

CARBON SHARING THROUGH PHYSIOLOGICAL INTEGRATION IN THE THREATENED SEAGRASS *HALOPHILA JOHNSONII*

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ABSTRACT

Carbon sharing among ramets of the clonal marine angiosperm *Halophila johnsonii* Eiseman was investigated in plants collected from Biscayne Bay, Florida, USA, and cultured in a seawater-supplied greenhouse facility in Wilmington, North Carolina. Different ramets along four-ramet segments (genets) were shaded for 3 d. At the end of the shading period, neighboring ramets were supplied with $\text{NaH}^{14}\text{CO}_3$ ($5\mu\text{Ci}$) to determine rates of carbon fixation and patterns of carbon allocation. The direction and degree of carbon support among ramets were determined by employing whole-plant autoradiography and scintillation spectrophotometry. Genets with shaded ramets were not statistically different in terms of carbon uptake per gram dry weight than genets with un-shaded ramets, suggesting that genet carbon uptake is unaffected by shading. Autoradiograms indicated a trend towards *H. johnsonii* allocating more carbon to younger parts of the genet. However, scintillation analysis showed no significant directionality in photosynthate allocation to ramets with respect to age or shading. Photosynthate was allocated to ramets proportional to their proximity to the source ramets rather than because of the condition of the neighboring ramets. The fast turnover and short lived deterministic leaves of *H. johnsonii* suggest that no advantage may be gained by selectively supporting ramets based on condition or age. The physiological strategy we describe indicates that *H. johnsonii* does not support stressed ramets along its genet. Along with this species' low capacity for storage, our data suggest that vegetative growth over large unsuitable patches may be unlikely and that *H. johnsonii*'s ability to recover from widespread habitat loss may be limited.

Halophila johnsonii Eiseman is the most endemic seagrass known (Virnstein et al., 1997), having been observed only from Sebastian Inlet (27°51'N, 80°27'W) south to Virginia Key in Biscayne Bay (25°45'N, 80°07'W) on the east coast of Florida, United States (Eiseman and McMillan, 1980). It often occurs intertidally and in waters subject to high sedimentation and moderate currents where the distribution of other marine plants is restricted (Dawes et al., 1989; Virnstein et al., 1997; Durako et al., 2003). *Halophila* species are relatively small plants with fast turnover rates (Kenworthy et al., 1989) and 2–3 orders of magnitude less biomass per unit area than other seagrass genera (Terrados et al., 1999). The growth habit of *H. johnsonii* is rhizomatous with a single root and leaf pair occurring at each node and meristems may arise at the base of each leaf pair. Kenworthy (1993) found that meristem densities of *H. johnsonii* are 1–2 orders of magnitude higher than for larger seagrasses indicating that frequent branching may occur. This branching appears to be its only means of reproduction as no male flowers have been observed. These characteristics suggest that *H. johnsonii* is a poor competitor with other marine flora under stable conditions, which may contribute to the fact that *H. johnsonii* is one of the rarest seagrasses known. In 1998, *H. johnsonii* gained status as a Threatened Species under the Endangered Species Act (US Federal Register, 1998). It is the only marine plant so listed.

Halophila johnsonii, like all seagrasses, is clonal, which refers to the phenomenon of many semi-independent units (ramets) acting together as a single organism (genet) (Cook, 1983). Clonal plants are, to varying extents, physiologically integrated (see Magda et al., 1988). Consequently, resource-starved ramets of clonal plants may be supported by unstressed ramets (Noble and Marshall, 1983; Watson, 1984; Slade and Hutchings, 1987; Oborny et al., 2001). Shading or removing leaves from ramets with intact rhizome connections to surrounding untreated ramets results in no significant change in growth rate from that of untreated plants for seagrasses adapted to live in stable conditions (Tomasko and Dawes, 1989; Terrados et al., 1997). In contrast, plants with shaded or defoliated ramets and severed rhizomes exhibited slower growth when compared to treated plants with un-severed rhizomes. Therefore, connection to other ramets may ameliorate shade stress on the genet.

This phenomenon of unstressed ramets supporting stressed ramets suggests that supported ramets provide benefits that outweigh their costs (Caraco and Kelly, 1991; Stuefer et al., 1994). If physiological integration does not contribute to the fitness of the organism, it may become detrimental. Consequently, some clonal plants facultatively modify their integration characteristics. Ramet interdependence is high in plants growing in patchy areas and low in plants growing in environments with homogeneous resource availability (Tomasko and Dawes, 1990; Kembell et al., 1992). This phenomenon occurs via a division of labor strategy where different ramets are responsible for different types of resource acquisition (Callaghan, 1976). Additionally, plants may respond to habitat patchiness with changes in growth and physiology to exploit favorable conditions or to escape unfavorable ones (Sutherland and Stillman, 1988).

Halophila johnsonii genets occasionally encounter heterogeneous light availability. Sediment overlay, epiphyte growth, self-shading or shading by other competing marine flora may stress ramets. Because its ramets are short lived and have little capacity for storage, carbon deficiencies due to shading may be particularly detrimental. Under these conditions, it may be beneficial for unshaded ramets to modify their physiology in order to support shaded ramets.

Clonal plants employ different physiological strategies when competing for resources. These strategies are described as either "guerilla" or "phalanx" (Lovett-Doust, 1981). Guerilla competition is a strategy where plants exhibit fast growth to quickly exploit resources. In this case, older ramets tend to provide support for new ramets, allowing the plant to direct resources to points of new growth, while providing little support to extant biomass. Slow growth and a reliance on new ramets to support existing biomass characterize the phalanx strategy. Stable-selected plants tend to employ the phalanx strategy, while opportunist species tend to exhibit the guerilla strategy. Because *H. johnsonii* exhibits fast growth and a high turnover rate, it may be beneficial to direct support selectively to new growth.

