of each growth metric was tested with a 1-way, fixed-factor ANOVA. The composite mean is calculated as the mean of individual sporophyte means (n = 6) in each treatment from July to November 2011. Significant pairwise comparisons (bold, p < 0.05) are shown with the use of |. Treatments on the same side of the | are not significantly different, as are comparisons not shown. Control: C; cut control: CC; canopy removal: CR; full removal: FR

Variable	Factor	df	MS	*F*	p	Pairwise comparisons
No. of initials	Treatment	3	10.84	8.16	**<0.001**	C CC CR | FR
	Residuals	20	1.33			
Frond initiation rate	Treatment	3	0.13	13.62	**<0.001**	C | CR FR, CC | FR
	Residuals	20	0.001			
New fronds per initial	Treatment	3	0.89	12.72	**<0.001**	C CC | CR FR
	Residuals	20	0.7			
Total fronds produced	Treatment	3	274.93	8.712	**<0.001**	C | CR FR, CC | FR
	Residuals	20	31.56			

and were reduced by 28% relative to the control (Fig. 2b, Table 1). The mean frond initiation rate of the CR treatment was less than half of that in the CC treatment, but there was only a marginal effect (Tukey's HSD p = 0.08, Fig. 2b, Table 1).

Biomass loss significantly reduced the growth of new fronds as a function of available frond initials (Fig. 2c, Table 1). Thus, the efficiency of frond growth and therefore recovery potential was negatively impacted by the loss of vegetative biomass in *Macrocystis*. Tukey's pairwise comparisons showed significant differences between treatments with an intact canopy (C and CC) and those without a canopy (CR and FR) (Table 1). Overall, sporophytes in the CR and FR treatments produced significantly fewer fronds than control sporophytes (Table 1). There was no statistically significant difference between the CC and CR treatments (Tukey's HSD p = 0.14, Table 1). Only 2 of 6 sporophytes in the CR treatment produced more than 5 fronds over the experiment, while only 1 of 6 in the CC treatment produced fewer than 10. The percentage of original biomass remaining on an individual sporophyte explained 49% of the variation in total frond initiation, and regrowth was substantially restricted in sporophytes that retained less than 30% of their original biomass (Fig. 3a; regression: $F_{1,16}$ = 15.24, p = 0.001, r^2 = 0.49; fronds produced = 0.241 ± 0.06 [SE] × percent biomass + 1.14 ± 1.83).

Tissue chemistry of juvenile blades

The mean $\delta^{13}C$ and $\delta^{15}N$ of juvenile blade tissue in June, prior to biomass manipulation, were $\delta^{13}C$ = −15.42‰ ± 0.33 (SE) and $\delta^{15}N$ = 10.63‰ ± 0.26 and did not differ across treatments (Table S1 in the Supplement). The mean %C and %N were 27.74% C ± 0.36 and 1.85% N ± 0.018, respectively, and were similar across treatments (Table S1 in the Supplement). The percentage of original biomass on a sporophyte explained 53 and 39% of the variation in the $\delta^{13}C$ and $\delta^{15}N$ values of juvenile blades, respectively (Fig. 3b,c). Sporophytes with less than 50% of their original biomass had lower mean $\delta^{13}C$ values relative to the control treatment (Fig. 3b; regression: $F_{1,16}$ = 18.17, p = 0.001, r^2 = 0.53; $\delta^{13}C$ = 0.06 ± 0.02 × percent biomass − 20.12 ± 0.47). Biomass loss reduced the mean $\delta^{15}N$ value of all sporophytes below the control treatment (Fig. 3c; regression: $F_{1,16}$ = 10.09, p = 0.01, r^2 = 0.39; $\delta^{15}N$ = 0.04 ± 0.01 × percent biomass + 7.87 ± 0.35).

