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Effects of physiological integration on the survival and growth of ramets and clonal fragments in the seagrass *Syringodium filiforme*

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ABSTRACT: Mesocosm experiments examined the importance of clonal integration to the growth of *Syringodium filiforme* ramets (short shoots with their associated section of rhizome and roots) and clonal fragments. High mortality and reduced growth following isolation from parent clones indicated that young ramets are dependent on clonal integration for survival and growth. In contrast, older ramets survived isolation, grew, and branched forming a new rhizome apical meristem (RAM) and additional ramets. Similar results were obtained from an experiment examining the survival and growth of clonal fragments. Older fragments produced longer branches and more new ramets than did younger fragments during the same time period. Cutting the rhizome behind a ramet led to a decrease in ramet growth compared to cutting the rhizome in front of a ramet. These results indicate that *S. filiforme* RAMs and young ramets act as physiological sinks, drawing resources from and reducing the growth potential of older ramets. These results also suggest that restoration projects should utilize planting units comprised of older clonal fragments due to increased survival and growth potential. Using older portions of clones as planting units negates the need for an intact RAM, as these fragments are capable of producing multiple new growth axes. Finally, our results indicate that in order to gain an accurate picture of seagrass meadow health in disturbance prone environments, rhizome condition and ramet position relative to the RAM should be considered in addition to the standard measurements of ramet productivity.

KEY WORDS: Clonal integration · Resource sharing · Ramet growth · *Syringodium filiforme* · Seagrass

INTRODUCTION

Clonal integration, defined as the physiological linkage between the constituent parts of a clonal plant (Little & Jones 1980), implies that resources are translocated from one region to another to support respiration, growth or reproduction (Alpert & Mooney 1986, Tomasko & Dawes 1989). Translocation of resources in clonal plants may include the movement of carbohydrates generated in the leaves, starch reserves mobilized from the rhizomes, and nutrients from uptake sites in the leaves and roots, and involves some physiological costs (Jonsdottir & Watson 1997). Among these are the expenses associated with the respiratory demands of the connective tissues, and the localized reduction of resources that results from resource sharing (Jonsdottir & Watson 1997). The internal movement of resources also has important implications to the survival and growth of clonal plants which are commonly found growing in heterogeneous environments where resource availability is highly variable (Harper 1985, Alpert & Mooney 1986, Slade & Hutchings 1987, Stuefer et al. 1994, 1996, Alpert & Stuefer 1997). Nutrients acquired through foliar or root-rhizome uptake in pockets of higher availability can be translocated to support growth in nutrient poor regions (Alpert & Mooney 1986, Duarte & Sand-Jensen 1996, Stuefer et al. 1996, Marba et al. 2002). In areas of high nutrient

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availability, ramet (i.e. a short shoot with its associated segment of rhizome and roots; Fig. 1) densities are typically high, resulting in self-shading and the reduction of light reaching individual ramets, such that light may become the limiting resource. In nutrient poor areas where ramet densities are lower, light availability is higher, and ramets positioned in these areas may support the growth of ramets in high nutrient areas through the translocation of carbohydrates (Harper 1985, Slade & Hutchings 1987, Stuefer et al. 1994). Therefore, the transfer of resources between ramets enables clonal plants to optimize growth in heterogeneous environments, increasing fitness by allowing for colonization and growth over large areas (Harper 1985, Alpert & Mooney 1986, Slade & Hutchings 1987, Stuefer et al. 1994, Jonsdottir & Watson 1997, Marba et al. 2002).

Tomlinson (1974) described the morphology and growth of the 12 major seagrass genera, and concluded that seagrass growth is dependent on the proliferation of rhizome apical meristems (RAMs). The RAMs, in turn, require the translocation of carbohydrates and nutrients from the attached ramets to support their growth. The majority of studies that have examined clonal integration in seagrasses have focused on the relationship between clonal integration and RAM growth. Translocation of nitrogen and phosphorus have been demonstrated to reduce the effects of nutrient limitation in the seagrass *Cymodocea nodosa*, and augment apical growth in nutrient poor areas (Duarte & Sand-Jensen 1996). From experimental manipulation of *C. nodosa* rhizome runners (sections of rhizome with a RAM, a series of consecutively older ramets and an intact connection to the larger clone), Terrados et al. (1997a) concluded that both apical growth and the growth of young ramets were supported by translocation of energy resources from older ramets, and that