Here we investigated how *H. johnsonii* responds to shading with respect to its physiological-integration attributes. We hypothesized that *H. johnsonii*: (1) is physiologically integrated; (2) would undergo a physiological response to shading in that more carbon would be assimilated by unshaded ramets in stressed genets than in unstressed genets; (3) would respond to shading of ramets via support from un-stressed ramets; and (4) would exhibit a guerilla strategy in that it would allocate support from older ramets to newer ramets.

METHODS

PLANT MATERIALS.—Sediment plugs containing *H. johnsonii* were collected in August 2001 from the lower intertidal to upper subtidal zones in Haulover Park (25°55'N, 80°04'W) in northern Biscayne Bay, Florida, United States, and placed in 10-cm³ peat-pots. The pots were transported in coolers to the Center for Marine Science in Wilmington, North Carolina, and placed in 50-L aquaria in a seawater-supplied greenhouse. Prior to each replicated experimental manipulation, 24 genets consisting of an apical ramet and three older ramets were excised from genets in the peat pots and transplanted into 12 plastic runway-type troughs containing fine-sand sediment collected in Bradley Creek at Wrightsville Beach, North Carolina. Care was taken to minimize root damage. Constituent plant parts are described in terms of ramets with the youngest tissues, the apical meristems, labeled as (RA), and ramets increasing in number from R2 (younger) to R4 (oldest) and rhizomes increasing in number from RzA-2 (youngest rhizome, connecting RA to R2) to Rz3-4 (oldest rhizome, connecting R3 to R4; Fig. 1).

EXPERIMENTAL DESIGN.—On the day following transplanting, genets were randomly assigned to different treatment regimes in order to determine the effects of ramet shading on carbon allocation. Twelve shaded treatments with 12 control treatments were constructed for each of the 5 replicate runs (Fig. 2). For the shaded group, three plants had the apical-ramet (RA) shaded and had ramet-two (R2), ramet-three (R3), or ramet-four (R4) supplied with radioactive carbon. Three plants had (R2) shaded and either (RA), (R3), or (R4) supplied with radioactive carbon. Three plants had (R3) shaded and either (RA), (R2), or (R4) supplied with radioactive carbon. Three plants had (R4) shaded and each plant had either (RA), (R2), or (R3) supplied with radioactive carbon. A corresponding control group was assembled for each replicate and was manipulated in exactly the same way, but without shading.

Selected ramets were shaded for 2 d while genets were rooted in sediment. Shading was performed by placing 1-dram glass vials covered with black electrical tape over the leaves of selected ramets. The shading vials were fixed to four 4-cm plastic stakes attached with rubber bands. The plastic stakes held the vials approximately 5 mm above the sediment surface allowing water circulation and gas exchange around the ramet while minimizing light exposure. Measurements with a cosine-corrected quantum sensor indicated that shaded vials

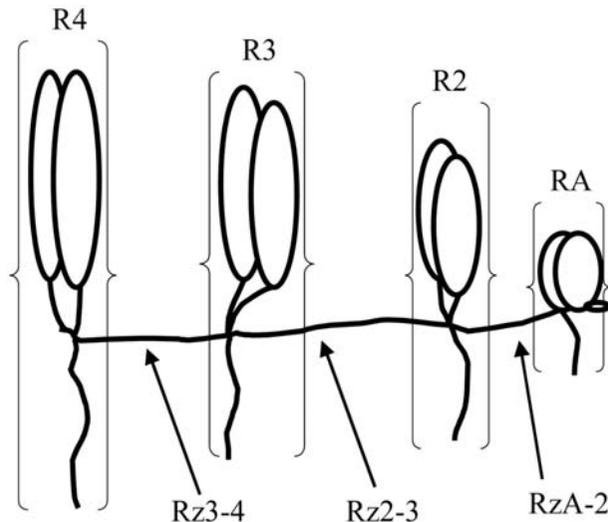


Figure 1. Diagram of a *Halophila johnsonii* genet. R4 = Ramet 4, R3 = Ramet 3, R2 = Ramet 2, RA = Apical Ramet, Rz3-4 = Rhizome 3-4, Rz2-3 = Rhizome 2-3, RZA-2 = Rhizome Apical-2.

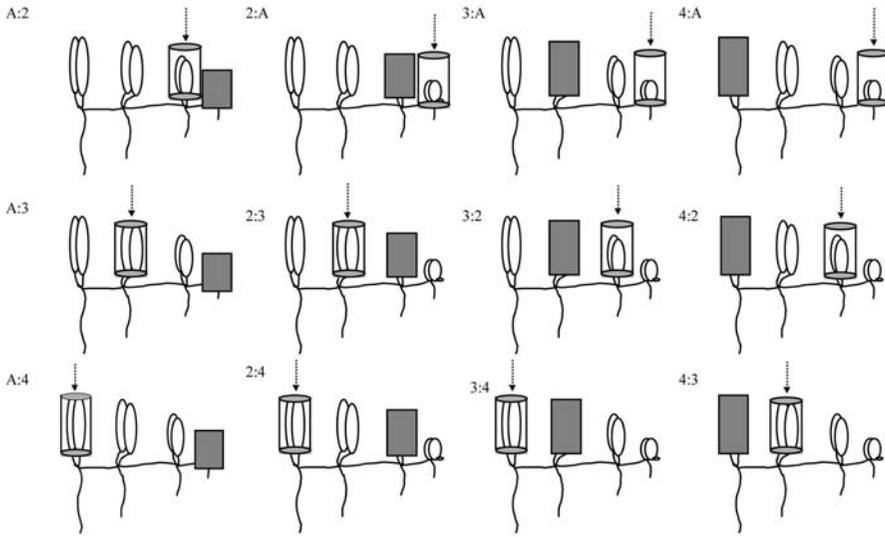


Figure 2. Experimental treatment regimes. Darkened box represents shading; cylinder with dashed arrow represents ^{14}C supplied. (A:2) RA shaded, R2 supplied; (A:3) RA shaded, R3 supplied; (A:4) RA shaded, R4 supplied. (2:A) R2 shaded, RA supplied; (2:3) R2 shaded, R3 supplied; (2:4) R2 shaded, R4 supplied. (3:A) R3 shaded, RA supplied; (3:2) R3 shaded, R2 supplied; (3:4) R3 shaded, R4 supplied. (4:A) R4 shaded, RA supplied; (4:2) R4 shaded, R2 supplied; (4:3) R4 shaded, R3 supplied. For each treatment a corresponding control treatment was performed whereby control genets were manipulated in exactly the same manner, but without shading.

reduced photosynthetically active radiation (PAR) by 95%. The control vials (no black tape) reduced PAR by < 5%.