Control sporophytes exhibited the least amount of variation from the pre-manipulation means in $\delta^{13}C$ and $\delta^{15}N$ between July and November 2011 relative

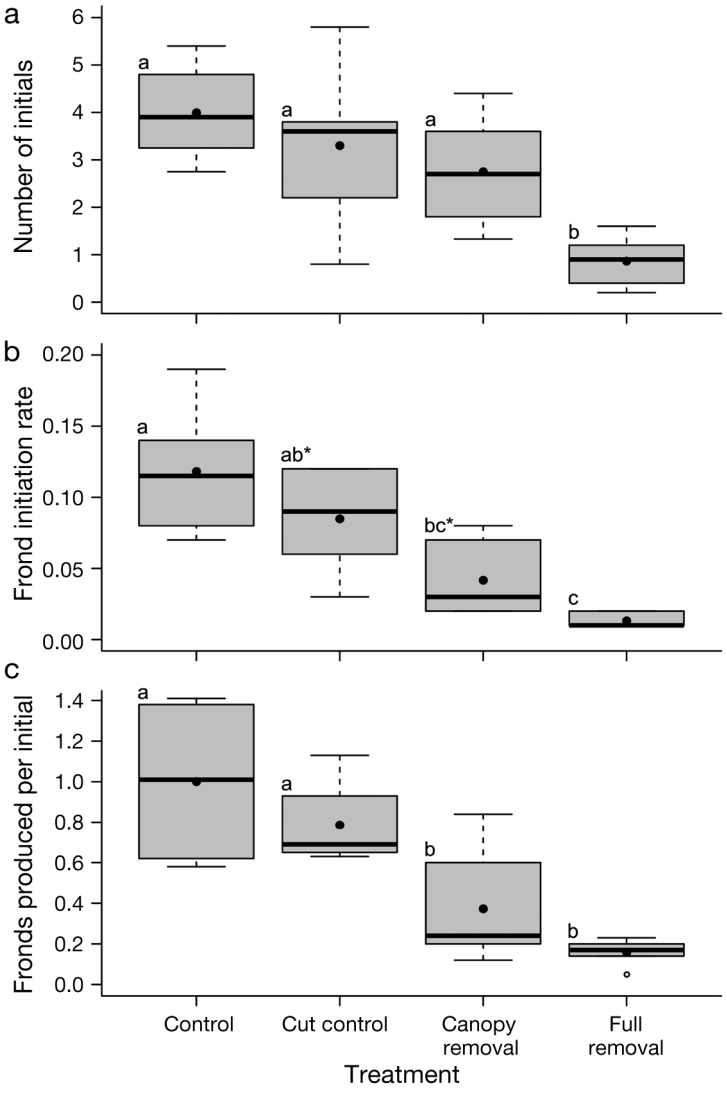

Fig. 2. The 5 mo composite means of growth response metrics by treatment. Data are calculated as the mean of individual sporophyte means (n = 6) in each treatment from July to November 2011. (a) Number of frond initials observed per sporophyte, (b) frond initiation rate expressed as the number of new fronds produced per day, (c) number of new fronds produced as a function of the number of frond initials present in the preceding month. Treatment means are displayed with a black dot. Box represents the lower and upper quartiles with the median value of the data shown with the black line. Whiskers represent the minimum and maximum values of the data that are not greater than 1.5 times the difference between the upper and lower quartiles. All data beyond this limit are displayed as points. Different letters denote significant differences at p < 0.05; asterisks denote significant difference at p < 0.1

