January 2012

Effects of Short Shoot Number and Presence of an Apical Meristem on Rhizome Elongation, New Short Shoot Production, and New Rhizome Meristem Production of Thalassia Testudinum Banks and Solander Ex König Planting Units in Tampa Bay.

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By

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science
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Date of Approval:
July 11, 2012

Keywords: seagrass, rhizome growth, long shoot meristem, plastochrone interval, planting unit size

ACKNOWLEDGEMENTS

The field studies were possible only with the help of Mathew Brown, Jennifer Sneed, and many undergraduate students, as this project was quite labor intensive. Benjamin Watkins, in particular, is thanked for his invaluable assistance during nearly all of the field work. Dr. Lesley Baggett and Dr. Bruce Cowell are thanked for their guidance in the statistical analysis.

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ABSTRACT

Thalassia testudinum Banks and Solander *ex* König is the dominant seagrass in the Gulf of Mexico, Caribbean and the West Coast of Florida, yet little rhizome elongation, new short shoot production, or new rhizome meristem production data has been collected via direct measurement. A study of the rhizome growth of *T. testudinum* was completed in December 2004 in southern Tampa Bay that determined growth after 26.5 months. Two PVC planting frames each containing four rhizomes with 2 short shoots, two rhizomes with 4 short shoots, and two rhizomes with 8 short shoots were planted next to existing *T. testudinum* beds at 5 sites (n = 10 planting frames). The rhizome apical meristem was removed from half of each set of short shoot units on each planting frame. Plants initially lacking a rhizome meristem produced more new long shoot meristems than those planted with an intact meristem, and larger planting units produced more new rhizome meristems than smaller ones, $P = 0.001$ and $P < 0.001$, respectively.

The total number of rhizome meristems per planting unit (new meristems + initial meristem) was greater in plantings initially lacking a long shoot meristem in the 2, 4 and 8 short shoot size classes. Only the two short shoot plants benefited from an intact rhizome meristem at planting time, elongating 66.4 cm versus 60.4 cm for plants initially lacking a rhizome meristem at 26.5 months. In the 4 and 8 short shoot classes, plants that lacked a rhizome meristem at planting outpaced those with a meristem, producing 192.1

and 277.9 for 4 and 8 short shoot plants compared to 120.9 cm and 177.7 cm for plants with a meristem during the same time period. The greatest growth rate increases were due to lateral branching on planting units that lacked a rhizome meristem in the two largest size classes (4 and 8 short shoots); the differences between plants with an intact rhizome meristem and those without with the size classes pooled did not prove to be statistically different, $P = 0.112$. Differences among the size classes were significant, however, $P < 0.001$. Analysis of new short shoots was analogous to the results for rhizome elongation, with the presence of an initial rhizome factor proving insignificant, $P = 0.401$, and the initial number of short shoots factor proving significant, $P < 0.001$.

The rhizome growth, new short shoot production, and new rhizome meristem production data determined by direct measurements in this study appear to be the first planting unit measurements for this species under natural conditions.

INTRODUCTION

Thalassia testudinum Banks *ex* König (turtle grass), the dominant species in mature Caribbean seagrass meadows (Patriquin, 1973; van Tussenbroek, 2002), grows more slowly than other species common to the West Coast of Florida, making studies of patch expansion or planted *T. testudinum*, meadow development a lengthy commitment. For example, Dawes *et al*. (1997) noted that *T. testudinum* requires as long as 7.6 years for complete regrowth from edges into relatively narrow, 0.25 m, propeller scars, a function of the infrequent formation of new rhizome (long shoot) meristems, the source of vegetative expansion in seagrasses. *Thalassia testudinum* growing in the Mexican Caribbean, branches every 6.3 m on average**.** In contrast, *Syringodium filiforme* Kützing (manatee grass) and *Halodule wrightii* Ascherson (shoal grass), branch every 94 cm and 22 cm, respectively (Gallegos *et al.*, 1994). Therefore, *T. testudinum* has less capacity to fill in space via branching than *S. filiforme* and *H. wrightii*, given its lower branching rate.

Studies comparing features of natural reference seagrass beds with those of seagrass planting units (PUs) using quantitative data are rare. Fonseca *et al*. (1996) observed newly planted beds of the relatively fast growing pioneer species *H. wrightii* and *S. filiforme* and reference seagrass meadows over a three-year period in Tampa Bay. Using regression analysis of shoot density over time, they concluded 3.4 years were required to reach reference bed densities for both planted species combined, for plantings of 15-25 shoots, 0.5m on center. Data for the much slower growing climax species *T. testudinum*, is nonexistent, despite its rank as the third most commonly planted seagrass species, behind the temperate species *Zostera marina* L. and the tropical pioneer species *H. wrightii* (Fonseca *et al*., 1998). In addition, restoration efforts often employ plantings of *H. wrightii* to insure a more rapid coalescence of planting units (PUs), despite the ephemeral nature of the fast growing species. The high growth rate of *H. wrightii* is associated with a high mortality rate (Fonseca et al., 1998), although it is true that this pioneer species will form dense meadows much more quickly than *T. testudinum*, patches of *H. wrightii* present one season may be absent the next. In contrast, *T. testudinum* shoots persist 6 to 9 years, while the life expectancy of *H. wrightii* shoots averages a mere 3 months (Gallegos *et al*., 1993, 1994).

Thalassia testudinum seed production within Tampa Bay is relatively low compared to plants in the Keys and Biscayne Bay, and is insufficient to supply restoration efforts (Grey and Moffler, 1978; Lewis *et al.*, 1985; Witz and Dawes, 1995). Restoration efforts must, therefore, rely on planting adult material taken from donor beds, although data on the development rates of various types of planting units (PUs) is limited. Vegetative expansion for all seagrass beds relies on rhizome elongation along with short shoot, ramet production at regular intervals. Little work on the below ground productivity of *T. testudinum*, specifically rhizome growth rates, has been published. The data that have been gathered focus on established meadows, not PUs, as shown by studies from the Mexican Caribbean (Gallegos *et al.*, 1993) and South Texas (Kaldy and Dunton, 2000).

One of the features of *Thalassia testudinum* that has implications for plant growth is that genets are physiologically integrated (Dawes, 1998; Marbá et al, 2002); that is, the short shoots, or ramets, share resources via a common rhizome. Plants frequently span multiple microenvironments, where more productive ramets provide photosynthate for stressed shoots (Tomasko & Dawes, 1989; Andorfer, 2000). Because larger PUs have more resources for rhizome development, they produce new short shoots more rapidly, (Tomasko *et al*., 1991). Other seagrasses also benefit from clonal integration, as evidenced by small (< 5 Short Shoots plant $^{-1}$) *Cymodocea nodosa* (Ucria) Ascherson growing in bare patches of sediment displaying lower relative leaf growth rates than larger runner plants growing outward at the leading edge of established seagrass patches (Nielsen and Pedersen, 2000).