After sunset on the second day of shading, all plant tissue was carefully scraped by hand to remove epiphytes. In order to minimize breakage of the plants during manipulation, all shading vials were detached and plants were carefully removed from the sediment and placed in filtered seawater inside of 300 ml Magenta™ Tissue Culture flasks. Previously shaded ramets were then re-shaded by placing aluminum foil envelopes over each leaf-pair. The foil envelopes were constructed with a small square piece of commercially available aluminum foil and a small piece of nylon screen. The screen was placed inside the foil envelope to prevent collapse of the envelope which could result in damage to plant tissue. A foil envelope and nylon screen was placed in the bottom of each control group flask to control for any material effects. Each flask with newly re-shaded genets and their corresponding controls were then placed together in a water bath inside a large 20-L Nalgene™ tray to maintain ambient temperatures.

Ramets to be supplied with radioactive carbon were isolated from other ramets by sealing them inside 5-cm diameter incubation chambers constructed of clear silicone tubing (I.D. 12.5 mm, O.D. 17.0 mm) with 90% PAR transmittance. Each chamber had a gray butyl serum stopper at one end and a split 15-d silicone stopper placed around the petioles of the experimental ramet at the other end. The inside of the split silicone stopper was lightly covered with silicone grease to prevent leakage. Care was taken to ensure that no grease covered any parts of the plant other than those in direct contact with the silicone stopper. Isolated leaf-pairs were incubated in 4-ml of an Instant Ocean™ medium with 0.17 g $\text{NaHCO}_3 \text{ L}^{-1}$ added to raise the bicarbonate concentration at pH 8 from 2.0 mM to 4.0 mM inside the chamber. The bicarbonate level was raised to ensure no carbon limitation was encountered during the experimental incubations. The salinity of the incubation medium was 35. In order to maintain constant light during incubations, the tray containing vials and plants was transferred to the laboratory immediately prior to administration of radioactive carbon. During ^{14}C incubation, plants were irradiated by approximately $600\text{-}\mu\text{M}$ quanta $\text{m}^{-2}\text{s}^{-1}$ PAR from two 500-w halogen

light fixtures arranged at opposite ends of the tray. All treatments for each replicate were incubated at approximately the same time of day (between 1000–1400). Replicates were performed 1 wk apart. ^{14}C in the form of solid $\text{NaH}^{14}\text{CO}_3$ (11.4 mCi mM^{-1} , $1.0 \text{ mCi } 7.4 \text{ mg}^{-1}$) was dissolved into 100 ml of deionized water to yield $10 \mu\text{Ci ml}^{-1}$. A 1-ml syringe was then used to inject $50 \mu\text{l}$ of this medium, with an activity of $5\text{-}\mu\text{Ci}$, through the serum stopper into the 4.0 ml Instant Ocean/bicarbonate solution inside of the sealed tubing yielding a specific activity of $1.2 \mu\text{Ci mM}^{-1}$ bicarbonate.

At initiation of the incubation, a $50\text{-}\mu\text{L}$ aliquot from each chamber was immediately taken and placed into a separate plastic scintillation vial. One drop of 1.0 N NaOH solution was added to the aliquot to raise the pH of the aliquot to > 9.0 to ensure no loss of carbon due to off gassing. The genets were incubated in the light for 2 hrs at which point a second $50\text{-}\mu\text{L}$ aliquot was taken. Both media aliquots were frozen at -25°C and stored until analysis by scintillation spectrophotometry to determine the total amount of radioactive carbon taken up by the plant.

AUTORADIOGRAPHY.—At the end of the 2-hr incubation, each genet was carefully removed from the culture media. The entire plant was then rinsed with deionized water and frozen at -25°C for at least 1 hr in order to cease biological activity. Genets were then placed in a herbarium press and dried in a fume hood for 3 d. To visualize the translocation of photosynthate from the ^{14}C incubations, dried plants were placed onto sheets of Kodak Biomax™ MR Brand x-ray film. The plants and film were then placed in a lightproof exposure holder and stored at -25°C for an exposure time of 1 wk (after Gahan, 1972).

When x-ray film is subjected to radiation it becomes exposed. Portions of the plant with high concentrations of radioactive carbon expose the film to a greater degree than portions with little or no radioactive carbon. More exposure equates to a darker image on the developed film. This attribute allowed the direction and magnitude of the translocation of radioactive photosynthate to be qualitatively visualized. At the end of the exposure period, film was developed and the resulting image was scanned into a computer graphics program and compared to scans of the pressed plants.

SCINTILLATION ANALYSIS.—After autoradiographic analysis, plant tissues were separated into constituent parts and each fraction was weighed. Tissue samples were then placed in plastic 20-ml scintillation vials. Plant samples were prepared for scintillation spectrophotometric analysis by digesting with Scintigest™ brand tissue digester for 24 hrs then bleaching with a commercially available 4% sodium hypochlorite solution (see Sun et al., 1988). Five ml of Scinti-safe Gel™ scintillation cocktail was then added to each sample. The samples were then analyzed for quantity of radioactive disintegrations via a Wallac Rack-Beta™ scintillation counter. Values for radioactive carbon for the incubation media, in terms of disintegrations per minute, were obtained from the final aliquot and subtracted from the first. These values were multiplied by the specific activity of the media and then converted into grams carbon assimilated per gram dry weight of tissue supplied per hour to allow us to determine the total amount of radioactive carbon taken up by the plant.