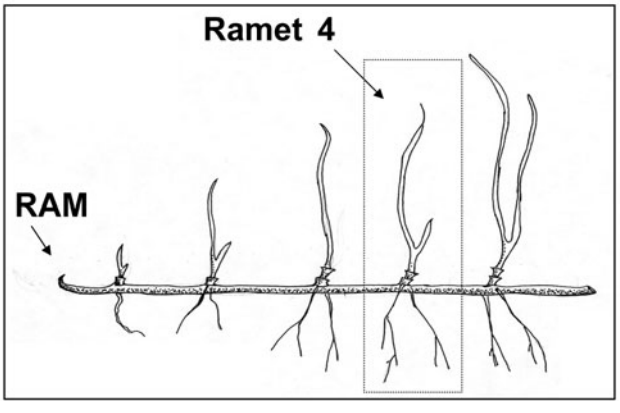

Fig. 1. *Syringodium filiforme* clonal fragment. RAM: rhizome apical meristem. Ramet 4 is the 4th oldest short shoot with its associated segment of rhizome and roots

resource translocation occurred over distances exceeding 50 cm. Schwarzschild & Zieman (2008) described similar results for the seagrass *Syringodium filiforme*. Evidence for the translocation of carbohydrates from *S. filiforme* ramets growing under normal light conditions to support the growth of shaded ramets has also been reported (Rey & Stephens 1996), as have observations of long distance transport of carbon within the seagrass *Posidonia oceanica* (Libes & Boudouresque 1987). Additional evidence for the importance of clonal integration to seagrass growth can be derived from studies of transplanted seagrasses. Tomasko et al. (1991) monitored the survival and growth of *Thalassia testudinum* planting units (clonal fragments) comprised of a RAM attached to 1, 2, and 4 ramets. Transplant survival was directly related to size, with 1 ramet planting units averaging only 33% survival, compared to 60 and 85% survival of the 2 and 4 ramet planting units, respectively. These results suggest that the RAM and young ramets are dependent on the translocation of resources from older ramets.

Newly formed ramets are small, with shoots and leaves growing below the leaf canopy, and therefore may be highly dependent on resources translocated from older ramets. Additionally, the rhizomes and root systems of young ramets have not fully developed and may not be capable of supplying all of the nutrients required for growth. However, older, larger ramets may be less impacted by clonal integration, since they have well-developed root systems, leaves extending through the leaf canopy, and may have starch reserves stored in their rhizomes.

The fact that the older ramets on a rhizome supply resources to the RAM and young ramets suggests that either the older ramets are producing in excess of what they require for active growth, or their growth is reduced due to the physiological demand on their resources exerted by the younger parts of the clone. Tomasko & Dawes (1989) examined the importance of resource sharing in *Thalassia testudinum* by shading and partially isolating individual ramets. Their results indicated that an unshaded ramet could support the growth of an adjacent, shaded ramet. The cost of this support may be a reduction in the growth of the unshaded ramet. This suggests that separating an older ramet from the physiological drain of younger ramets should result in increased growth of the older ramet.

This study used mesocosm experiments to examine the effects of clonal integration on the growth of isolated ramets and clonal fragments in the seagrass *Syringodium filiforme* (Fig. 1). The relationship between age and growth were examined to determine when ramets transition from functioning as (1) resource sinks, dependent on resources translocated from older ramets to

(2) sources of resources for younger ramets, an aspect of physiological integration that has not been previously considered in seagrasses and which may have important implications for growth in heterogeneous and disturbance-prone environments.

