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LETTER

RESOURCE TRANSLOCATION DRIVES $\delta^{13}C$ FRACTIONATION DURING RECOVERY FROM DISTURBANCE IN GIANT KELP, *MACROCYSTIS PYRIFERA*

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Resource allocation and translocation are fundamental physiological functions for autotrophs. The mobilization and use of resources drive population dynamics by regulating growth and recovery of individuals, but also influences ecosystem-level processes such as primary productivity and carbon cycling. This study provides the first observation of translocation-driven gradients of $\delta^{13}C$ in macroalgae, a critically important phenomenon recognized in vascular plants for decades. A ~10‰ $\delta^{13}C$ increase in new giant kelp (*Macrocystis pyrifera*) fronds relative to mature canopy blades was produced after 5 weeks following a biomass removal experiment, more than twice the variation typical for macroalgae. The observed $\delta^{13}C$ patterns are consistent with tissue enrichment following resource translocation in vascular plants. The analogous source-sink relationships and consistent translocation patterns in *Macrocystis* and vascular plants suggest that translocation of stored resources is critical for structuring productivity and recovery from disturbance in important, habitat-forming macroalgae such as kelps and fucoids.

The conspicuous surface canopy of *Macrocystis pyrifera* (L.) C. Agardh is sustained by the production and elongation of frond initials from the base of the sporophyte. Resources are translocated from the canopy to support the growth of new fronds, which suggests that canopy biomass may play a critical role in recovery from disturbance (Lobban 1978). Translocation of carbohydrates in excess of 1 $m \cdot h^{-1}$ has been estimated in *Macrocystis* (Schmitz and Srivastava 1979) and the cellular structures involved in translocation are considered analogous to the phloem system of vascular plants (Parker and Huber 1965). Isotopic discrimination during photosynthesis is commonly used to distinguish photosynthetic pathways in vascular plants (i.e., C_3 vs. C_4; Farquhar et al. 1989), although plants regularly display isotopic variation that cannot be explained by photosynthetic fractionation alone. Carbon allocation and resource translocation have been shown to drive isotopic gradients across tissue types in vascular plants (Hobbie and Werner 2004, Gessler et al. 2009, Werner and Gessler 2011). Unfortunately, the high temporal, spatial, and directional variability of such gradients among species (Gessler et al. 2004, 2007, 2008, Brandes et al. 2006, 2007, Rascher et al. 2010, Salmon et al. 2011) have precluded a thorough understanding of their physiological basis (Werner and Gessler 2011). One proposed mechanism is the continual offloading and reloading of sugars along the phloem pathway, which is believed to affect the isotopic composition of translocated materials, thereby enriching sink tissues (Van Bel 2003, Gessler et al. 2009). To my knowledge, no physiological studies have examined this phenomenon in macroalgae despite the well-documented importance of resource translocation in large foundation species, such as *Macrocystis* (Graham et al. 2007).

In *Macrocystis,* the direction of translocation is driven by the proximity of sinks to the source material (Schmitz and Srivastava 1979). Therefore, if the translocation mechanisms in *Macrocystis* are similar to those in vascular plants and the growth of new fronds is supported primarily by stored resources, frond initials should become enriched in $\delta^{13}C$ relative to canopy blades and the magnitude of this enrichment should be biomass dependent. 'This study was designed to quantify the effect of biomass loss on resource translocation to frond initials, which represent the recovery potential of an individual. Twelve *Macrocystis* sporophytes in Stillwater Cove, CA (12 m depth; 36°33′ 38.63″ N, 121°56′ 45.24″) were standardized to 11 non-senescent canopy fronds, in July 2012, by removing excess fronds 1 m above the holdfast. Mean water column temperature and NO_3 + NO_2 ranged from 10.5°C to 13.8°C and 8.1–20.6 $\mu mol \cdot L^{-1}$, respectively (Figs. S1 and S2 in the Supporting Information); such conditions are indicative of upwelling and considered optimal for growth in *Macrocystis* (Gerard 1982, Foley and Koch 2010). After standardization, sporophytes were given 1 week to equilibrate and were then trimmed to a randomly assigned frond number ranging from 0 to 11 fronds per sporophyte (the sporophyte with 9 fronds was lost). Frond number was converted to biomass using a predictive length to biomass model (Fig. S3 in the Supporting