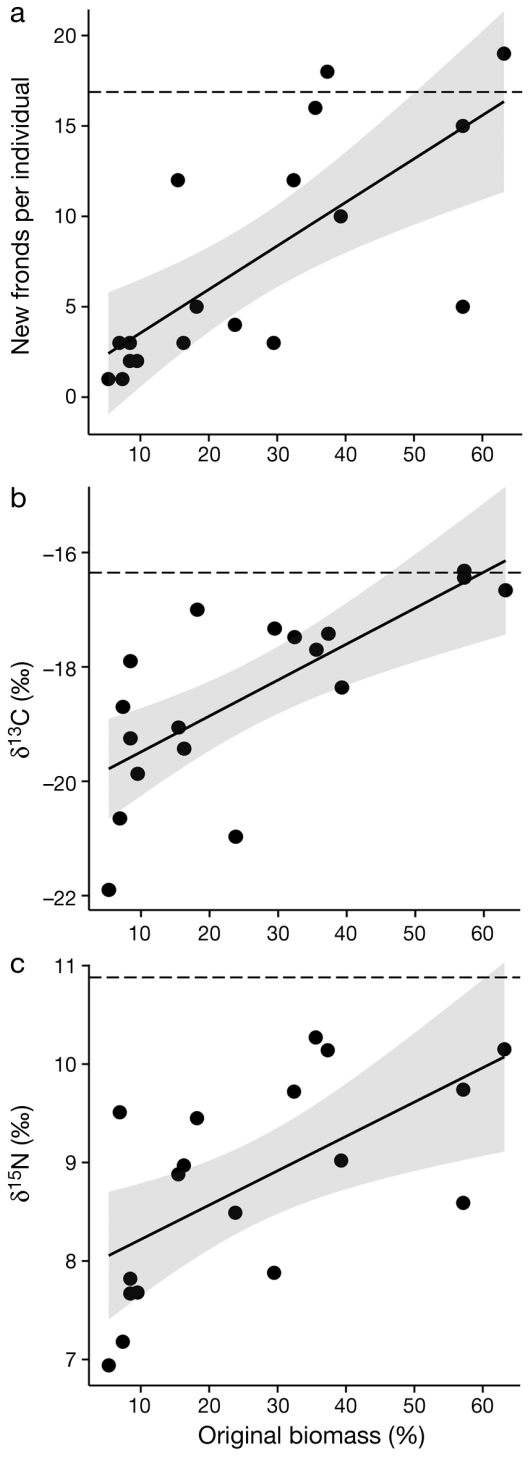

Fig. 3. Effects of biomass removal on the long-term mean of growth and the isotopic composition of juvenile blades. Individual sporophytes are presented as the percent of original biomass remaining after biomass manipulations (*x*-axis). Lines represent best-fit linear regressions. Mean value of the control treatment on the *y*-axis is plotted as a horizontal dashed line. 95% confidence intervals for each regression line are shown in gray. (a) Mean number of fronds >1 m grown by each sporophyte. (b) Mean $\delta^{13}C$ value of juvenile blade tissue. (c) Mean $\delta^{15}N$ value of juvenile blade tissue

to the other treatments (Fig. 4a). Biomass loss caused significant temporal shifts in the isotopic composition of the other treatments relative to pre-disturbance values and generally led to a reduction of both $\delta^{13}C$ and $\delta^{15}N$ (Fig. 4b–d, Table 2, Fig. S2 in the Supplement). All treatments (including the control) exhibited a significant decrease in $\delta^{15}N$ during July; however, the magnitude of the reduction in the CC, CR, and FR treatments was more than 1‰ greater than the control. Similarly, all manipulated treatments experienced an initial decrease in $\delta^{13}C$, and the magnitude of the depletion increased as a function of biomass loss (Fig. 4b–d). While the control treatment quickly returned to pre-treatment isotopic levels after 1 mo, the trajectory of the CC treatment required 4 mo to stabilize at the pre-disturbance levels. Neither the CR nor the FR treatments returned to pre-disturbance isotopic values, and they remained in a permanently depleted state following experimental biomass removal. The plants in the FR treatment died 3 mo after biomass manipulation.

The successive depletion of $\delta^{13}C$ and $\delta^{15}N$ as a function of biomass loss is evident in the plots of the resampled 5 mo means for each treatment (Fig. 5, Table S2 in the Supplement). Isotopic depletion was greatest in the FR and CR treatments (Fig. 5, Table 2, Fig. S2), but there was significant overlap between the CC and CR treatments. Contrary to the other treatments, the control treatment remained significantly enriched in ^{15}N. Variability in the $\delta^{15}N$ values appeared consistent across the CC, CR, and FR treatments, but the variability in $\delta^{13}C$ increased proportionately as a function of biomass loss (Fig. 5). The magnitude of depletion in ^{13}C was greatest in the CR and FR treatments, yet high variability among the sporophytes within the CR treatment weakened the statistical difference from the control (Tukey's HSD $p = 0.055$, Table 2, Fig. S2a). The FR treatment was significantly depleted in ^{15}N for the duration of the study relative to the control and CC treatment but was only marginally different from the CR treatment (Tukey's HSD $p = 0.07$, Table 2, Fig. S2b).