Tomasko *et al.* (1991) observed that PUs possessing rhizome meristems outpaced those lacking meristems in new short shoot production at the end of nine months, and concluded, as did Fonseca *et al.* (1998), that for transplant projects, plants with an intact rhizome apical meristem are the most productive. This is certainly true initially, but may not be so in the long term. Tomasko *et al*. (1991) collected, and planted in June, during active summer growth in Tampa Bay. However, newly transplanted *T. testudinum* PUs undergo a period of shock, in which roots die back, lasting up to two months (Dawes & Meads, personal observation); therefore, the plants in Tomasko *et al.* (1991) missed a large fraction of the peak growing season, with the study ending in March before the next period of productive summer growth. In this regard, *Thalassia testudinum* growing in Tampa Bay has an inter- annual pattern. Generally, leaf lengths increase in May and decline in November (Lewis *et al*., 1985; Dawes et al., 1997); similarly, *T. testudinum* growing in South Texas has higher rhizome growth rates during the same time period (Kaldy & Dunton, 2000). A study in which established transplanted PUs proliferate for the entire growing season has yet to be attempted.

Thalassia testudinum plants exhibit strong apical dominance; small PUs (e. g. rhizomes with two short shoots) with intact rhizome apical meristems do not form new meristems readily. New meristems are produced only by the short shoot apical meristems and then most commonly on rhizomes that have had the apical meristems removed (Dawes and Andorfer, 2002). Frequently, both short shoots of a two short shoot PU that lacks an apical meristem will produce new rhizome meristems (Dawes, personal observations). Plants with multiple rhizome meristems may eventually outpace single meristem plants in total rhizome growth and short shoot production and density.

Although it is accepted that larger *T. testudinum* PUs (e. g. 4 short shoots per rhizome versus 2 short shoots) have increased survivorship and produce new ramets more rapidly than smaller ones (Tomasko et al., 1991), the rhizome elongation, rhizome meristem production and short shoot production of different sized PUs have not yet been determined. Models of seagrass growth currently focus on the dynamics of already established meadows. A better understanding of PU development is needed in order to forecast planted bed development and plan restoration efforts with an emphasis on efficient resource utilization in PU preparation.

This study examines the effect of plant size, the number of short shoots, and the presence or absence of a rhizome meristem on rhizome elongation in *T. testudinum* PUs. The following aspects of *T. testudinum* PUs were examined:

Rhizome elongation: Gains in rhizome length are acquired through extensions of the primary axis meristem and any newly produced rhizome meristems arising from mature short shoots. Increases in rhizome length for PUs lacking a rhizome meristem are delayed until new apices are produced. Do larger planting units produce meristems more rapidly? In addition, Dawes and Meads (personal observations) observed smaller PUs (2-3 short shoots) that initially lacked a rhizome meristem produce new side branches within 6 months. Can PUs that produce multiple apices outpace single meristem plants in terms of total rhizome lengthening rate, or do multiple apices only share the same pool of resources? Finally, how does plant size (e.g. number of short shoots PU^{-1}) influence total rhizome elongation and meristem production in PUs?

Short shoot additions: Distances between short shoots along *T. testudinum* rhizomes vary on individual plants; and, more importantly, the distance between the youngest shoot and the tip of the rhizome meristem must be considered. Thus, will the ratio of new short shoot additions to the ratio of gains in rhizome length remain constant for different sized plants? Measures of rhizome elongation alone lend an upward bias to growth rates of slower growing plants, and short shoot additions may be a more reliable measure when comparing plants with widely varying growth rates. For example, one short shoot addition accompanied with two units of rhizome extension have a ratio of 1:2, while seven short shoots emerging from eight units of new rhizome exhibit a 7:8 ratio. Comparing growth rates using the values in this example gives a 1:4 comparison when considering rhizome length alone; the new short shoot comparison yields a value of 1:7. Otherwise, questions regarding short shoot additions are analogous to questions focusing on rhizome elongation.

Based on these questions, two null hypotheses were proposed regarding the development of *Thalassia testudinum* planting units:

- Increasing numbers of short shoots on intact rhizomes of *Thalassia testudinum* will not enhance rhizome meristem production, short shoot production, or total rhizome elongation.

- The absence of rhizome apices at planting will not enhance rhizome meristem production, short shoot production, or total rhizome elongation when compared to PUs planted with an active long shoot meristem.

MATERIALS AND METHODS

Site: The study was conducted northwest of Joseph's Island in lower Tampa Bay (27º35'30" N, 82º36'00" W). The area is characterized by low wave energy with average annual breaker heights less than 10cm (Tanner, 1960) and sandy beaches with a series of sand bars parallel to the beach that are exposed at low tide. The bars protect a continuous dense turtle grass meadow bordering the entire bayside of the island (Figure 1). Located at the Bay/Gulf margin, water clarity is superior to that of interior bay segments, and the sediment is coarse silica sand (0.125 mm, median grain size). Five sites were established between 120 and 220 m offshore among patchy seagrass beds northwest of a protected lagoon. Sites were selected to span a narrow range of water depths beginning just below the intertidal/subtidal fringe at the shallowest site:

1) 27º35'31.4'' N, 82º36'01.4" W, ca. -6 cm at MLLW
2) 27º35'32.6N, 82º36'02.1" W, ca. -21 cm at MLLW
3) 27º35'28.4" N, 82º36'11.5" W, ca. -35-50 cm at MLLW
4) 27º35'33.4" N, 82º36'03.5" W, ca. -48 cm at MLLW
5) 27º35'29.1'' N, 82º36'09.1" W, ca. -30 cm at MLLW

Figure 1: Aerial photograph of research area, showing Joseph's Island (J), the South Skyway rest area on I-275 (RA), the collection site for planting unit material (C), and research sites (1-5). Photo taken May 10, 2002. Image courtesy of the U.S. Geological Survey and TerraServer USA.

Experimental Units: Between July 24, 2002 and September 15, 2002 segments of *Thalassia testudinum* rhizome having two, four, or eight short shoots and intact rhizome apical meristems were collected southwest of the study site to avoid impacting reference meadows. Main axis apices were removed from half of the plants, thus all plants were of equivalent age. This precaution was taken because short shoot age will influence meristem production in *Thalassia testudinum* (Andorfer and Dawes, 2002; Dawes and Andorfer, 2002). Any secondary meristems arising from individual short shoots were removed. Four 2SS, two 4SS, and two 8SS PUs, half of each with and half lacking a meristem, were each affixed with plastic cable ties to a PVC burial frame (Figures 2 and 3). PVC burial frames were constructed with a 35 cm by 80 cm rectangle of 1.3 cm PVC pipe supporting a taught net of a 1 cm plastic mesh material. A colored cable tie identified the youngest ramet on each plant at the time of planting and the distance from this short shoot to the apical was measured.