MATERIALS AND METHODS

Clonal integration in the seagrass *Syringodium filiforme* was examined through 3 independent experiments conducted in April through September 2002 using a mesocosm system that had been used for a previous set of experiments examining apical dominance and the importance of clonal integration to RAM growth in *S. filiforme* (Schwarzschild & Zieman 2008). Plant material used in the experiments was collected from a nearly monospecific grassbed in the vicinity of Sprigger Bank (24° 54.73′ N, 80° 56.19′ W), on the western margin of Florida Bay, USA. The mesocosms used for the experiments were rectangular plastic tubs (approximate dimensions of 80 × 50 × 20 cm) positioned in the center of a hard bottom bare area in Sunset Cove (25° 04.46′ N, 80° 27.36′ W), Florida Bay and anchored in place with rebar poles. The tubs were filled with carbonate screening sand (Florida Rock and Sand) with a grain size similar to sediments collected from *S. filiforme* patches growing on Sprigger Bank, Red Bay Bank and the Florida Keys Reef Tract. Water depth at the mesocosm site was approximately 1.5 m, and all work was done using SCUBA. Light levels and water temperature at the experimental site were similar to those of the area around Sprigger Bank; however, salinity was occasionally lower than that observed at the Sprigger Bank donor site (Schwarzschild 2004).

Approximately 10 d prior to the initiation of an experiment, clonal fragments (Fig. 1) were harvested from soft bottom bare areas in the vicinity of Sprigger Bank. Only unbranched rhizomes with a RAM and a minimum of 10 robust, intact, consecutive ramets were collected. Care was taken not to damage the rhizome or ramets, and to maintain as many intact roots as possible. Following selection, 15 fragments were planted in each of the mesocosms and allowed to acclimate prior to the initiation of experiments.

Ramet isolation experiment. This experiment was designed to examine the relationship between ramet age and the survival and growth of isolated ramets. This 37 d experiment was run from April 27 to June 3, 2002 and utilized 4 of the mesocosm tubs. To start the experiment, clonal fragments in the tubs were examined and the 4 most robust in each tub were selected for use; the remaining fragments were discarded. Criteria for selecting experimental fragments were the presence of (1) an intact, healthy RAM, and (2) the youngest 6 consecutive ramets, intact and not showing signs of branching. Once a fragment was selected, the rhizome was cut midway between the 6th and 7th ramet, and the tissue behind the 6th ramet was discarded. The RAM was also cut from the fragment and discarded. The rhizome connections between each of the remaining 6 ramets on the fragment were then cut midway between ramets (Fig. 2A). Ramets were replanted, in age consecutive order, using a wire grid

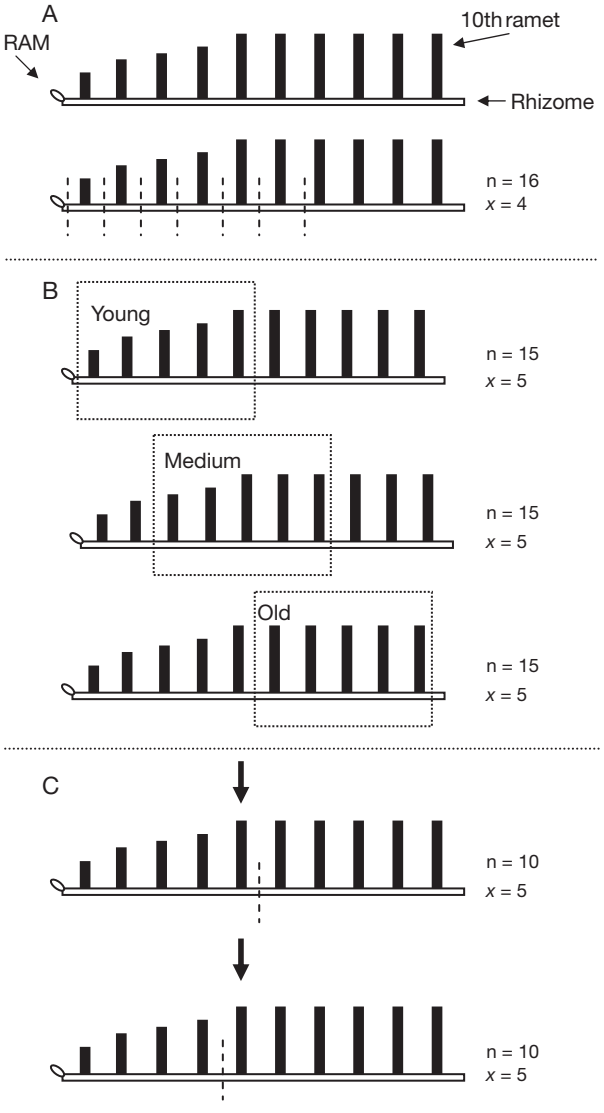

Fig. 2. Schematic representation of experimental treatments. n: no. of replicates of each treatment, *x*: no. of mesocosm tubs. (A) Unmanipulated clonal fragment comprised of a rhizome apical meristem (RAM) and 10 consecutive ramets, and a fragment used in the ramet isolation experiment showing how the rhizome is cut (dashed lines) between the 6 youngest ramets on the fragment. (B) Generation of young, medium and old clonal fragments for the fragment age experiment. (C) First ramet integration experiment. Dashed lines: point of rhizome severance either behind or in front of the 5th ramet (arrow)