STATISTICAL ANALYSES.—Allocation was described in terms of percent of total radioactive carbon that was translocated from the supplied leaf pair, or the relative amount of radioactive carbon that moved from the supplied ramet to a constituent part. In order to detect any differences in relative radioactive carbon allocation between treatment and control groups, a series of one-way analyses of variance (ANOVA) were performed on the scintillation data, with the dependent variable being the relative amount of radioactive carbon present in the constituent part and the independent variable being the distance of that constituent part to the supplied ramet. Where the data failed to exhibit equal variance, a Kruskal-Wallis One-Way Analysis of Variance on Ranks (K-W ANOVA) was performed. Carbon allocation for each constituent part in the experimental group was compared to its corresponding control constituent part. To determine whether supplied tissue took up more or less carbon in genets with one ramet shaded vs unshaded control genets (i.e., whether supplied tissues undergo any facultative increase in carbon uptake in response to shading on other parts of the genet),

values for grams carbon taken up per gram dry-weight of supplied leaf tissue of control genets were compared to the uptake of supplied leaves on genets subjected to having a ramet shaded using ANOVA.

The magnitude of directionality of carbon translocation from the oldest ramet to younger tissues was compared to that of the youngest ramet to older tissues using simple regression analyses: percent carbon allocation was plotted against distance, with distance described in terms of constituent parts away from the supplied ramet. The slopes of the two regression lines were then compared via analysis of covariance (ANCOVA; Sokal and Rohlf, 1995).

RESULTS

TRANSLOCATION AMONG RAMETS.—Percentages of carbon translocated were highly variable in both the experimental and control groups. Uptake values were also highly variable, as were data describing allocation to R4 (oldest ramets) vs RA (youngest ramets). For all treatments, carbon was translocated among ramets at a rate over two orders of magnitude greater than would be expected for simple Fickian diffusion of sucrose: $t_{c/2} = L^2/D_s$, where t_c is the time in seconds a solute takes to achieve half of its original concentration at a given distance L and D_s is the diffusion coefficient of the solute (Taiz and Zeiger, 2002). Sucrose has a diffusion coefficient (D_s) of 0.71×10^{-9} and the average length of the genets was ~ 4.0 cm. Therefore, one would predict a simple diffusion rate of approximately 26 d for half of the concentration of sucrose to disperse through the entire genet. Photosynthate was translocated throughout *H. johnsonii* genets over the course of 2 hrs in our experiments, suggesting physiological integration.

Ramet shading was not observed to have any significant effect on magnitude of carbon uptake or direction of carbon allocation. Scintillation analysis showed that the control Rz2-3 exhibited a significantly higher percent radioactive carbon allocation when R2 was shaded and RA supplied, as did Rz3-4 in the shaded group when R3 was shaded with R4 supplied. These findings were contradictory in terms of allocation based on proximity to the shaded ramet, proximity to supplied ramet, and age of constituent part. Additionally, no other significant difference in carbon allocation between shaded and unshaded constituent parts was observed. Autoradiography exposures (see Figs. 3–6) showed a slight trend of plants selectively supporting younger tissues (Fig. 3A panels 1,2; 3B panels 1–4; Fig. 5B panels 1–4; Fig. 6A panels 1–4; 6C panels 1–4). However, neither scintillation analysis nor regression analysis supported this observation.

APICAL RAMET SHADED.—In plants with RA shaded and R2 supplied, autoradiographic analysis (Fig. 3A panels 1, 2) showed most translocated radioactive carbon moved to the shaded RA. These results were similar to the control group (Fig. 3A panels 3, 4). Scintillation analysis showed that less carbon was translocated to the shaded RA than the control RA (Fig. 3A panel 5). One-way ANOVA indicated no significant differences in the relative percent carbon allocation between the shaded constituent parts and their corresponding controls.

In plants with RA shaded and R3 supplied, autoradiographic analysis (Fig. 3B panels 1, 2) indicated that most translocated radioactive carbon moved to adjacent leaf-pairs, rhizomes, and the apical ramet, which were all equally exposed. Control plants showed a similar pattern of exposure (Fig. 3B panels 2, 3). Scintillation analysis indicated that less radioactive-carbon was translocated to the shaded RA than the control RA (Fig. 3B panel 5). However, there were no significant differences in the