Bulk carbon and nitrogen concentrations of juvenile blade tissue were not directly influenced by experimental biomass removal. Total tissue %N ranged from 1.38 to 2.10% across all sporophytes and did not differ between treatments (Table 2). Therefore, it is unlikely that environmental conditions, specifically DIN availability, reached stressful levels during the experiment. Only %C of the juvenile blades in the FR treatment was reduced relative to control values (Table 2). Overall, minimal variation was observed in the 5 mo composite means of %C, %N, and C:N values across all treatments (Fig. S3 in the Supplement, Table 2).

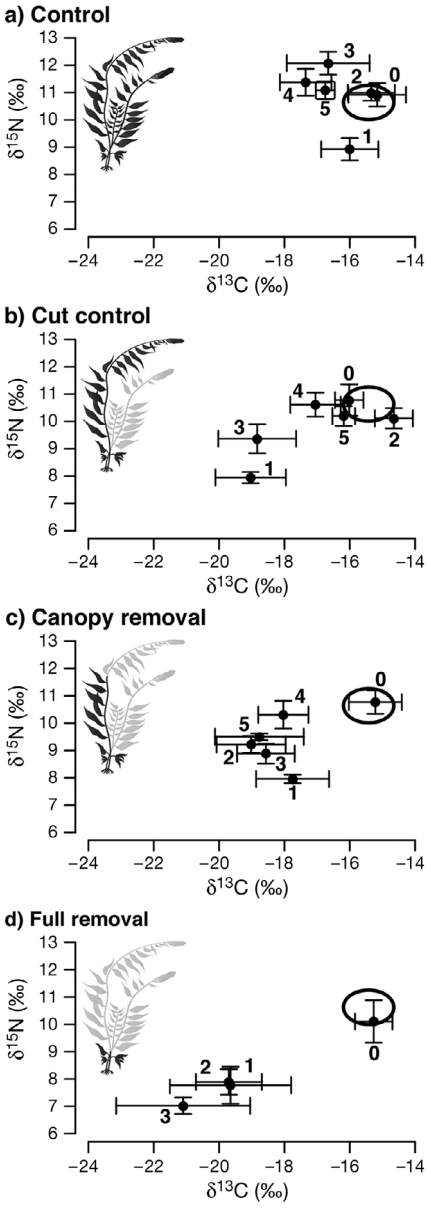

Fig. 4. Temporal trajectories of the mean isotopic ratios of juvenile blades for each treatment. Each data point is numbered with the corresponding month of the experiment: 0 = before manipulation (June 2011); 5 = end of experiment (November 2011). Circle represents the bootstrapped 95% confidence interval of the isotopic ratios of all 24 experimental sporophytes prior to the start of the experiment (June). These data were combined and resampled 10 000 times to more accurately represent the possible variability of juvenile blade tissue within the experimental population at that time. Any data point that falls beyond the boarder of the circle can be considered to be significantly different from the isotopic composition in June at the $p < 0.05$ level. Each panel depicts the temporal changes of the isotopic composition of an average juvenile blade over the course of the experiment for each treatment. (a) Control; (b) cut control; (c) canopy removal; (d) full removal. Image in each graph depicts the extent and location of biomass removal associated with that treatment. Error bars are ±1 SE

DISCUSSION

The results of this study demonstrate that biomass loss can constrain the growth of new fronds in *Macrocystis* and may thus limit recovery potential. Moderate levels of biomass loss did not stimulate regrowth, unlike some terrestrial grasses (Hilbert et al. 1981). Instead, growth appeared to be directly proportional to the amount of biomass that remained on a sporophyte following a physical disturbance. The importance of near-surface biomass to the observed shifts in the isotopic composition of juvenile blade tissues indicates that the translocation of carbon and nitrogen to juvenile fronds is critical for regrowth in *Macrocystis.*

Experimental removal of biomass in the CR and FR treatments severely limited regrowth in *Macrocystis* sporophytes. The loss of surface canopy biomass did not result in sporophyte mortality but significantly reduced frond initiation rates relative to the control treatment. Only slight differences in frond initiation were observed between the CC and