placed on the surface of the sediments to locate the positions for ramet planting. A total of 16 replicate ramets from each age group were prepared, with 4 replicates planted in each of the tubs. The number of leaves on each ramet, leaf length, and the presence or absence of intact leaf tips was recorded. Ramets were monitored every 4 to 10 d to note survival, rhizome condition, branch formation, and the formation of new ramets. At the termination of the experiment all of the surviving ramets were collected and measured. Chi-square analysis was used to determine if there was a significant difference in the numbers of ramets that survived and branched in each of the age classes. Regression analysis was used to examine the relationships between ramet age, rhizome growth and the production of new ramets.

Fragment age experiment. This experiment was designed to examine the relationship between age, and the survival and growth of clonal fragments. This 20 d experiment was run from June 6 to June 26, 2002, and utilized 5 of the mesocosm tubs. At the start of the experiment, the 9 most robust fragments in each tub were selected and randomly assigned to one of 3 treatment groups. This experiment required healthy fragments with a RAM and 10 consecutive, intact ramets, with no signs of branching. Each tub contained 3 replicates from each group, yielding a total of 15 replicates for each treatment group. Experimental treatments were classified as young, medium and old clonal fragments, and were comprised of a clonal fragment with 5 healthy, consecutive ramets. Once assigned to a treatment group, fragments were trimmed to the appropriate length. To generate young fragments the RAM was removed and the rhizome was cut behind the 5th ramet. Medium age fragments were comprised of the 3rd through 7th ramets, and old fragments were comprised of the 6th through 10th ramets (Fig. 2B). Color-coded cable ties were placed around the rhizomes between the 2 youngest ramets on each fragment and used to indicate treatment groups. At the termination of the experiment the fragments were harvested and transported to the lab for analysis, where the position, length and number of ramets on any branches produced during the experiment were recorded. The number of new ramets produced and rhizome growth were compared across treatments using 1-way ANOVA followed by Student-Newman-Keuls test (SNK, $\alpha = 0.05$).

Ramet integration experiment. This experiment was designed to determine the effect of clonal integration on the growth of an individual ramet connected to a clone. The first ramet integration experiment was initiated on July 3, 2002. Due to vandalism, and the destruction of all test plants, this experiment did not run to completion, and was terminated on July 18 after only 15 d. A second experiment was initiated on August 22, and completed after 22 d on September 13, 2002. At the start of the first experiment, the 6 healthiest fragments in each of 5 mesocosm tubs were selected for use and assigned to one of 3 treatment groups, with 2 replicates of each group per tub, for a total of 10 replicates in each treatment group. Runners used in the experiment had to meet the criteria of having an intact, healthy RAM and 10 consecutive, healthy, unbranched ramets. Individually numbered, color-coded cable ties were placed around the rhizomes in front of Ramet 5 to indicate treatment group and mark the position of the ramet to be monitored. Treatments were (1) cut in front = rhizome cut in front of the 5th ramet, (2) an unmanipulated control, and (3) cut behind = rhizome cut behind the 5th ramet (Fig. 2C). Leaf length and presence of an intact leaf tip were recorded for each leaf on the 5th ramet at the start of the experiment. Ramets were monitored and leaf lengths measured every 2 to 4 d until the end of the experiment. Leaf growth rates and the total length of new leaf tissue produced by the monitored ramets during the experiment were compared across treatment groups using 1-way ANOVA followed by SNK ($\alpha = 0.05$).