Information) and a site-specific mean frond length to improve ecological relevance. Biomass production was calculated by converting the number of new fronds (>1 m in length) produced per sampling period to biomass. All sporophytes were sampled before biomass manipulation and 1, 3, and 5 weeks following manipulation. Due to the high diel variability in isotopic signatures observed in vascular plants (Werner and Gessler 2011), tissue samples were collected mid-morning during each sampling period to standardize for such variability. Blades were collected from mature and initial fronds and cleaned of epiphytes. A 2–5 g subsample was dried at 60°C for 72 h, homogenized in a ball mill and analyzed for $\delta^{13}C$, $\delta^{15}N$, %C and %N at the University of Wyoming Stable Isotope Facility. Changes in chemical constituents of blade tissue were used to identify changes in bulk composition (%C, %N) and $\delta^{13}C$ and $\delta^{15}N$ were used to examine physiological changes between tissues. Given the direct relationship between resource translocation from mature blades to frond initials and the biomass-dependent nature of resource availability, ANCOVA was used to detect significant interactions between tissue type (mature vs. initial fronds) and sporophyte biomass. The interaction term provides the most accurate metric to test for translocation and was therefore the focus of this study. A tissue effect would likely indicate biomass-independent ontogenetic differences in tissue chemistry and a biomass effect would likely indicate changes driven by growth rates, neither of which were the specific focus of this study. Relationships between additional variables were examined using linear least-squares regression to elucidate drivers of change in tissue chemistry.

Heterotrophic tissues (sinks), such as stems and roots, are typically ^{13}C-enriched relative to leaves (sources) in vascular plants (Cernusak et al. 2009). The magnitude of these gradients is typically 1‰–3‰ (Cernusak et al. 2009, Salmon et al. 2011), but daily variations of up to 14.8‰ have been observed in leaf $\delta^{13}C$ (Werner and Gessler 2011). In this study, the dominant sinks (initials) in *Macrocystis* were enriched in $\delta^{13}C$ on average up to 10‰ relative to canopy blades (sources) over 5 weeks (Fig. 1). The magnitude of this shift was directly proportional to biomass loss and was driven by significant interactions between tissue type and remaining biomass. This pattern suggests that carbon allocation to frond initials is regulated by the amount of extant biomass (Table S1 in the Supporting Information). $\delta^{13}C$ enrichment