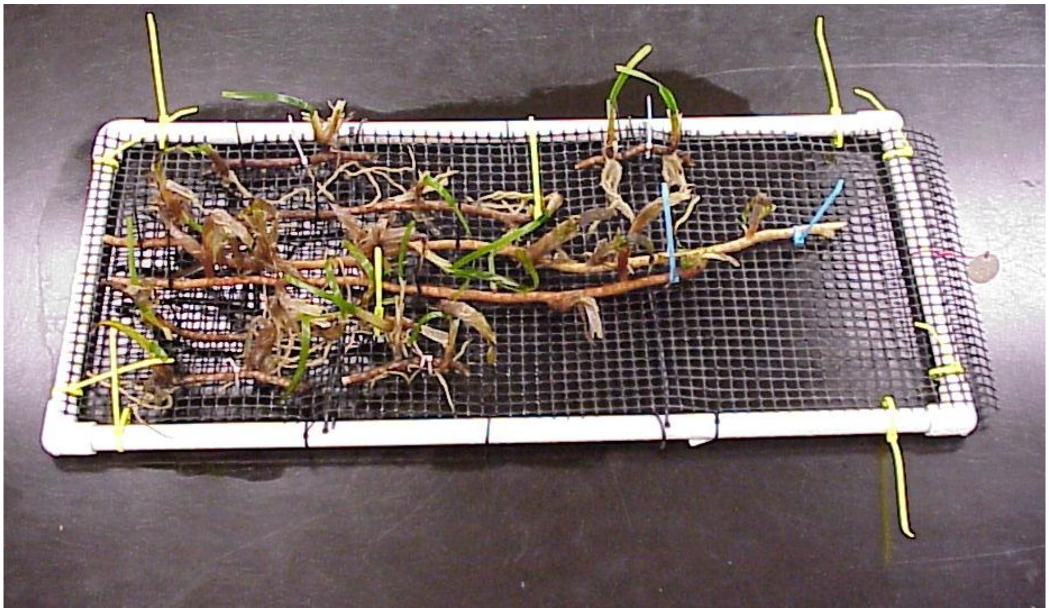

Figure 2: PVC Burial frame with attached and tagged *T. testudinum* rhizome segments.

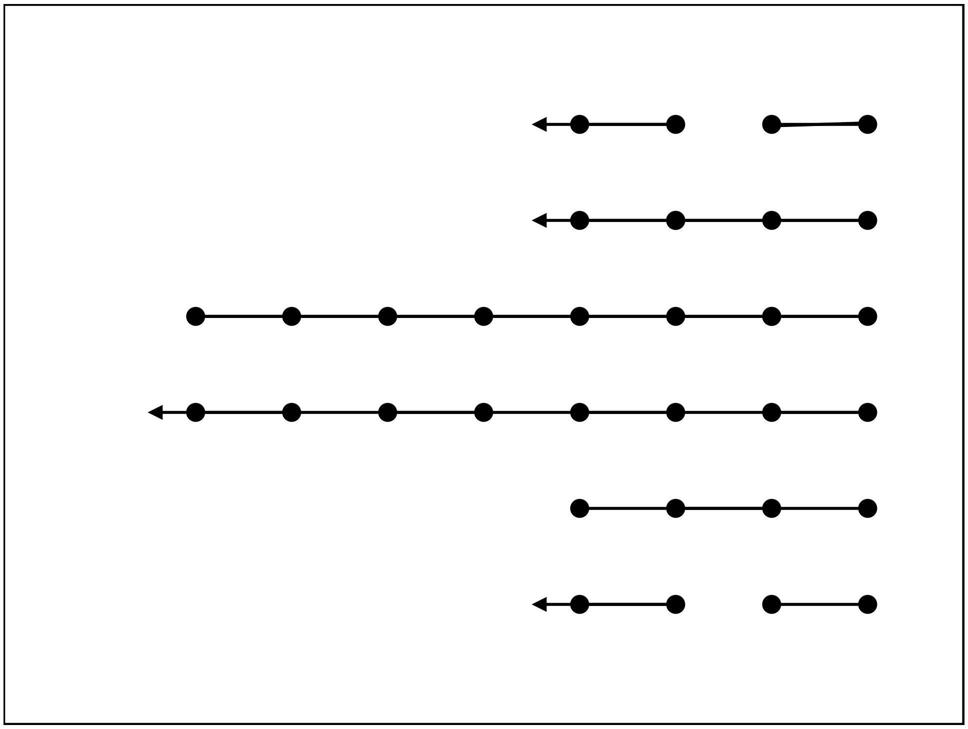

Figure 3: Diagram of typical PVC planting frame PU arrangement; circles represent short shoots and arrows represent rhizome meristems.

Ten PVC frames were transported to the five study sites, two to each site, and planted at a uniform depth (-6 to -50 cm MLLW) in areas of bare sediment near established *T. testudinum* beds. The apices were oriented away from seagrass beds towards areas of bare sediment (Figure 4).

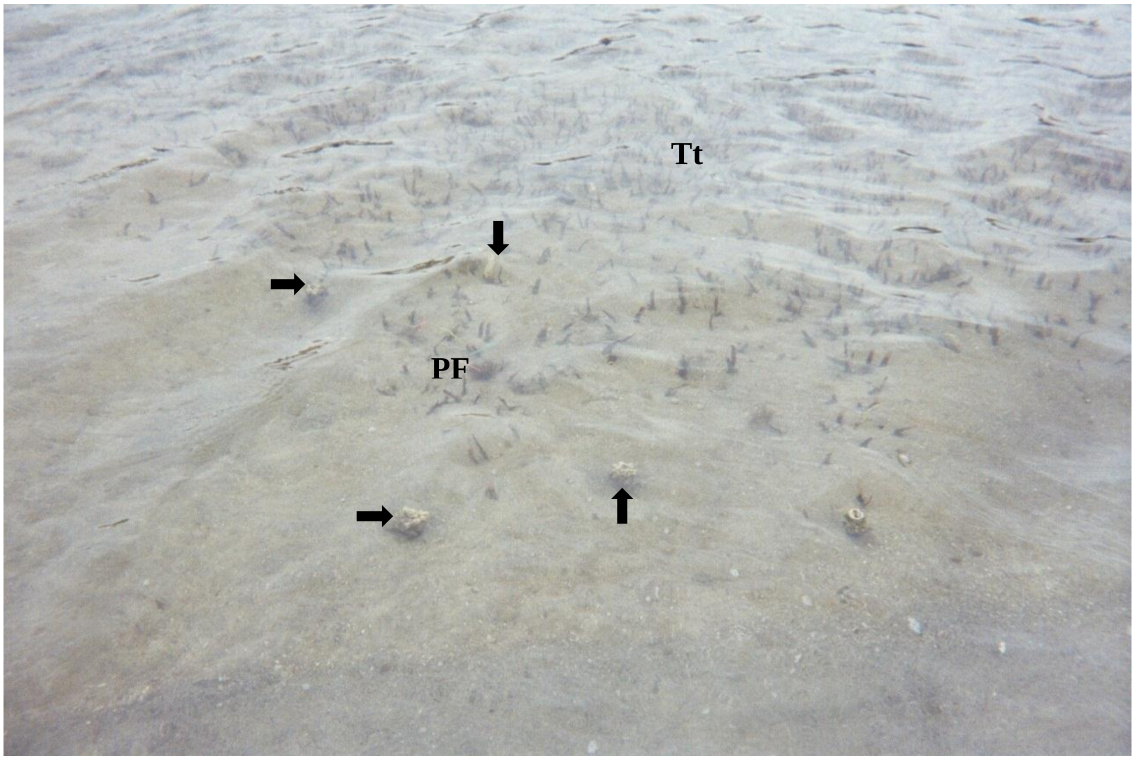

Figure 4: Planting frame (PF) adjacent to natural *Thalassia testudinum* bed (Tt). The four stakes marking the corners of the planting frame are indicated by arrows.

Analysis: After planting between July 24 and September 15, 2002, plants were harvested for analysis in November/December 2004 (ca. 26.5 months). Harvesting also occurred after 8 and 14.5 months but the data are not presented due to the success of the 26.5 month harvest. Two experimental units per treatment per site were collected (n=10), except for the smallest PUs where four were collected per site (n=20). PUs attached to PVC frames were carefully excavated to minimize breakage and placed intact in a plastic chest filled with seawater for transport back to the laboratory for the initial analysis, including measures of primary rhizome axis elongation, secondary rhizome elongation, short shoot additions, and PU survivorship.