Determination of leaf growth rates required considerable effort and SCUBA bottom time to collect the repeated measurements of the plants in the field, and the physical disturbance of measuring the ramets has the potential to alter growth rates (Schwarzschild 2004). Analysis of leaf growth data in short-term experiments is further complicated by the fact that *Syringodium filiforme* leaf growth rates vary as a function of leaf age, with maximum growth rates observed in young leaves (Fry 1983, Schwarzschild 2004). A simpler metric for determining the potential effects of clonal integration on ramet growth is the formation of new leaves by the manipulated ramets. Determination of this metric can be made by counting the number of replicate ramets in each treatment that produced new leaves during the experiment. Monitoring requires little time, results in minimal disturbance to the ramets, is not affected by the advent of branch formation, and the results can be compared using a Tukey modified χ^2, which allows for multiple comparisons of proportional data (Zar 1984). This method was used in the first ramet integration method, along with the analysis of leaf growth rates.

The design and set-up for the second ramet integration experiment was the same as that for the previous experiment except that in this experiment (1) the clonal fragments were shorter, comprised of only 8 consecutive ramets, (2) the rhizome manipulation and monitoring were focused on Ramet 3, and (3) the formation of new leaves was the only metric used for determination of treatment effects.

Table 1. *Syringodium filiforme*. Ramet isolation experiment. Ramet 1 is the youngest ramet, and Ramet 6 is the oldest ramet. The experiment lasted 37 d with 16 replicates in each age class. Survived / branched: no. of ramets in each age group that survived or branched during the experiment; new ramets: mean (SE) no. of new ramets produced by the ramets in each age group; branch length: mean (SE) length of the branches produced by the ramets that branched in each age group. p-values are from a Pearson chi square test. n: total no. of ramets observed; r: r-value of the regression

	Ramet 1	Ramet 2	Ramet 3	Ramet 4	Ramet 5	Ramet 6	p
Survived	3	11	14	14	16	14	≤0.001
Branched	0	7	10	14	15	14	≤0.001
New ramets	0	1.7 (0.5)	2.1 (0.5)	2.6 (0.3)	2.7 (0.2)	2.5 (0.1)	0.004 (n = 72, r = 0.34)
Branch length (cm)	0	2.1 (0.8)	2.5 (0.7)	3.9 (0.8)	4.7 (0.7)	4.5 (0.5)	≤0.001 (n = 60, r = 0.44)

RESULTS

Ramet isolation experiment

The results of the ramet isolation experiment (Table 1) clearly illustrate the importance of ramet age for survival and growth. Mortality was highest for the youngest ramets, and declined rapidly with increasing ramet age. The youngest ramets, Ramet 1 located directly behind the RAM at the start of the experiment, suffered 81% mortality when isolated, compared to 31% mortality of the second youngest ramets. Mortality was minimal in older ramets, with 13% mortality in Ramets 3, 4 and 6. All of the 5th ramets survived to the end of the experiment. Branching, branch growth, and the formation of new ramets increased with increasing ramet age (Table 1). None of the youngest ramets branched during the experiment compared to near 100% branching of the older ramets, those located 4, 5 and 6 positions behind the RAM at the start of the experiment. Younger ramets tended to die more quickly than older ramets following the severing of the rhizome, whereas older ramets tended to branch earlier than younger ramets. Leaf growth of the youngest ramets continued until death, which was observed to occur as a result of the breakdown of the ramet–rhizome connections. The rhizome sections of these young ramets became progressively softer and more translucent over time, while the rhizomes of older ramets remained firm and opaque. As ramet age increased from Ramets 2 to 6, branching occurred earlier, and both the branch length and the number of new ramets produced increased with increasing ramet age.

Fragment age experiment

The pattern of increasing growth with increased age observed in the ramet isolation experiment was supported by the results of the fragment age experiment, in which a similar relationship between fragment age and branching (Table 2) was observed. The mean number of new ramets, and branch length produced by young fragments (comprised of Ramets 1 to 5 on a rhizome section) were lower than those of medium age fragments (Ramets 3 to 7) and old fragments (Ramets 6 to 10). The ramet formation rate (plastochrone interval, PI) of the oldest fragments was 5 to 7 d, equivalent to the ramet PI observed in the area around Sprigger Bank, where the plants were collected (Schwarzschild 2004). The youngest ramets on these growing clonal fragments typically had 2 leaves, indicating an initial leaf PI of 2.5 d (Leaf PI = Ramet PI/Number of leaves). The next youngest ramet generally had 2 leaves, and 1 leaf scar, indicating that the leaf PI for the 3rd leaf was 5 d. Older ramets did not generate additional leaves or leaf scars during the course of the experiment, indicating that the leaf PI for the 4th leaf was longer than 5 d.