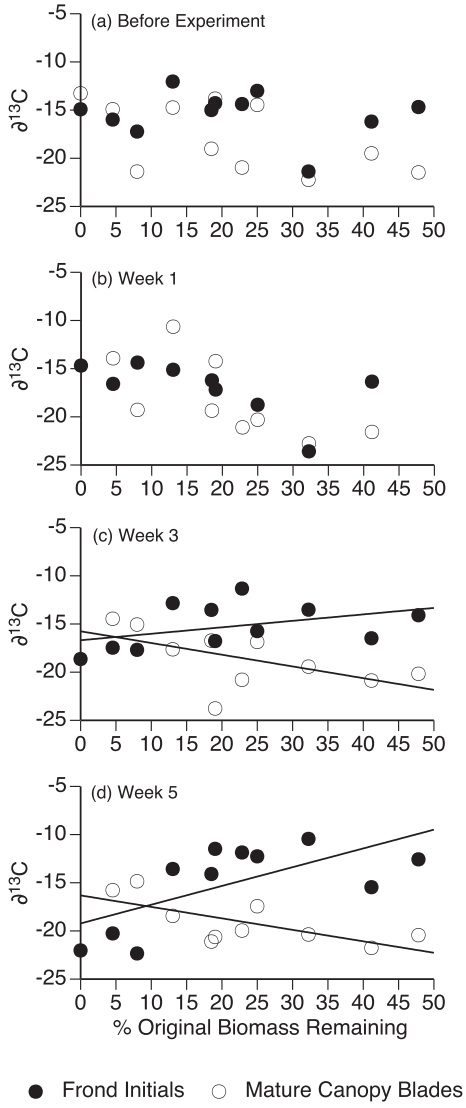

FIG. 1. Comparison of $\delta^{13}C$ in frond initials and mature canopy blade tissue. Tissue comparisons were made against the percentage of original biomass left on individual sporophytes after experimental manipulations prior to week 1. (a) before biomass manipulations, (b) week 1 of the experiment, (c) week 3, and (d) week 5 (end of the experiment). Tissue chemistry comparisons were made using an analysis of covariance (ANCOVA) to detect significant interactions between tissue type and remaining biomass. Trend lines were used to help illustrate the interaction terms and are only presented for time periods in which a significant interaction effect was detected and not when individual factors were significant (biomass, week 1, $P = 0.009$). Such a distinction was made because only significant interaction terms relate directly to translocation of resources. The trend lines were not intended to signify significant regressions, see the text for details. Significant interaction terms were only detected in c ($P = 0.025$) and d ($P = 0.003$).

was dependent on the production of new biomass; no new biomass was produced during week 1 and therefore no difference in $\delta^{13}C$ values was observed between initial and mature blades until week 3 (Fig. 1, a and b). However, a significant biomass effect was detected in week 1 (Table S1). This effect was likely strengthened by tradeoffs between growth of mature blades and translocation to frond initials that occurred in response to biomass loss. Sporophytes that lost more biomass likely used existing resources to produce more canopy biomass during week 1 instead of translocating resources to frond initials, reducing their overall depletion in $\delta^{13}C$. This pattern is consistent with what was observed in weeks 3 and 5, but no interaction term was detected because the $\delta^{13}C$ signature of the frond initials had not yet changed significantly. By week 3, frond initials had slightly higher $\delta^{13}C$ on sporophytes with greater biomass (Fig. 1c; Regression: $F_{(1,9)} = 1.946$, $P = 0.196$, $r^2 = 0.178$ (initials); $F_{(1,8)} = 4.018$, $P = 0.080$, $r^2 = 0.334$ [mature]). By week 5, frond initials on sporophytes possessing <10% of their original biomass had lower $\delta^{13}C$ values relative to mature blades, while initials on sporophytes with >10% of original biomass had significantly higher $\delta^{13}C$ values (Fig. 1d; Regression: $F_{(1,9)} = 7.244$, $P = 0.025$, $r^2 = 0.446$ (initials); $F_{(1,8)} = 7.710$, $P = 0.024$, $r^2 = 0.491$ [mature]). This observed enrichment of frond initials (Fig. 1), the primary sinks in this experiment, is indicative of increased translocation of ^{13}C-enriched compounds from mature blades to frond initials and is highly consistent with the fractionation observed during translocation in vascular plants. Interestingly, C/N ratios did not produce this pattern suggesting that resource translocation within *Macrocystis* is best tracked by changes in $\delta^{13}C$ (Table S1).

Stored reserves are critical for recovery from disturbance in autotrophs. Maunoury-Danger et al. (2010) blocked the supply of carbon from leaves to stems in sessile oaks, *Quercus petraea*, and found that tissues below the block became enriched in $\delta^{13}C$ due to reliance on starch reserves. Similarly, frond initials on *Macrocystis* sporophytes with <10% of their original biomass lacked access to reserves and consequently had lower $\delta^{13}C$ values, likely due to increased uptake of CO_2 relative to HCO_3^- as photosynthetic substrate (Raven et al. 2002). Depletion in $\delta^{13}C$ of frond initials directly reduced biomass production thereby inhibiting recovery (Fig. 2a; Regression: $F_{(1,2)} = 370.852$, $P = 0.003$, $r^2 = 0.995$). Conversely, initials on sporophytes with >10% of their original biomass were actively enriched in $\delta^{13}C$ through the translocation of stored reserves.