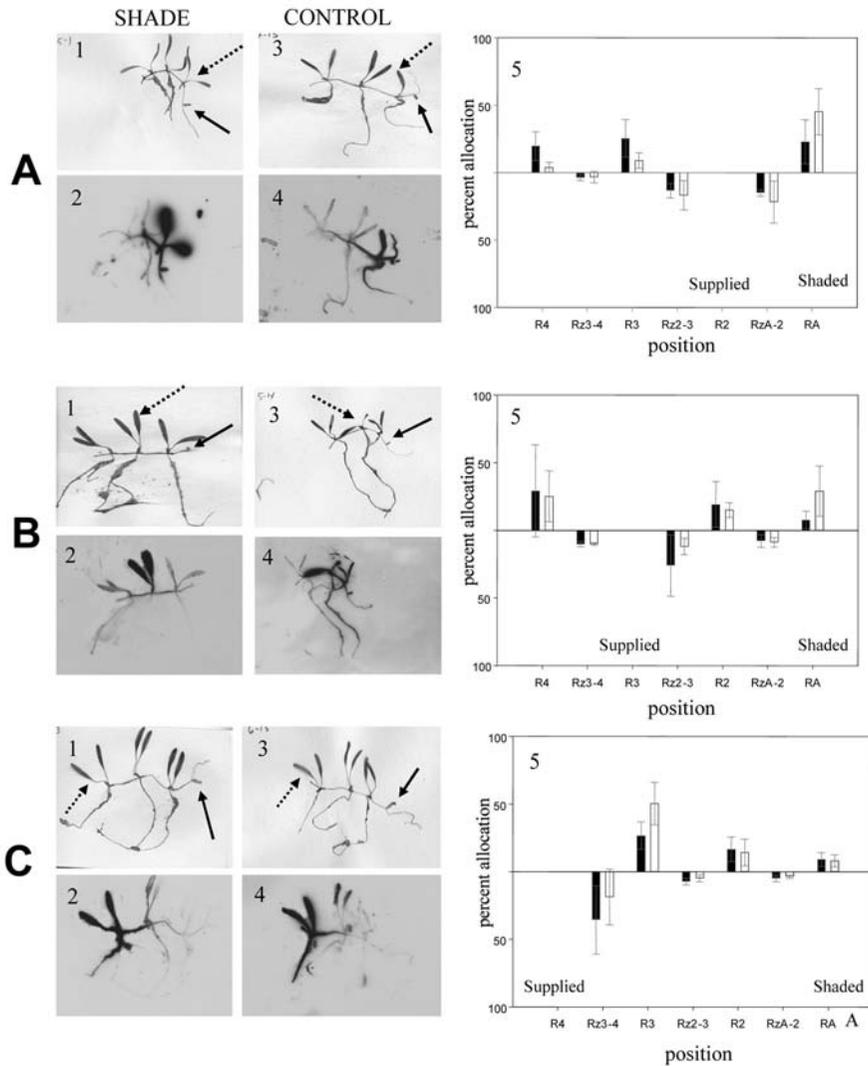


Figure 3. (A) Apical ramet shaded, ramet two supplied with radioactive carbon, (B) apical ramet shaded, ramet three supplied, (C) apical ramet shaded, ramet four supplied. Photographs of shaded plants are positioned on the left (1, 2) and control-plant images are located on the right (3, 4). Pressed plants are in the top panels (1, 3) and radiograms in the lower panels (2, 4). Histograms showing allocation of percent-translocated carbon are to the right of the photographs (5). Results are described as allocation to constituent parts. Translocated radioactive carbon values for above-ground ramets are represented above the x-axis and values for below-ground rhizomes are represented below this axis. Hatched arrow = ¹⁴C supplied, solid arrow = shaded. Filled bars represent experimental data, open bars represent control data. Error bars are standard deviation.

relative percent carbon allocation between the shaded constituent parts and their corresponding controls (ANOVA: $P > 0.05$).

In plants with RA shaded and R4 supplied, autoradiographic analysis indicated less radioactive carbon moved to the shaded RA (Fig. 3C panels 1, 2) than in the control RA (Fig. 3C panels 3, 4), but scintillation analysis showed that a similar amount of translocated radioactive carbon moved to the shaded RA and the corresponding

controls (Fig. 3C panel 5). There were no significant differences in the relative percent carbon allocation between the shaded constituent parts and their corresponding controls (ANOVA: $P > 0.05$).

RAMET 2 SHADED.—In plants with leaves on R2 shaded and RA supplied, autoradiographic analysis (Fig. 4A panels 1, 2) indicated that most translocated radioactive carbon moved to the youngest ramet and its corresponding rhizomes in both the shaded plant and the control (Fig. 4A panels 3, 4). Scintillation analysis showed that in the shaded group, most translocated radioactive carbon moved to RA followed by the shaded R2 (Fig. 4A panel 5). In the control group, most translocated radioactive carbon was found in R2 followed by RZA-2, in contrast to the autoradiogram for the shaded group which showed most radioactive carbon remaining in or near RA (Fig. 4A panels 1, 2). The RZ2-3 on the control plants had a significantly higher percentage carbon allocation than did the shaded, with 8% and 2% respectively (ANOVA: $F_{1,9} = 8.53$, $P = 0.04$). There were no other significant differences in relative percent carbon allocation between the remaining shaded constituent parts and their corresponding controls (ANOVA: $P > 0.05$).

In plants with leaves on R2 shaded and the leaves on R3 supplied, autoradiography analysis (Fig. 4B panels 1, 2) indicated that most translocated radioactive carbon moved to the RA, whereas most translocated radioactive carbon in the control group moved to R4 (Fig. 4B panels 3, 4). Ramet-2 from the shaded group exhibited less exposure than did R2 from the control group. Scintillation analysis showed a higher percentage of carbon moved to the shaded R3 than the corresponding control (Fig. 4B panel 5). Differences in the relative percent carbon allocation between the shaded constituent parts and their corresponding controls were not significant (ANOVA: $P > 0.05$).

In plants with leaves on R2 shaded and the leaves on R4 supplied, autoradiography analysis showed most translocated radioactive carbon moved to RZ3-4 (Fig. 4C panels 1, 2). Similarly, most translocated radioactive carbon moved to RZ3-4 in the control group (Fig. 4C panels 3, 4). There appeared to be little difference in the exposure intensity between the shaded leaf-pair on R2 and the corresponding control R2. Scintillation analysis indicated that in the shaded group, most translocated radioactive carbon moved to the shaded R3 (Fig. 4C panel 5). Similarly, in the control group, most translocated radioactive carbon also moved to R3. However, there were no significant differences in the relative percent carbon allocation between the shaded constituent parts and their corresponding controls (ANOVA: $P > 0.05$).

RAMET 3 SHADED.—In plants with leaves on R3 shaded and RA supplied, autoradiographic indicated that in both the shaded and control groups, most translocated radioactive carbon moved to the adjacent RZA-2 (Fig. 5A panels 1–4). There appeared to be little difference in the exposure intensity between the shaded leaf-pair on R3 and the corresponding leaf pair on the control R3. Scintillation analysis showed that the shaded group had a higher percent radioactive carbon allocation than the corresponding controls (Fig. 5A panel 5). However, there were no significant differences in the relative percent carbon allocation between the shaded constituent parts and their corresponding controls (ANOVA: $P > 0.05$).