Table 2. *Syringodium filiforme*. Fragment age experiment. Young fragments included Ramets 1 to 5, medium fragments Ramets 3 to 7, and old fragments Ramets 6 to 10. Values are means (SE); superscript letters indicate significant differences determined by 1-way ANOVA followed by Student-Newman-Keuls test (SNK, $\alpha = 0.05$). SNK p-values are presented

	Young	Medium	Old	p
Replicates	15	15	15	
New ramets	1.6 (0.2)^a	2.5 (0.3)^b	3.4 (0.3)^c	<0.001
Branch length (cm)	2.7 (0.7)^a	5.4 (0.7)^b	6.8 (0.6)^c	<0.001

Ramet integration experiments

The effects of clonal integration on ramet growth were also observed in the results of the ramet integration experiments. The mean leaf growth rate of 0.52 cm d^{-1} determined for ramets from the Cut Behind treatment was significantly lower than the rates of 0.82 and 0.89 cm d^{-1} determined for the Control and Cut in Front treatments respectively (Fig. 3A).

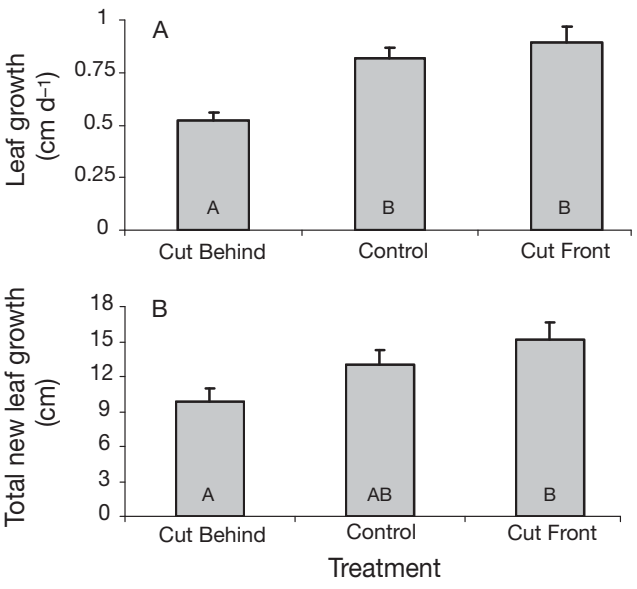

Fig. 3. *Syringodium filiforme.* Results from the first ramet integration experiment, monitoring growth of the 5th oldest ramet on clonal fragments. Cut Behind: rhizome cut behind the monitored ramet; Control: rhizome intact; Cut Front: rhizome cut in front of the monitored ramet (see Fig. 2C). Letters in the bars indicate statistically significant differences (1-way ANOVA and Student-Newman-Keuls test, $\alpha = 0.05$). (A) Mean leaf growth rates for the monitored ramet. (B) Mean amount of new leaf tissue produced by the monitored ramet