Mobilization of stored carbohydrates has been well documented in kelps (Black 1954, Chapman and Craigie 1978, Gagné et al. 1982). Excess photosynthate is typically stored as laminarin and converted to mannitol before being translocated to areas of active growth (Kremer 1981). Stored starches can be enriched up to 4‰ in $\delta^{13}C$ relative to recently assimilated carbohydrates (Gleixner et al. 1998, Tcherkez et al. 2004) and result in $\delta^{13}C$ enrichment of sink tissues when metabolized in vascular plants (Damesin et al. 1998, Badeck et al. 2005, Maunoury-Danger et al. 2010). Translocation of mannitol to frond initials should therefore drive an increase in $\delta^{13}C$ and in

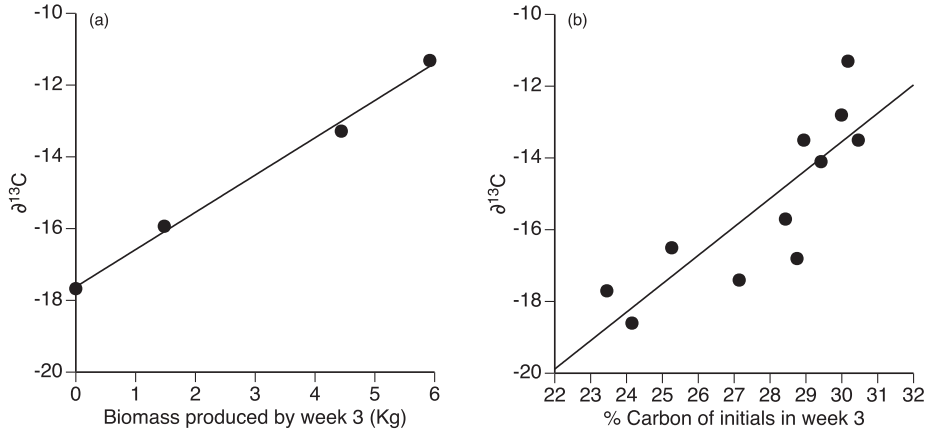

FIG. 2. (a) Production of biomass from weeks 1 to 3 of the experiment compared with the $\delta^{13}C$ enrichment of frond initials during week 3. Data points represent the mean of independent plants that produced identical biomass during the time period. The rough estimates of biomass used in this experiment do not capture small variability in biomass and are based off the number of fronds produced; in cases where sporophytes produced the same number of fronds, their biomass production was identical. As such, the results of this analysis are non-conservatively biased, however, the direction and magnitude of the relationship are more important than the predictive abilities of the model for this particular study. From left to right, the means were calculated from three, four, and three sporophytes, respectively; data for the plant that produced ~6 kg ($\delta^{13}C = -11.3$) are for a single sporophyte. Standard error for all mean values was <0.72‰ $\delta^{13}C$. Error bars were not visible on the data points and therefore omitted from the figure. The line represents best fit (Least-squares linear regression: $\delta^{13}C = -17.623$ (±0.203) + 1.038 (±0.054) * biomass; $F_{(1,2)} = 370.852$, $P = 0.003$, $r^2 = 0.995$). (b) Relationship between $\delta^{13}C$ and% bulk carbon [C] in frond initials during week 3. The line represents best fit (Least-squares linear regression: $\delta^{13}C = -37.306$ (±4.779) + 0.792 (±0.171) * [C]; $F_{(1,9)} = 21.431$, $P = 0.001$, $r^2 = 0.704$).