In plants with R3 shaded and R2 supplied, autoradiographic analysis indicated that most translocated radioactive carbon moved to the younger tissues, which were all heavily exposed (Fig. 5B panels 1, 2). The control plant showed a similar pattern (Fig. 5B panels 3, 4). The shaded plant exhibited a weaker signal at R3 than did the control

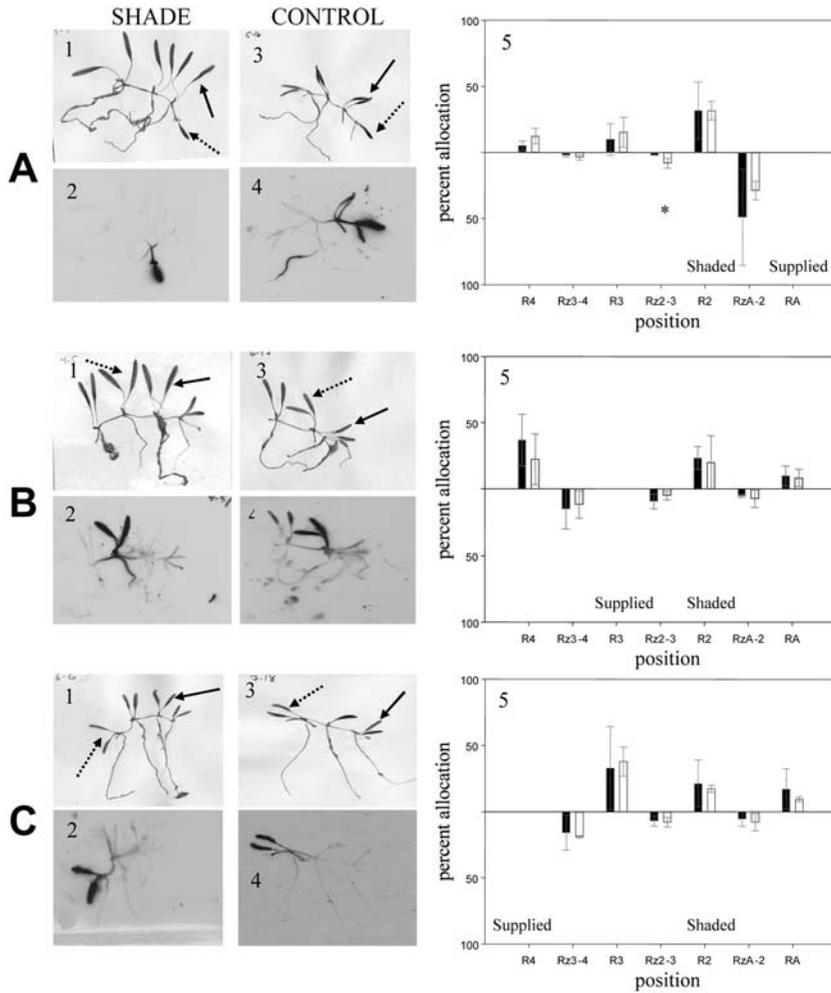


Figure 4. (A) Ramet two shaded, apical ramet supplied with radioactive carbon, (B) ramet two shaded, ramet three supplied, (C) ramet two shaded, ramet four supplied. Photographs of shaded plants are positioned on the left (1, 2) and control-plant images are located on the right (3, 4). Pressed plants are in the top panels (1, 3) and radiograms in the lower panels (2, 4). Histograms showing allocation of percent-translocated carbon are to the right of the photographs (5). Results are described as allocation to constituent parts. Translocated radioactive carbon values for above-ground ramets are represented above the x-axis and values for below-ground rhizomes are represented below this axis. Hatched arrow = ¹⁴C supplied, solid arrow = shaded. Filled bars represent experimental data, open bars represent control data. Error bars represent standard deviation. Asterisk represents statistical significance between experimental and control treatments.

group. Scintillation analysis showed that in both the shaded and control groups, most translocated radioactive carbon moved to R3, with less radioactive carbon moving to the shaded R3 than in the corresponding control (Fig. 5B panel 5). There were no significant differences in the relative percent carbon allocation between constituent parts on shaded plants and their corresponding controls (ANOVA: $P > 0.05$).