Statistical Analysis: Two-Way Factorial Analysis of Variance (ANOVA) were used to test for differences in new short shoot production, new long shoot meristem production, and total rhizome elongation using SPSS 20.0 software. Data were transformed when necessary using the square root of the dependant variable to satisfy Levene's Test of Equality of Error Variances and analyzed at the $P < 0.05$ significance level. When necessary and no factor interactions were present, multiple comparisons were made using the Bonferroni adjustment with a 0.05 alpha level. Planting unit size (2, 4, or 8) and presence or absence of the rhizome meristem (M or X) were the independent factors for analysis.

RESULTS

Means of seagrass metrics were calculated from survivors only of 2 subsamples for each of the five sites, $6 \leq n \leq 11$ (Table 1). Survivorship of PUs was very similar across treatments from experimental units collected in November/December 2004, 26.5 months after planting. Table 2 presents data on new short shoots, new rhizome apices, and total rhizome elongation of plants tied to PVC racks after collection based on presence (M) or absence (X) of the rhizome meristem. After 26.5 months, total rhizome elongation including side branches of PUs ranged from 60.41 (2X) to 277.92 (8X) cm. Short shoots on plants with 4 or 8 initial short shoots with the rhizome apex removed (X) produced more side branches than those that retained the original apical meristem and had the highest level of rhizome elongation except for 2M (68.14 cm) versus 2X (60.41 cm). For both plant types (M and X plants), rhizome elongation, short shoot additions, and new meristem production increased as planting unit size increased.

Table 1: Survivorship of *Thalassia testudinum* planting units after 26.5 months in Tampa Bay. Treatment indicates the original number of short shoots on each planting unit and the presence of a long shoot apical meristem: M = plants with the initial rhizome meristem; X = plants that had the rhizome meristem removed.

Treatment	n	Survivors	Survivorship (%)
2M	20	11	55
2X	20	11	55
4M	10	7	70
4X	10	6	60
8M	10	6	60
8X	10	6	60
Total	80	47	59

Table 2: Means for new short shoot production, new rhizome meristem production, and rhizome elongation for the 26.5 month growth period. Means in each category are derived from n=2 subsamples from each of the 5 sites. M = plantings with rhizome meristems**;** X = plantings lacking a rhizome meristem.

Plant Size	N	New Short Shoots	Number New Long Shoot Meristems	Total Rhizome Elongation (cm)
2M	11	6.82	0.18	66.41
2X	11	4.91	1.18	60.41
2 (X+M)	22	5.86	0.68	63.41
4M	7	13.71	1.29	120.93
4X	6	17.67	3.00	192.08
4 (X+M)	13	15.54	2.08	153.78
8M	6	18.83	2.83	177.67
8X	6	29.17	5.67	277.92
8 (X+M)	12	24.00	4.25	227.79
M	24	11.83	1.17	110.13
X	23	14.57	2.83	151.50

Rhizome Elongation: At 26.5 months the total rhizome elongation mean for M plants, 110.13 cm, was not significantly different ($p = 0.112$) than the mean for X plants, 151.50 cm using the transformed (square root) data (Tables 2 and 3, Figure 6). Among the size classes, significant differences were detected among the means for 2SS, 63.41 cm; 4SS, 153.78 cm; and 8SS PUs, 227.79 cm ($p < 0.001$), (Table 3, Figure 5). There was no interaction between independent variables, Initial SS Number and Initial Meristem ($p = 0.870$).

In two of three sets of multiple comparisons, the larger size class produced significantly more new rhizome than the smaller size class at the $\alpha = 0.05$ level (Table 4). Total elongation means for 2SS plants (63.41 cm) were significantly different than 4SS (153.78 cm, $p = 0.001$) and 8SS (227.79 cm, $p < 0.001$) plants, but 4SS plants (153.78 cm) were not significantly different than 8SS (227.79 cm, $p = 0.242$).

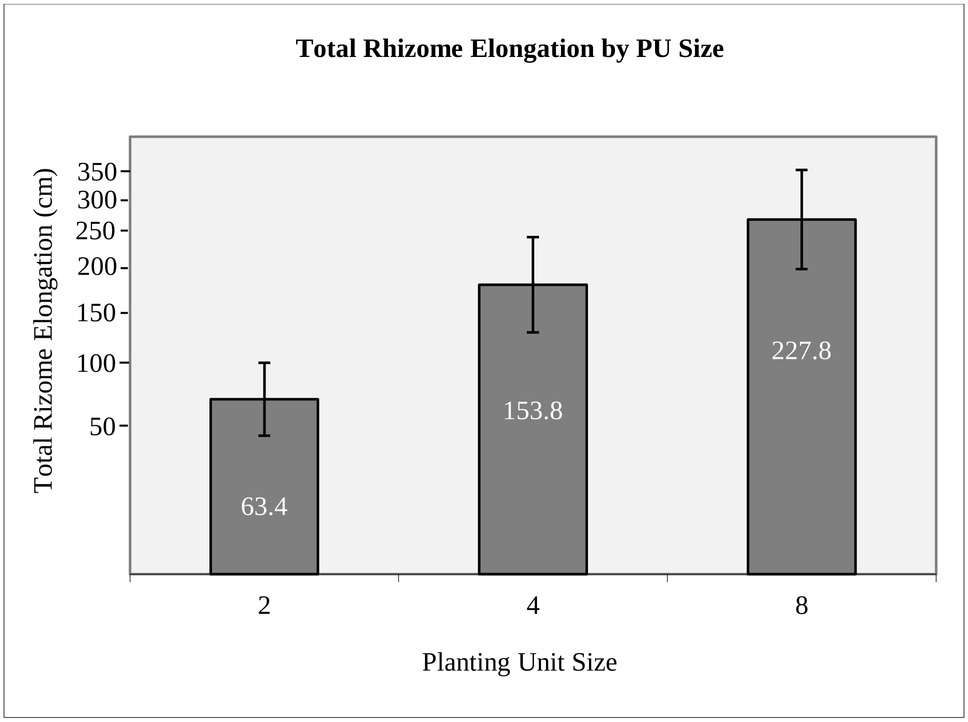

Figure 5: Total rhizome elongation by planting unit, PU, size after 26.5 months; PU sizes are the number of short shoots present at planting time. Recalibrating the Y-axis scale was necessary to reflect the true values, also represented in white lettering within the columns, after the square root transformation. Error bars represent the 95% confidence interval.