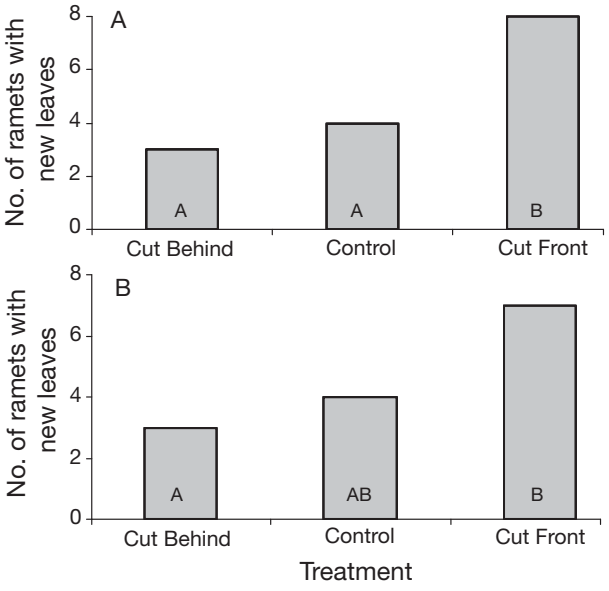

Fig. 4. *Syringodium filiforme.* Number of ramets producing new leaves in (A) the first ramet integration experiment, monitoring the 5th oldest ramets on clonal fragments; and (B) the second ramet integration experiment, monitoring the 3rd oldest ramets on clonal fragments. Cut Behind: rhizome cut behind the monitored ramet; Control: rhizome intact; Cut Front: rhizome cut in front of the monitored ramet. Letters in the bars indicate statistically significant differences (Tukey-type multiple comparison for proportions, $\alpha = 0.05$)

Comparing the total length of new leaf tissue generated during the experimental period provides results similar to the leaf growth rate analysis (Fig. 3B). Ramets in the Cut Behind group generated 9.8 cm of new leaf growth during the experiment, which is significantly less than the 15.2 cm of new leaf material produced by the Cut in Front group. Neither of these values, however, was significantly different from the 13.0 cm of new leaf material generated by ramets in the Control group. This analysis was complicated, however, by the fact that many of the ramets in the Cut in Front treatment branched and generated new ramets during the course of the experiment.

Analysis of the number of new leaves produced by ramets in the varying treatment groups during the experimental period provides results similar to those generated through analysis of leaf growth. A significantly lower number of ramets forming new leaves were observed for the Cut Behind treatment group relative to the Cut in Front treatment (Fig. 4A).

The results of the second ramet integration experiment were determined solely through the analysis of ramets producing new leaves, and show a similar pattern to that observed in the earlier experiment (Fig. 4B). Only 3 of the 10 ramets in the Cut Behind treatment produced new leaves during the 22 d experiment. In comparison, 7 out of 10 ramets from the Cut in Front treatment produced new leaves.

DISCUSSION

Results obtained from the experiments demonstrate the importance of clonal integration to the survival and growth of *Syringodium filiforme* ramets, supporting the hypothesis that young ramets are dependent on resources translocated from older ramets. When isolated from the parent clone, the youngest ramets experienced 81% mortality. Mortality of isolated ramets declined with increasing ramet age, with near 100% survival observed for ramets located 4 positions behind the RAM. Additionally, most of these older ramets branched, generating a new RAM and new ramets. These results show that older ramets are not only self sufficient, but also capable of supplying resources to support the growth of a RAM and younger ramets. In contrast, the youngest isolated ramets appeared to mobilize carbohydrate resources from their rhizome segments in order to support leaf growth as evidenced by the observation that the rhizomes became progressively softer and more translucent over time, until they eventually degraded entirely. A similar response was

observed in the 31% of the 2nd ramets that died during the course of the experiment, unlike the rhizomes of surviving ramets which remained firm and opaque. This suggests that the relatively small leaves of young ramets do not generate sufficient photosynthetic output to support their growth, and that these young ramets are dependent on resources translocated from older ramets. A future study examining the levels of soluble carbohydrate concentrations in the rhizome sections of isolated ramets is warranted.

The results of the fragment age experiment further illustrate the relationship between ramet age and clonal growth. Clonal fragments cut from older sections of a rhizome runner (1) branched earlier, (2) branched more often, and (3) produced more robust branches than did similar size fragments comprised of the youngest ramets from a runner. These observations, in conjunction with the results from the ramet isolation experiment, suggest that fragments comprised of old ramets either have ample energy reserves in their rhizomes or sufficient energy generating potential from the ramets to (1) maintain ramet growth and (2) support branching and the production of a new growth axes, including the formation of a new RAM and young ramets. In comparison, fragments comprised of young ramets must first mobilize resources to ensure survival and growth of the young ramets on the fragment. Once the fragment has aged, and the young ramets have become more self sufficient, energy resources can be diverted to branching and fragment growth.