overall% carbon (C). This hypothesis is supported by the ~7% increase of carbon observed in enriched initials (Fig. 2b; Regression: $F_{(1,9)} = 21.431$, $P = 0.001$, $r^2 = 0.704$). The $\delta^{13}C$ of initials ranged from −11.3‰ to −18.6‰ with corresponding [C] of 30.5% and 23.5%, respectively. A $\delta^{13}C$ shift of ~8‰ is very large and such variability has significant implications for understanding trophic interactions in algal-dominated systems (Dethier et al. 2013). Additionally, the strong correlation between biomass production during weeks 1–3 and $\delta^{13}C$ enrichment of frond initials (Fig. 2a) could be explained by a rapid sink-to-source transition in juvenile fronds. Such transitions have been observed in new tree leaves (Keel and Schädel 2010) and would allow surplus resources to be diverted to adjacent frond initials.

This study provides the first empirical evidence of translocation-mediated recovery in macroalgae and a novel approach to understanding the physiological mechanisms behind it. To validate the hypothesized mechanisms in kelps, future research should develop compound-specific stable isotope values for mannitol and laminarin. Sporophyte biomass is one of the most important factors driving variability in primary production of *Macrocystis* populations (Reed et al. 2008, 2011) and an improved understanding of isotopic fractionation during translocation in kelps will provide critical insights to the processes governing population dynamics and productivity in *Macrocystis* forests worldwide. The importance of resources stored within active biomass also has substantial implications for *Macrocystis* harvesting and farming. While further work is necessary to determine optimum harvesting strategies and direct impacts of harvesting, the results of this study suggest that considering the percentage of biomass removed from individual sporophytes is important for maintaining sustainable harvests. Finally, given the complex source-sink relationships, high rates of translocation, and similarities to vascular plants, *Macrocystis* may serve as a model species to elucidate the basic physiological mechanisms regulating post-photosynthetic fractionation in marine and terrestrial autotrophs.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. Relationship between ocean temperature and dissolved inorganic nitrogen concentration (DIN) (NO_2 + NO_3) for Stillwater Cove, Pebble Beach, California. Temperature data were obtained from an array of four Onset StowAway Tidbits (±0.2°C) positioned at 0.5, 4, 8, and 13.5 m along a mooring line at the study site. Nutrient data were obtained from 50 discrete water samples collected throughout the water column, adjacent to the temperature loggers between 2010 and 2012. Water samples were frozen after collection and analyzed at the Oceanic Nutrient Laboratory at the University of South Florida. The line represents best fit (Least-squares linear regression: DIN = 59.68 (±2.94) + −3.75 (±0.25) * temperature; $F_{(1,48)}$ = 216.346, $P \ll 0.001$, $r^2 = 0.818$).

Figure S2. Time series from the Stillwater Cove mooring from July 13 to August 22, 2012. (a) Daily mean water column temperature derived from the measurements collected at five minute intervals from 0.5, 4, 8, and 13.5 m depth, (b) daily mean (NO_2 + NO_3) concentration calculated using the linear relationship presented in Fig. S1.

Figure S3. Frond biomass as a function of frond length determined from 39 individual fronds collected from adult *Macrocystis* sporophytes at the study site between March and August 2012. Intact fronds were collected by divers from randomly selected sporophytes, rehydrated to a constant weight at the lab, and wet weight was determined to the nearest 0.01 kg. The equation derived from the quadratic fit was used to calculate biomass in this experiment using a site-specific mean frond length calculated from 85 randomly selected fronds >1 m between 2010 and 2012. The line represents best fit (Least-squares polynomial regression: Biomass = 0.182 (±0.123) − 0.01 (±0.044) * frond length + 0.017 (±0.003) * frond length^2; $F_{(2,36)}$ = 232.119, $P = \ll 0.001$, $r^2 = 0.924$).

Table S1. Summary statistics for ANCOVAs run against all five examined chemical variables. Degrees of freedom are reported in parentheses for all dates for $\delta^{13}C$ and are consistent for all other variables tested. For all factors except tissue, deviations from 21 degrees of freedom are due to sample loss. Bold values indicated $P < 0.05$.