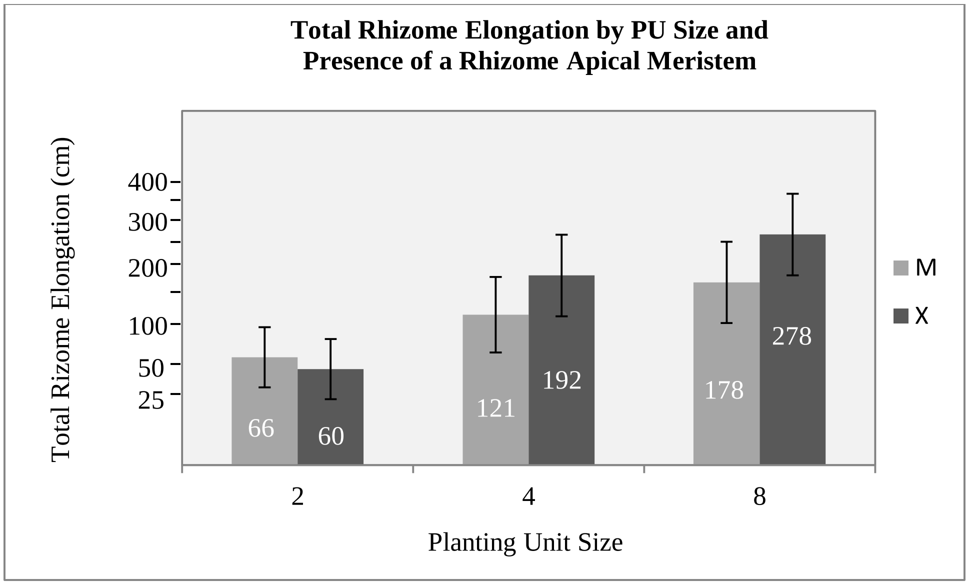

Figure 6: Total rhizome elongation by planting unit, PU, size and type, those with (M) and without (X) an intact rhizome meristem, after 26.5 months; PU sizes are the number of short shoots present at planting time. Recalibrating the Y-axis scale was necessary to reflect the true values, also represented in white lettering within the columns, after the square root transformation. Error bars represent the 95% confidence interval.

New Short Shoot Production: Results for new short shoot production were analogous to the results for rhizome elongation; with size classes pooled, means for M and X plants were 11.83 and 14.57 new short shoots produced, respectively. Using the transformed data (square root) the two-way factorial ANOVA showed no significant difference between M and X PUs for the factor Initial Meristem, $p = 0.401$ (Table 3, Figure 8) Significant differences were found among the size classes ($p < 0.001$); the means were 5.86 for 2SS, 15.54 for 4SS, and 24.00 for 8SS PUs (Tables 2 and 3, Figure 7). Again, there was no interaction between the two factors ($p = 0.091$).

Multiple comparisons revealed significant differences among treatments similar to that found for rhizome elongation (Table 4). Newly produced short shoot means for 2SS plants (5.86) were significantly different than 4SS (15.69, $p < 0.001$) and 8SS (24.00, $p < 0.001$) plants, but 4SS plants (15.69) were not significantly different than 8SS (24.00, $p = 0.145$).

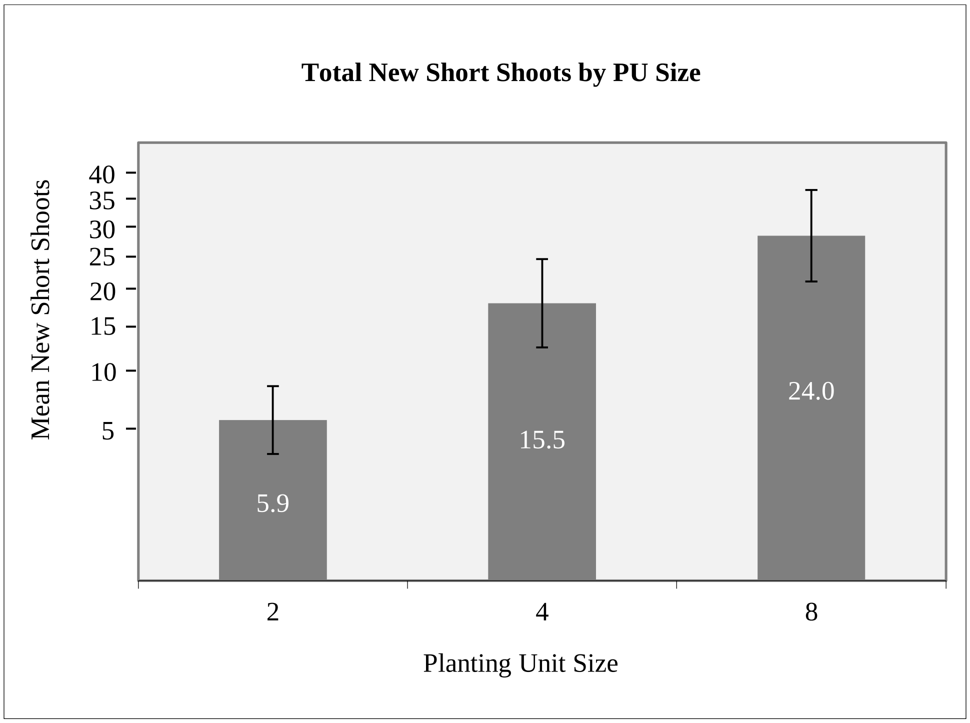

Figure 7: New short shoots produced by planting unit, PU, size after 26.5 months; PU sizes are the number of short shoots present at planting time. Recalibrating the Y-axis scale was necessary to reflect the true values, also represented in white lettering within the columns, after the square root transformation. Error bars represent the 95% confidence interval.

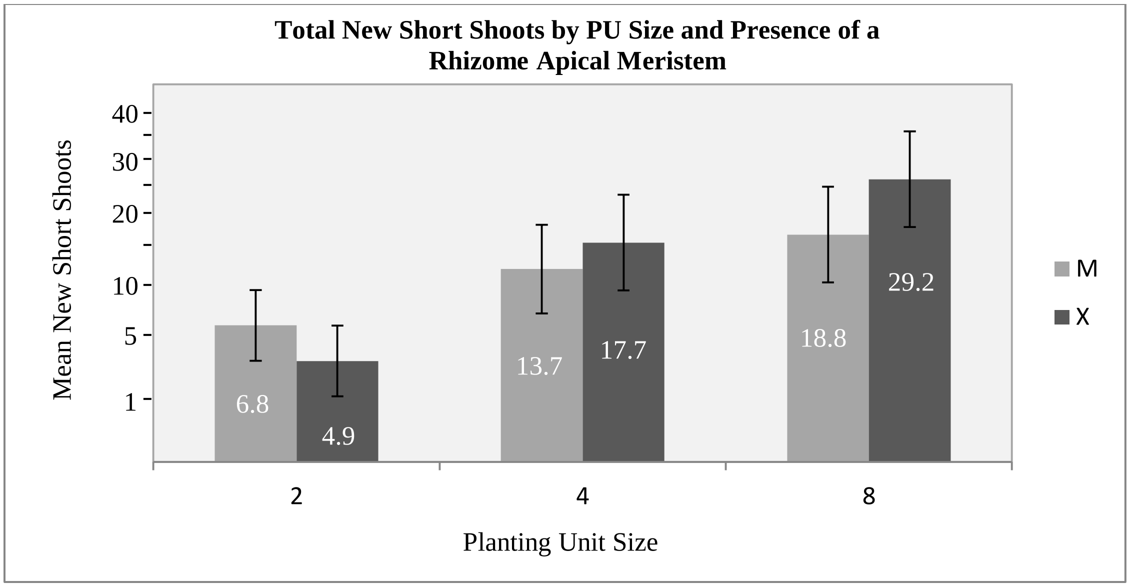

Figure 8: New short shoots produced by planting unit, PU, size and type, those with (M) and without (X) an intact rhizome meristem, after 26.5 months; PU sizes are the number of short shoots present at planting time. Recalibrating the Y-axis scale was necessary to reflect the true values, also represented in white lettering within the columns, after the square root transformation. Error bars represent the 95% confidence interval.