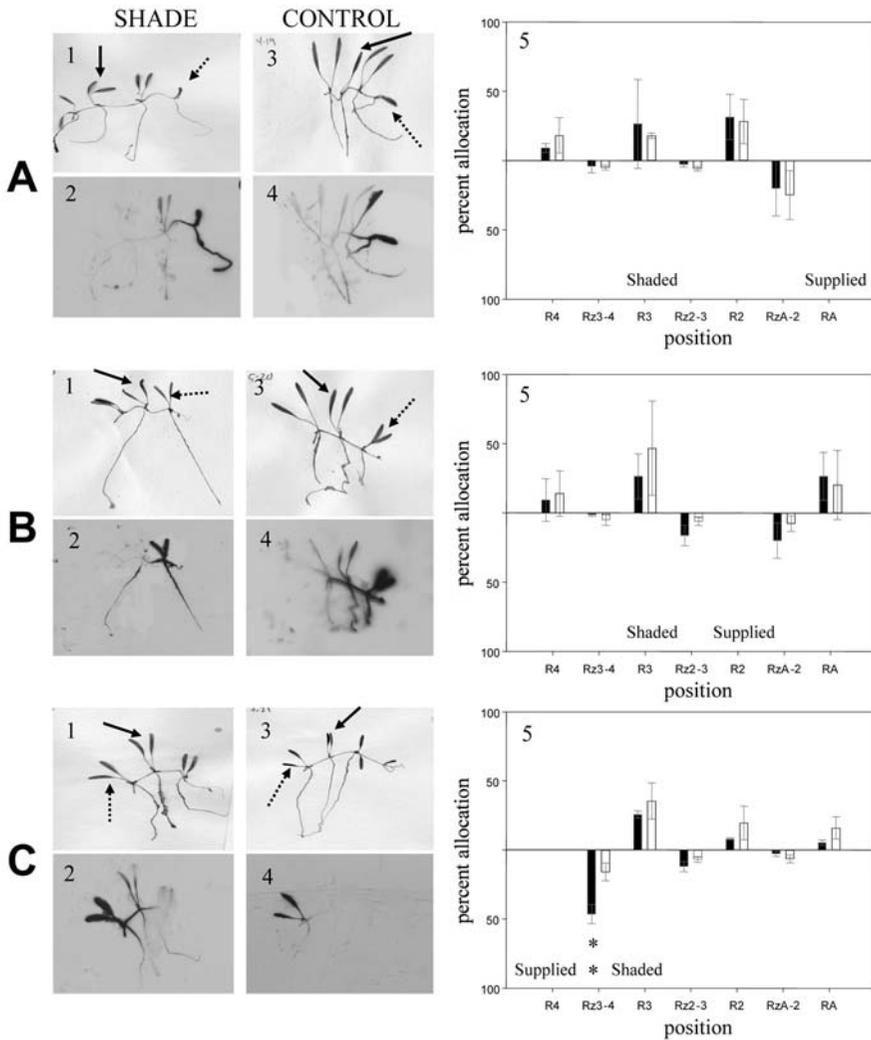


Figure 5. (A) Ramet three shaded, apical ramet supplied with radioactive carbon, (B) ramet three shaded, ramet two supplied, (C) ramet three shaded, ramet four supplied. Photographs of shaded plants are positioned on the left (1, 2) and control-plant images are located on the right (3, 4). Pressed plants are in the top panels (1, 3) and radiograms in the lower panels (2, 4). Histograms showing allocation of percent-translocated carbon are to the right of the photographs (5). Results are described as allocation to constituent parts. Translocated radioactive carbon values for above-ground ramets are represented above the x-axis and values for below-ground rhizomes are represented below this axis. Hatched above = ¹⁴C supplied, solid arrow = shaded. Filled bars represent experimental data, open bars represent control data. Error bars represent standard deviation. Asterisk represents statistical significance between experimental and control treatments.

In plants with leaves on R3 shaded and the leaves on R4 supplied, autoradiography analysis indicated that most translocated radioactive carbon moved to Rz3-4 (Fig. 5C panels 1, 2). In the control group, most translocated radioactive carbon was weakly distributed throughout the plant (Fig. 5C panels 3, 4). The shaded-R4 exhibited a stronger exposure signal than the corresponding control. Scintillation analysis showed that in the shaded group most translocated radioactive carbon moved into Rz3-4 followed by the shaded R3 (Fig. 5C panel 5). In the control group, most

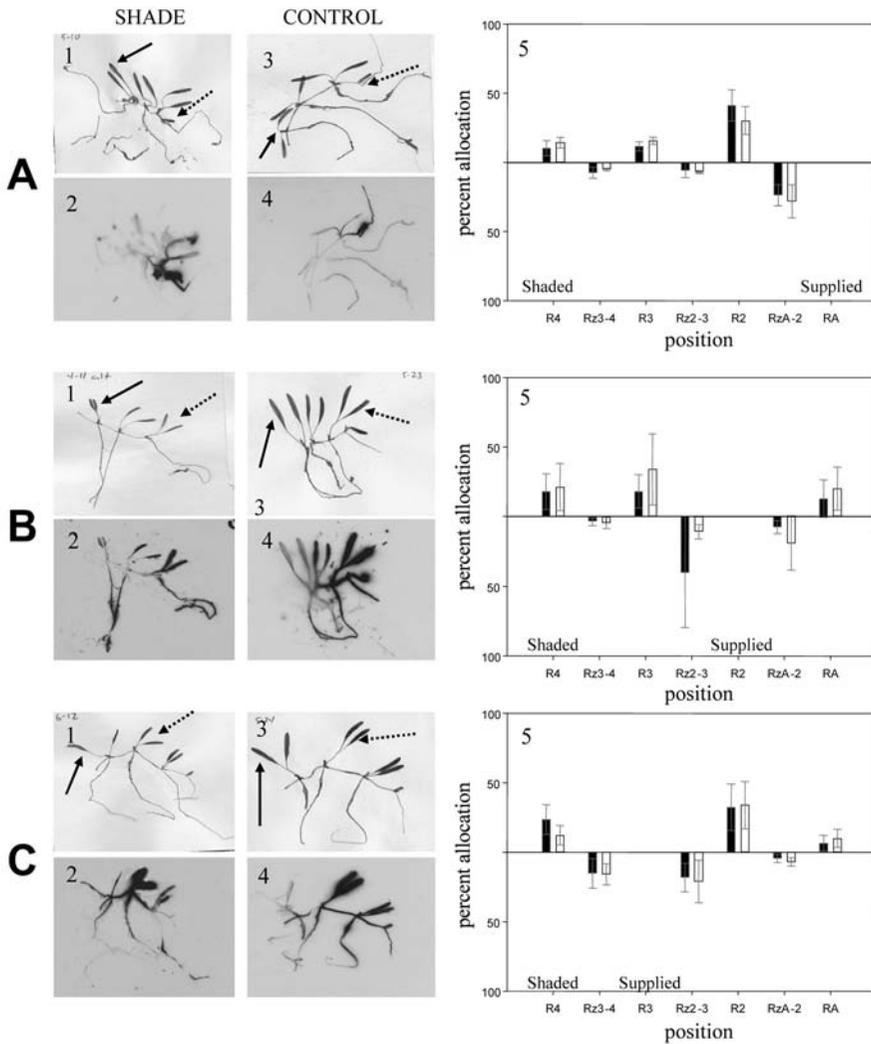


Figure 6. (A) Ramet four shaded, apical ramet supplied with radioactive carbon, (B) ramet four shaded, ramet two supplied, (C) ramet four shaded, ramet three supplied. Photographs of shaded plants are positioned on the left (1, 2) and control-plant images are located on the right (3, 4). Pressed plants are in the top panels (1, 3) and radiograms in the lower panels (2, 4). Histograms showing allocation of percent-translocated carbon are to the right of the photographs (5). Results are described as allocation to constituent parts. Translocated radioactive carbon values for above-ground ramets are represented above the x-axis and values for below-ground rhizomes are represented below this axis. Hatched arrow = ^{14}C supplied, solid arrow = shaded. Filled bars represent experimental data, open bars represent control data. Error bars represent standard deviation.

translocated radioactive carbon moved to R3. The Rz3-4 on the shaded plants had a significantly higher percentage carbon allocation than did the controls (ANOVA $F_{1,9} = 31.87, P = 0.01$). There were no significant differences in the relative percent carbon allocation between the remaining shaded constituent parts and their corresponding controls (ANOVA: $P > 0.05$).