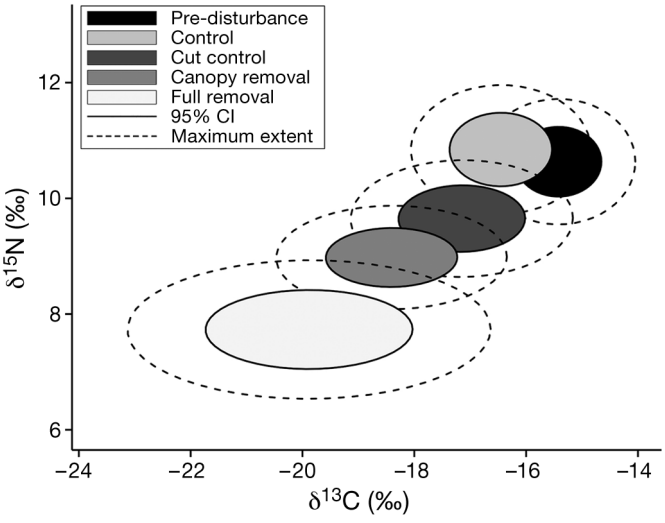

Fig. 5. Total variation of the isotopic composition of juvenile blades within each treatment expressed relative to the 5 mo mean centroid. Solid black line around each filled ellipse denotes the 95% confidence interval around the centroid of the 5 mo means for all individual sporophytes within a treatment. Thus, these circles represent the most likely mean isotopic composition of juvenile blade tissue for a sporophyte that has experienced a particular degree of biomass loss. Data for each treatment were resampled 10 000 times. Dashed line: most extreme values in both dimensions generated through resampling. Solid black ellipse and associated 95% confidence interval: centroid of means from all 24 experimental sporophytes prior to biomass manipulation at the beginning of the experiment. Non-overlapping shaded ellipses can be considered significantly different at the $p < 0.05$ level

Table 2. Analysis of variance (ANOVA) outputs testing for variations in tissue chemistry in juvenile blades. The 5 mo composite mean for each parameter was examined using a 1-way, fixed-factor ANOVA. Significant pairwise comparisons (bold, $p < 0.05$) are shown with the use of |. Treatments on the same side of the | are not significantly different, as are comparisons not shown. Control: C; cut control: CC; canopy removal: CR; full removal: FR

Variable	Factor	df	MS	*F*	p	Pairwise comparisons
$\delta^{13}C$	Treatment	3	13.3	6.984	**<0.01**	C CC | FR
	Error	20	1.91			
$\delta^{15}N$	Treatment	3	10.04	19	**<0.001**	C|CC CR FR, CC | FR
	Error	20	0.53			
%C	Treatment	3	7.338	6.31	**<0.01**	C | FR
	Error	20	1.16			
%N	Treatment	3	0.06	2.17	0.12	
	Error	20	0.03			
C:N	Treatment	3	0.98	0.523	0.67	
	Error	20	1.88			

CR treatments. This suggests that an overall reduction in biomass is the main driver of reduced growth rather than the loss of the surface canopy. The differences observed between treatments in this experiment indicate that a loss of biomass can restrict growth, but more work is needed to examine the importance of canopy biomass relative to biomass in the upper water column.

Frond initials represent the growth potential of an individual sporophyte and are the primary mechanism for regrowth following biomass loss in *Macrocystis,* yet little is known about the mechanisms of frond initial growth. Some *Macrocystis* tissues, particularly embryonic and juvenile sporophytes (Dean & Jacobsen 1984, Kinlan et al. 2003), can exist in a state of arrested development prior to the onset of favorable environmental conditions. The maintenance of tissues in this state is not energetically costly; thus, the metabolic cost of maintaining a standing stock of frond initials is likely low and not strongly influenced by biomass loss. However, the number of initials that grew into juvenile fronds decreased with increasing biomass loss. This suggests the energetic requirements of growing a frond initial into a juvenile frond cannot be met without resources supplied by the canopy biomass, particularly during times of the year when light levels are limiting. The physiological trade-offs between the initiation of a new meristem and the growth of a new frond initial to replace it have not been studied but are likely critical determinants of the ability of *Macrocystis* sporophytes to recover following biomass loss.