Table 3: Results of Two-Way Factorial ANOVA for the dependent variables: Total Elongation, New Short Shoots, and New Long Shoot Meristems. Analysis of Total Elongation and New Short Shoots relied on the square root transformation of the raw data; New Long Shoot Meristem data were not transformed.

Source of Variation	df	MS	*F*	*P*
Total Elongation				
Initial SS #	2	235.10	18.20	< 0.001
Initial Meristem	1	34.17	2.65	0.112
Initial SS # x Initial Meristem	2	22.54	1.75	0.870
Error	41	12.92		
New Short Shoots				
Initial SS #	2	27.24	22.15	< 0.001
Initial Meristem	1	0.89	0.72	0.401
Initial SS # x Initial Meristem	2	3.13	2.54	0.091
Error	41	1.23		
New Long Shoot Meristems				
Initial SS #	2	49.62	17.12	<0.001
Initial Meristem	1	37.32	12.87	0.001
Initial SS # x Initial Meristem	2	3.27	1.13	0.334
Error	41	2.90		

New Long Shoot Meristem Production: Plants that had the rhizome meristem removed (X plants) produced more new meristems in all size classes when compared to plants with apices (Table 2). Further, the overall new meristem production for X plants increased with increasing plant size, with 2X, 4X, and 8X PUs producing 1.18, 3.00, and 5.67 new long shoot meristems, respectively.

Planting units with an active rhizome meristem (M plants) also produced new meristems, with larger plants producing more than smaller plants. After 26.5 months 2M PUs produced 0.18 new meristems, while 4M and 8M PUs produced 1.29 and 2.83 new long shoot meristems, respectively.

The analysis of new long shoot meristem production relied on untransformed data, as no transformations could be found that satisfied Levene's Test of Equality of Error Variances. As such, these results should be considered with caution.

Plants lacking an apical meristem (X PU's) produced more new rhizome meristems than (M PUs), with means of 2.83 and 1.17, P = 0.001 (Tables 2 and 3, Figures 9 and 10). Significant differences were detected among the size classes as well, p < 0.001 (Table 2 and 3, Figure 10). There was no interaction between the two factors (p = 0.334).

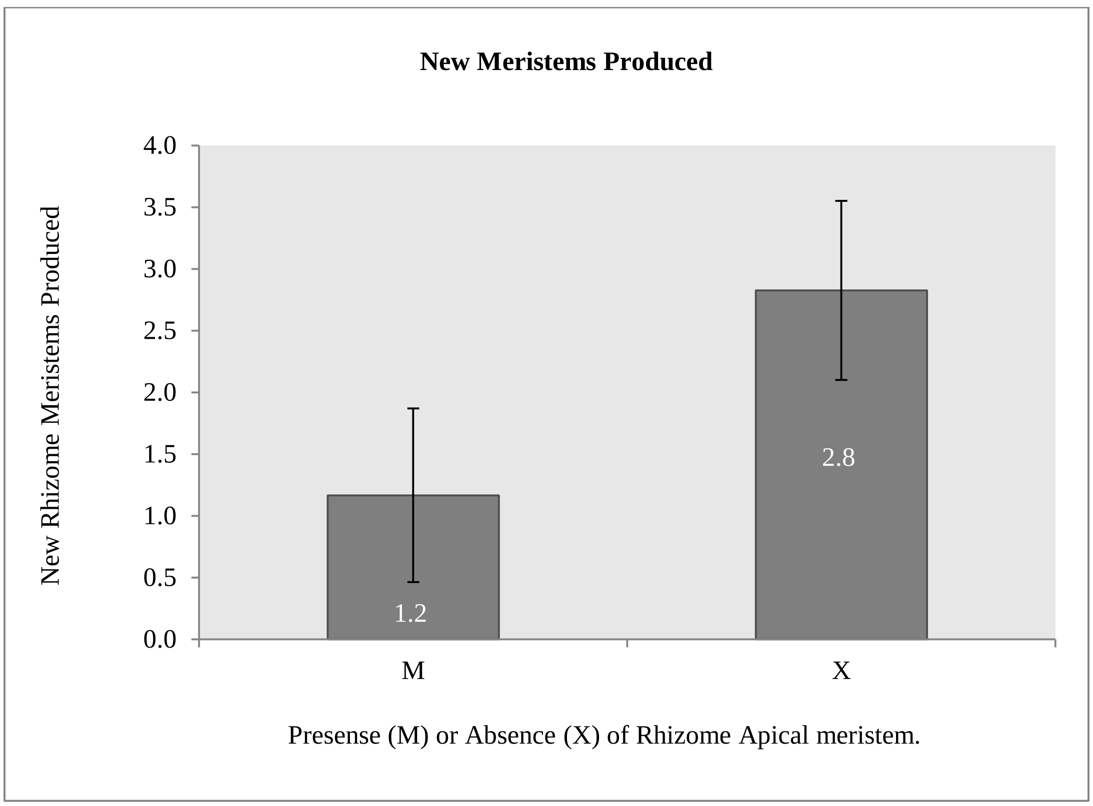

Figure 9: New rhizome meristems by planting unit, PU, type: those with and without an intact rhizome meristem, after 26.5 months. Error bars represent the 95% confidence interval.

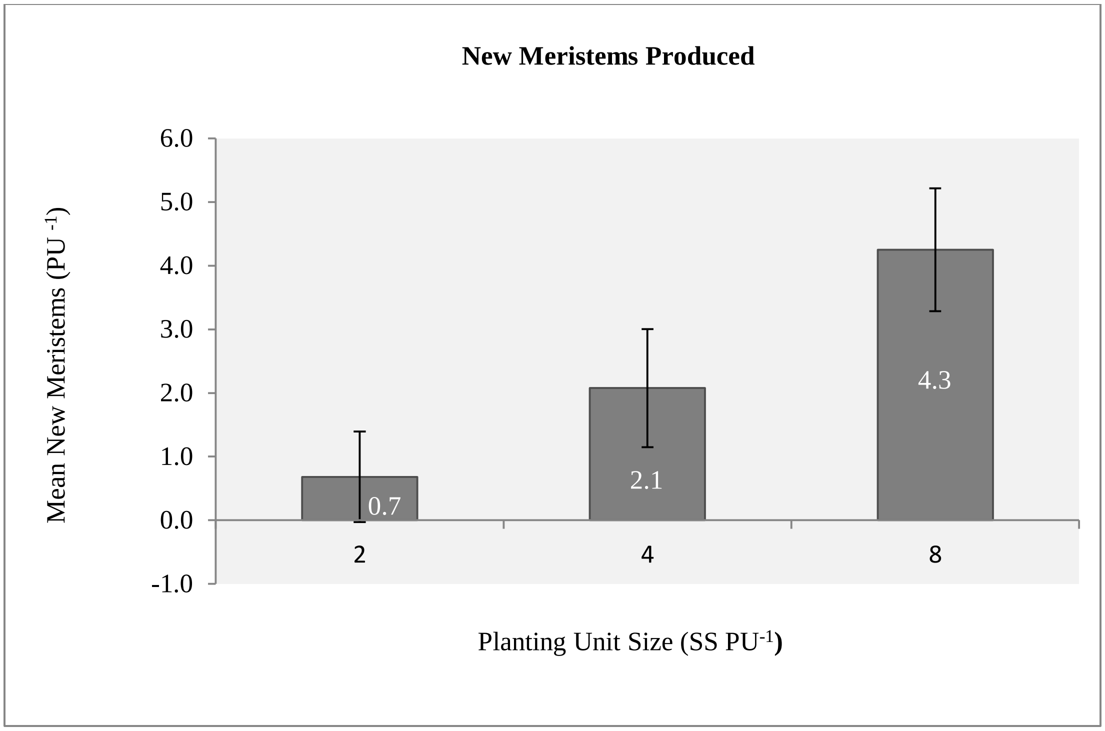

Figure 10: New rhizome meristems produced by planting unit, PU, size after 26.5 months; PU sizes are the number of short shoots present at planting time. Error bars represent the 95% confidence interval.