RAMET 4 SHADED.—In plants with R4 shaded and RA supplied, autoradiographic analysis showed a similar weak allocation of radioactive carbon at R4 to the control

plants (Fig. 6A panels 1–4). Scintillation analysis also showed that less radioactive carbon moved to the shaded R4 than in the corresponding control (Fig. 6A panel 5). However, ANOVA indicated no significant differences in the relative percent carbon allocation between the shaded constituent parts and their corresponding controls ($P > 0.05$).

In plants with R4 shaded and R2 supplied, the shaded and the control group showed similar exposure intensity at R4 (Fig. 6B panels 1–4), but the scintillation analysis showed that in shaded plants, less radioactive carbon was allocated to R4 than in the corresponding control group (Fig. 6A panel 5). There were no significant differences in the relative percent carbon allocation between the shaded constituent parts and their corresponding controls (ANOVA: $P > 0.05$).

In plants with R4 shaded and R3 supplied, autoradiography analysis indicated that the shaded plant exhibited stronger exposure intensity at the shaded R4 than did the control plant (Fig. 6C panels 1–4). In the shaded group, scintillation analysis showed that more translocated radioactive carbon moved to R4, than in the control group (Fig. 6C panel 5). However, ANOVA again revealed no significant differences in the relative percent carbon allocation between the shaded constituent parts and their corresponding controls ($P > 0.05$).

CARBON UPTAKE AND DIRECTIONALITY.—The control group had a slightly higher uptake of carbon per gram dry weight supplied ($1.35 \text{ mg C g}^{-1} \text{ hr}^{-1} \pm 2.12 \text{ SD}$) than did the shaded group ($1.26 \text{ mg C g}^{-1} \text{ hr}^{-1} \pm 1.43 \text{ SD}$), however, this difference was not significant (K-W ANOVA: $H = 0.0326$, $P = 0.857$). The magnitude of directionality of carbon to RA when R4 was supplied (mean regression slope = $3.70 \pm 6.54 \text{ SD}$) was not significantly different from the magnitude of directionality of carbon to R4 when RA was supplied (mean regression slope = $3.96 \pm 5.89 \text{ SD}$; ANCOVA: $F_s = 0.040$, $P > 0.05$).

DISCUSSION

Halophila johnsonii exhibits clonal integration, however, it does not significantly modify carbon translocation to support specific ramets. Rather, our results suggest that photosynthate is made available to all ramets regardless of their age or condition. While autoradiographic analysis showed a slight trend towards allocating support preferentially to younger tissues, there was no statistically significant difference in the directionality of carbon translocation to the apical ramets compared to the oldest ramets. *Halophila johnsonii* did not exhibit a higher carbon assimilation rate in response to ramet shading which suggests that it does not employ a facultative mechanism for the enhanced uptake of carbon to buffer shading stress. Because its ramets are short-lived and deterministic, *H. johnsonii* may not benefit from using its limited resources to increase active carbon uptake or to distribute this carbon selectively. This finding is in contrast to those strategies employed by larger seagrasses with slower turnover rates that show facultative and directional physiological support for light-stressed ramets (Tomasko and Dawes, 1989).

The cost of providing support to a shaded ramet by other ramets, which themselves live for a relatively short time, may outweigh benefits. Marbà et al. (2002) found that physiological support for *Halophila stipulacea* (Forsskål) Ascherson (a similar sized congener) was maintained for only 1.6 d, compared to 5.4 yrs for the stable-selected seagrass *Cymodocea nodosa* (Ucria) Ascherson. Unlike the larger seagrasses, car-

bon translocation in *H. stipulacea* exhibited little directionality. Similarly, some terrestrial clonal herbs transfer photosynthate to a "common-pool." Chapman et al. (1991a,b) and Kembell et al. (1992) found that the small ruderal clonal herb *Trifolium repens* L. makes surplus photosynthate available to all ramets and does not selectively supply resources to stressed ramets. *Halophila johnsonii* appears to employ a similar strategy.

Support for all local ramets may result in the preservation of links to future ramets (Caraco and Kelly, 1991; Stuefer et al., 1994). *Halophila johnsonii* branches frequently through apical meristems that may arise at the base of each leaf pair. Meristems are the points of all of the genet's new growth and subsequent vegetative reproduction through fragmentation in *H. johnsonii* might be a viable mechanism of dispersal, though possibly limited to short distances (Hall et al., 2006). Selective support of a stressed ramet by a fast-lived plant where new growth is critical could be disadvantageous.

Acquisition and holding of space are much more important attributes for longer-lived, slower-growing plants. In contrast, *H. johnsonii* seems to exploit unstable environments or newly-created unvegetated patches, with minimal resources allocated to the holding of space. These growth characteristics partially explain the patchy, non-contiguous distribution of *H. johnsonii* (Kenworthy, 1993; Virnstein et al., 1997). By exhibiting fast-growth and support for all local ramets, *H. johnsonii* may occur in areas where it could otherwise not compete. It may quickly exploit locally uninhabited patches, and through prolific lateral branching and fast horizontal growth, move once conditions become unfavorable. While these attributes may allow *H. johnsonii* to compete effectively in periodically perturbed areas such as shallow intertidal fringes, if the distribution of this species becomes limited to stable areas it may be out-competed by more stable-selected plants (Durako et al., 2003).

The physiological strategy described here shows that *H. johnsonii* does not appear to support stressed ramets along its genet. This characteristic, along with the species' low capacity for storage, suggest that vegetative growth over large unsuitable areas may be unlikely. Given that its geographic distribution is so endemic and that its capacity to disperse over large distances may be limited, widespread loss of suitable habitat may be especially detrimental to the distribution and abundance of *H. johnsonii* as well as its ability to recover from widespread disturbance.

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