Stable isotope analysis provides an indirect measurement of carbon and nitrogen translocation to actively growing tissue in kelps. The enriched $\delta^{13}C$ values of the juvenile fronds in all treatments relative to the control are consistent with the biomass-dependent enrichment of frond initial $\delta^{13}C$ that has been observed in *Macrocystis* (Fox 2013). Additionally, the consistent positive relationships between frond initiation and $\delta^{13}C$ as a function of sporophyte biomass suggest that increased $\delta^{13}C$ values are associated with increased growth (Carvalho et al. 2009). Therefore, enriched $\delta^{13}C$ signatures in juvenile fronds may represent a direct link between surface and subsurface biomass and serve as a reliable proxy for carbohydrate translocation. Less is understood about nitrogen translocation in kelps, but the temporal differences and relationship to sporophyte biomass observed with $\delta^{15}N$ suggests that stable isotopes may be a reliable proxy for nitrogen translocation.

The isotopic composition of macroalgal tissue can be influenced by numerous physiological (Farquhar et al. 1989, Raven et al. 2002) and environmental factors (Foley & Koch 2010, Dethier et al. 2013), which makes interpretation of fine-scale variability challenging. Perhaps the most important driver of the $\delta^{13}C$ ratios of marine macrophytes is the available dissolved inorganic carbon (DIC) pool and the primary carbon species (CO_2 vs. HCO_3^-) used in photosynthesis (Smith & Epstine 1971, O'Leary 1988). Sporophytes with surface biomass could have accessed atmospheric CO_2, but that would have resulted in a depletion of $\delta^{13}C$ (Raven et al. 2002), the opposite of what was seen in this experiment. The patterns of $\delta^{13}C$ values observed in this study were not likely driven by changes in the available DIC pool, as all treatments were homogenously distributed across a small area within the same kelp bed and experienced comparable flow at all depths. Increased water flow has been shown to decrease the $\delta^{13}C$ of marcoalgal tissue (France & Holmquist 1997), which could explain the decrease in $\delta^{13}C$ of the FR treatment. Increased flow due to the removal of adult fronds could have reduced the boundary layers

around the juvenile fronds of this treatment. Flow around the CC and CR treatments was likely similar, however, yet these treatments exhibited temporal differences in $\delta^{13}C$. Additionally, increased water flow can increase nitrogen uptake in *Macrocystis* (Hepburn et al. 2007), and there was no difference observed in %N across all treatments. This suggests that biomass may influence the isotopic shifts in juvenile blade tissues more than small-scale flow dynamics at this location.

The increased light availability and reduced self-shading associated with the removal of canopy biomass may have been insufficient to support growth in the CR and FR treatments due to a metabolic deficit in the juvenile fronds that may have been exacerbated by the absence of translocated resources from the canopy (Lobban 1978b, Wheeler & North 1980). It is important to note that the experimental removal of biomass was conducted during a time of peak canopy biomass at the study site (Reed & Foster 1984). Thus, it is possible that the growth rates observed during this experiment are not representative of regrowth that may occur in early spring. Large physical disturbances typically occur in winter along central and northern California, and rapid growth of fronds and juvenile sporophytes is commonly observed in spring, when light levels are higher under a reduced canopy (Dean & Jacobsen 1984, Graham et al. 1997, Stewart et al. 2009). As such, the observed mortality of the FR treatment and reduced growth of the CR treatment may be due more to the time of year than by the loss of biomass itself. Indeed, the canopy biomass of central California kelp forests is almost completely removed in the winter yet typically recovers by the summer and early fall (Reed & Foster 1984, Bell et al. 2015). This suggests that, while physical disturbance can dramatically impact growth rates, the increased light availability under a substantially reduced canopy can be sufficient to overcome the resource deficit in juvenile fronds at the end of winter.

Light availability and photosynthetic rates also influence the $\delta^{13}C$ ratio of autotrophic tissues (O'Leary 1988, Farquhar et al. 1989, Raven et al. 2002). Elevated light levels have been shown to increase the $\delta^{13}C$ ratio algae through the increased uptake of HCO_3^- to support the carbon requirements of high photosynthetic rates (Kübler & Raven 1995). Conversely, under light-limiting conditions macroalgae cannot meet the energetic costs of HCO_3^- uptake and defer to passive uptake of CO_2, which ultimately reduces $\delta^{13}C$ values (Wiencke & Fischer 1990, Hepburn et al. 2011). The seasonal light limitation caused by the dense surface canopy during this study likely limited all juvenile fronds. The reduction of self-shading by the removal of the canopy in the CR and FR treatments could have reduced the light limitation on these treatments relative to the C and CC treatments. Therefore, in the absence of translocation, juvenile fronds should have exhibited the most reduced $\delta^{13}C$ values in the C and CC treatments. The biomass-dependent enrichment of $\delta^{13}C$ observed across all treatments, however, is contrary to this expectation. Thus, the patterns of isotopic enrichment observed in this experiment were more strongly driven by processes other than light limitation and can be considered strong evidence of carbohydrate translocation from surface biomass.