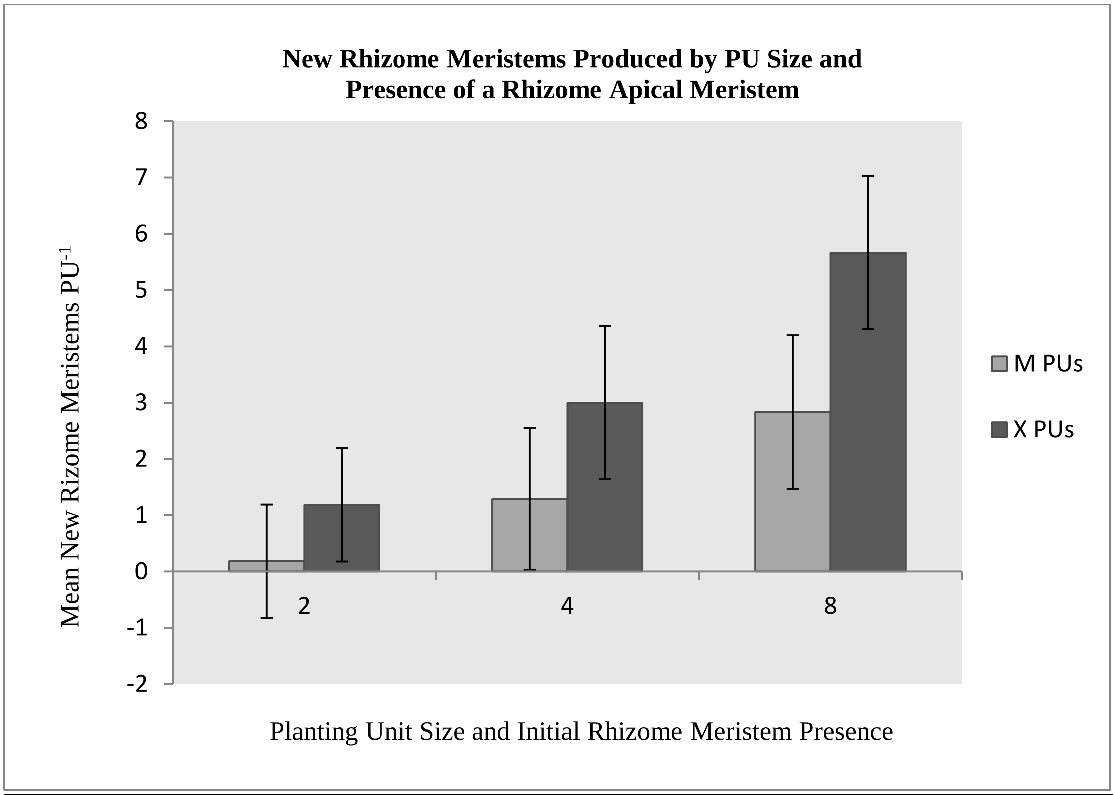

Figure 11: New rhizome meristems produced by planting unit, PU, size and type, those with (M) and without (X) an intact rhizome meristem, after 26.5 months; PU sizes are the number of short shoots present at planting time. Error bars represent the 95% confidence interval.

Significant differences were found between the size classes using the multiple comparison analysis. There was no significant difference in new long shoot meristem production for the 2SS/4SS, 0.68/2.08 new meristems, comparison, $p = 0.056$. The remaining comparisons were significant; the 2SS/8SS at $p < 0.001$, and 4SS/8SS at $p = 0.011$ (Table 4).

Table 4: Results of multiple comparison tests using the Bonferroni adjustment. Analysis of Total Elongation and New Short Shoots relied on the square root transformation of the raw data; New Long Shoot Meristem data was not transformed.

Dependent Variable	Comparison a/b	Mean Difference b-a	Means (Comparison a/b) a	b	Standard Error	p
Total Elongation	2/4	4.815	7.194	12.009	1.260	0.001
	2/8	7.396	7.194	14.590	1.290	< 0.001
	4/8	2.581	12.009	14.590	1.441	0.242
New Short Shoots	2/4	1.62	2.200	3.820	0.389	< 0.001
	2/8	2.525	2.200	4.725	0.398	< 0.001
	4/8	0.905	3.820	4.725	0.445	0.145
New Long Shoot Meristem	2/4	1.461	0.682	2.143	0.597	0.056
	2/8	3.568	0.682	4.250	0.611	< 0.001
	4/8	2.107	2.143	4.250	0.683	0.011

DISCUSSION

In contrast to estimates determined in previous studies (Patriquin, 1973; Gallegos *et al.*,1993; Kaldy and Dunton, 2000), rates of rhizome elongation, growth of new branches from short shoots, and short shoot production of *Thalassia testudinum* were directly measured in Tampa Bay, Florida using tagged plants.   The study design allowed comparisons of rhizome growth between PUs of different sizes and type, those with and without a long shoot meristem, tied to PVC racks. In contrast to the two null hypotheses, higher numbers of initial short shoots and absence of the primary long shoot meristem in planting units enhanced total rhizome growth, new short shoot production, and new long shoot meristem production.

Survival: *Thalassia testudinum* the dominant seagrass in Florida (Dawes et al., 2004), forms the climax seagrass communities in terms of habitat and faunal complexity (Zieman and Zieman, 1989). Unfortunately, unlike successful transplantation of more rapidly growing *Halodule wrightii* and *Ruppia maritima* (Fonseca et al., 1996, 1998), *T. testudinum* transplants require substantial time to establish and exhibit low survivorship unless the planting units initially have 4 or more short shoots (Tomasko et al., 1991). However, because survival data for *T. testudinum* is usually limited to less than a year, so it is difficult to measure relative long term success. The present study is aligned with results from a 9 month field study by Tomasko et al. (1991) because survivorship for all

PUs tied to PVC racks was 59% after 26.5 months (Table 1), very similar to values reported by Tomasko after 9 months 2SS PUs were 60% and 85% for 4SS PUs. Although, survivorship was a little higher in the 9 month Tomasko study, the PUs in the present study were left in place nearly 3 times as long, and thus were exposed to anthropogenic and bioturbation effects. In spite of the low survivorship at 26.5 months, the production of new short shoots (and rhizome growth) by X plants resulted in 2.5 to 4.4 times the initial number of short shoots (e.g. 2X: 4.9; 4X: 17.7; 8X: 29.2 short shoots) (Table 2). This suggests that larger X PUs are optimal for planting efforts; in this study the 4X PUs had the highest New SS/Initial SS ratio at 4.4.