Cutting the rhizome in front of a monitored ramet led to an increase in ramet growth, often resulting in branching and the formation of a new growth axis by the monitored ramet, compared to cutting the rhizome behind a monitored ramet, which resulted in decreased ramet growth and a reduction in the number of new leaves formed. No branches were generated by monitored ramets in the Cut Behind or Control treatments. These results support the hypothesis that the RAM and young ramets on a clone act as a physiological sink, drawing resources from and reducing the growth potential of older ramets. Severing the rhizome behind the monitored ramet terminated the flow of resources from older ramets to the RAM and young ramets, causing the monitored ramet to allocate more resources to support apical growth and ensure clonal survival, thereby reducing the growth potential of the monitored ramet. In contrast, cutting the rhizome in front of the monitored ramet released it from the need to send resources forward to younger parts of the clone, allowing for increased ramet growth. It is also possible that the increased growth observed in the Cut in Front treatments was augmented by resources supplied from older ramets on the clone that would have otherwise been used to support the growth of the RAM and younger ramets had the rhizome connection not been severed. In either case, the results demonstrate that acropetal resource translocation reduces the growth potential of older ramets.

The results and conclusions drawn from these mesocosm experiments are supported by field studies of *Syringodium filiforme* and other seagrasses presented in the literature. Apical dominance, i.e. the suppression of lateral or ramet branch formation exerted by the RAM (Cline 1991, Callaghan et al. 1997), has been demonstrated to exist in the seagrasses *Cymodocea nodosa* (Terrados et al. 1997b) and *S. filiforme* (Schwarzschild & Zieman 2008). By limiting the formation of ancillary branches, apical dominance focuses the majority of plant resources towards the primary growth axis. Removal of apical dominance, followed by prolific branching may result in the dilution of resources available to support the growth of branches and young ramets, reducing overall clonal fitness. Therefore, the existence of apical dominance can be seen as evidence for the importance of acropetal resource translocation to clonal survival and growth. The reliance of RAMs on acropetal resource translocation has also been documented through the manipulation of rhizomes from plants grown in the field. Terrados et al. (1997a) report that severing the rhizome as many as 11 ramets behind the RAM reduced growth in the seagrass *C. nodosa*. Similar results have been reported for *S. filiforme* (Schwarzschild & Zieman 2008).

In situ observations that the first few leaves generated by young ramets are produced at a rate significantly faster than subsequent leaves (Patriquin 1973, Brouns 1985, Kenworthy & Schwarzschild 1998) supports the hypothesis that the growth of young ramets is dependent on resources translocated from older ramets. A similar result was observed in the mesocosm experiments, with an initial leaf PI of 2.5 d for the 1st and 2nd leaves formed on a new ramet, increasing to 5 d for the 3rd leaf and exceeding 5 d for the 4th leaf. Mortality of the 3rd ramets was dramatically reduced when compared to the 1st and 2nd ramets (13 vs. 81 and 31%, respectively) following isolation from the parent clone, and nearly all of the 4th, 5th and 6th ramets branched, generating new RAMs and young ramets. These observations, coupled with the age-specific leaf PI suggest that once ramets were approximately 15 d (>3 PI) old, and located 3 ramets behind the RAM, they transitioned from being physiological sinks dependent on resources from older ramets, to independent entities capable of supporting their own growth. The age at which this transition occurs may be dependent on environmental condition, with ramets growing in favorable environments transitioning at a younger age than those growing under less favorable conditions. However, it is clear that as ramets age they shift

from being dependent on clonal integration to eventually becoming sources of resources, supporting the growth of younger portions of the clone.

The documentation of the importance of clonal integration to *Syringodium filiforme* growth has implications to future studies of seagrass productivity, particularly for plants affected by resource limitation or disturbance events. The results of this study show that *S. filiforme* clones preferentially allocate resources to the growth of the primary RAM and young ramets at the expense of the growth of older ramets. Therefore, monitoring leaf growth without consideration of rhizome condition or the position of ramets relative to the RAM will not accurately reflect the health and productivity of the clone. As a result, measurements of leaf growth may not prove useful for making predictions of meadow productivity or maintenance in heterogeneous and disturbance prone environments. The results also suggest that restoration projects should utilize planting units comprised of older clonal fragments due to their increased survival and growth potential. Furthermore, using older portions of clones as planting units negates the need to have an intact RAM, as these fragments are capable of producing multiple new growth axes. Finally, our results indicate that in order to gain an accurate picture of seagrass meadow health in disturbance prone environments, rhizome condition and ramet position relative to the RAM should be considered in addition to the standard measurements of ramet productivity.

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