Nitrogen isotopic ratios of macroalgae closely track differences in the available dissolved inorganic nitrogen pool and therefore may reflect changes in nutrient availability (Lapointe 1997, Cohen & Fong 2005). *Macrocystis* tissue nitrogen content typically lags environmental conditions by 1 mo (Zimmerman & Kremer 1986) and can have lower $\delta^{15}N$ values during times of high ambient DIN concentrations (Foley & Koch 2010). The observed declines in $\delta^{15}N$ across all treatments in July may therefore be partially driven by the selective uptake of ^{14}N under nutrient replete conditions (Foley & Koch 2010), but there were no consistent patterns between ambient DIN and $\delta^{15}N$ across treatments during the other months of the experiment.

All juvenile fronds sampled during this experiment were collected from near the bottom, at the depth with the highest DIN concentration observed throughout the study. Therefore, in the absence of other nitrogen sources, these fronds should have exhibited $\delta^{15}N$ values similar to the ambient signature of upwelled water in the Monterey Bay region, which is approximately 7.8‰ (Altabet et al. 1999, Foley & Koch 2010). The mean $\delta^{15}N$ value of the FR treatment was exactly 7.8‰, while the mean values of the other treatments ranged from 8.9 to 10.9‰ in order of increasing biomass. This biomass-dependent enrichment of $\delta^{15}N$ may represent increased translocation of nitrogen from surface waters. The high canopy biomass of the kelp forest at the time of the study could have drawn down the surface nitrogen pool, which may have enriched the $\delta^{15}N$ signature of canopy blades. The translocation of this enriched $\delta^{15}N$ nitrogen to juvenile fronds may explain the enrichment of juvenile fronds in the C, CC, and CR treatment above the background level of 7.8‰. Translocation of nitrogen from surface blades to juvenile blades may be the primary direction of export, as labeled ^{15}N export from the surface blades

of *Macrocystis* was found to only be in the direction of the holdfast and juvenile fronds (Hepburn et al. 2012). To accurately address possible links between ambient or depth-specific DIN concentration and patterns in $\delta^{15}N$, future studies should be conducted over longer time scales and across seasons of greater DIN variability. However, the consistent relationships between the enrichment of $\delta^{13}C$ and $\delta^{15}N$ suggest that enriched isotopic ratios in regions of new growth may be indicative of carbon and nitrogen exported from surface biomass.

CONCLUSIONS

Macrocystis is unique among kelps in that it has a highly differentiated morphology and numerous active meristems that span multiple biophysical environments. The results of this study show that the experimental removal of biomass directly reduced the growth rates of *Macrocystis* sporophytes in a central California kelp forest by reducing the translocation of carbon and nitrogen to juvenile fronds. Similarities to physiological mechanisms in vascular plants have long been observed in *Macrocystis* (Parker 1963, Wheeler 1980), and more recently this cross-systems approach has helped to shed light on important mechanisms regulating biomass dynamics in this species, such as progressive senescence (Rodriguez et al. 2013). This study confirms stable isotope ratios can reliably track resource translocation in macroalgae and may be influenced by physiological mechanisms similar to those of vascular plants (Werner & Gessler 2011). Further inquiry into physiological mechanisms analogous to vascular autotrophs, such as the rate at which juvenile fronds shift from resource sink to source (Keel & Schädel 2010), are likely to provide important information on the internal regulation of biomass dynamics within kelp-dominated systems. Given the strong relationship between isotopic composition of juvenile blades and biomass loss observed in this study, stable isotopes ratios can provide physiological insights that scale with large-scale patterns of *Macrocystis* biomass dynamics in wave-dominated systems.

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