Rhizome Elongation: When evaluating individual treatment means alone, plantings without an apical meristem produced more new rhizome than plantings with apical meristems in all but the 2SS size class. After 26.5 months, 4SS and 8SS plantings that lacked an apical meristem (X) produced 192.08 and 277.92 cm of new rhizome, while identically-sized plantings that had a meristem (M) produced 37.04% and 36.07% less of new rhizome than PUs without a meristem. These results follow observations by Dawes and Andorfer (2002), who noted a strong apical dominance in *T. testudinum* that inhibits production of side rhizome branches from existing short shoots. Further, this study suggests that loss of rhizome meristems or cutting of rhizomes may actually enhance vegetative expansion of turtle grass over the timescale examined here.

Means of total rhizome elongation rate for *Thalassia testudinum* planting units of varying sizes (2, 4, or 8 SS) and types (M or X) in south Tampa Bay, including lateral branches from short shoots, ranged from 27.36 cm yr^{-1} (2X) to 125.85 cm yr^{-1} (8X) for plants tied to PVC racks after 26.5 months. The majority of these values were within the range of previously reported rhizome extension rates for this species, while the highest value surpassed those previously reported (8X=125.85 cm yr^{-1}). Patriquin (1973) estimated rhizome extension rates for *Thalassia testudinum* of 80.3 cm yr^{-1} in Bermuda and 102.2 cm yr^{-1} in Barbados (originally expressed as mm d^{-1}), and Gallegos *et al.* (1993) estimated 22.3, 24.4, and 35.0 cm yr^{-1} in the Mexican Caribbean. The earlier work, however, relied on a method that estimates elongation from a single sampling event in established seagrass meadows. This method, first used by Patriquin (1973) in seagrasses, requires determination of the plastochrone interval (PI), or the time interval between the initiations of consecutive plant parts (e.g. leaves and short shoots) to determine production rates for these parts. Thus the methods formerly used to assess possible elongation may need to be carefully reconsidered.

In Patriquin (1973), the leaf PI was determined by observing leaf growth during summer 1969 for Barbados and August 1970 for Bermuda, both which are the most productive season for seagrasses. Because multiplying the rate blades are produced (from using summer data) increases estimated rhizome elongation rates by an equal multiple using this technique, the Patriquin study very likely provides an inaccurate comparison to this study. Extrapolating yearly growth rates based on summer data (Patriquin never intended a yearly rate, giving a mm d^{-1} rate instead, but did assume a constant rate of leaf production over time) overestimates rhizome elongation rates basing conclusions on a summer PI instead of using a more reliable yearly PI accounting for all seasons, including periods of low productivity, as noted by Kaldy and Dunton (2000) for *T. testudinum* in Laguna Madre, Texas. Gallegos *et al.* (1993) employed a yearly PI in determining growth rates making their work the better comparison. Further, using the PI to estimate rhizome extension approximates a single axis rate, not a total rhizome growth rate including growth from all apices (original + newly produced) as in this study. My data are expresses as "total rhizome elongation" in order to compare planting units that had a rhizome meristem with those that did not, and the single axis rhizome elongation rates can be determined. Here M plants after 26.5 months had primary rhizome elongation rates that ranged from 30 – 49 cm yr^{-1} which exceeds or overlaps the highest values of Gallegos *et al.* (1993) values (22.3-35.0 cm yr^{-1}).

Removal of the rhizome meristem at the beginning of the study (X PUs) resulted in the greatest total rhizome elongation as a result of growth of side branches arising from existing short shoots when considering individual treatment means, although analysis of all M and X plants via Two-Way Factorial ANOVA showed no significant difference, with elongation means of 110.13 cm for total M plants and 151.50 cm for total X plants (Table 2). Thus, X plants were not significantly more productive than M plants after 26.5 months, although the trend in the data shows otherwise. This demonstrates the importance of conducting studies that span longer time periods to understand more completely the productivity of different types of planting units. In addition, the experimental design for this study was inadequate to reveal a statistical difference between M and X PUs. In the future an increase in replication may address this issue.

Pairwise comparisons of the 3 size classes revealed significant differences in two of three comparisons. The 2SS/4SS and 2SS/8SS comparisons were significant, producing P values of 0.001 and < 0.001, respectively; the 4SS/8SS comparison was not significant, $p = 0.242$. However, the Two-Way Factorial ANOVA detected a difference among all size classes, $p < 0.001$. These findings indicate that larger PUs do produce new rhizome at a greater rate than smaller PUs.

Short Shoot Production: The number of short shoots produced over time is linked to rhizome growth, the number of initial short shoots, and the presence or absence of a primary rhizome apical meristem at planting. These results follow from the findings from this field study. For example, after 26.5 months 8X PUs produced more new short shoots than 4X PUs and X plants more than M plants (4X: 17.67 SS versus 4M: 13.71 SS; 8X: 29.17 SS versus 8M: 18.83 SS). In contrast, 2M PUs produced slightly more new short shoots than 2X PUs (2M: 6.82 SS versus 2X: 4.91 SS) after 26.5 months. The Two-Way Factorial ANOVA analysis of M/X planting units produced similar results to the total elongation analysis; M/X mean differences were not significant after the length of this study (Table 3).

A significant difference in short shoot production among all size classes was detected in this field study. When M and X plants were pooled within size classes, pairwise comparisons of new short shoot production were significantly different for 2 of the 3 comparisons. These results thus mirror those from rhizome elongation comparisons (Table 4).

Again, treatment means from the raw data showed clear trends; X PUs produced more new short shoots than M PUs and successively larger PUs produced more new short shoots than smaller ones. While these were not statistically different, future studies should improve upon the experimental design used here.

Long Shoot Meristem Production: Analysis of pooled size classes to compare long shoot production in M and X plants demonstrated the importance of rhizome meristem removal in planting units for greater new long shoot initiation. X plants displayed the ability to produce higher numbers of new long shoot meristems. Likewise when M and X plants were pooled; differences in size classes were recorded, with more long shoot initiation noted for each increasingly larger size classes. This suggests that removal of rhizome meristems and use of 4 SS PUs may be a valuable technique to maximize PUs' productive efficiency.

Conclusions: Rhizome growth, including production of lateral branches by *Thalassia testudinum* over 26.5 months in the Tampa Bay was high (60.4-277.9 cm) and greatest for plants that lacked a long shoot meristem, as demonstrated by the ranges of treatment means: 66.4-177.7 cm and 60.4-277.9 cm for plants with and without a primary rhizome meristem, respectively. A negative relationship existed between formation of rhizome branches and the presence of an initial intact long shoot meristem at planting time, indicating apical dominance. Larger planting units and planting units that initially lacked an intact long shoot apical meristem produced more total rhizome elongation, more new short shoots, and more new long shoot apical meristems. While statistical analysis supported most of these claims, an improved experimental design with more replication likely would have improved the ability to detect significant differences among treatments.

Statistical analysis did not verify a difference between PUs that lacked a long shoot meristem and PUs that had a long shoot meristem at planting time or support the idea that X PUs will produce greater total rhizome elongation and greater new short shoot production than M PUs**.** However, results presented here provide strong evidence that directly contradict claims made previously (Tomlinson, 1974; Tomasko et al., 1991; Fonseca *et al.*, 1998) that PUs with an intact rhizome apical meristem are more productive than PUs that lack an intact rhizome apical meristem.

LIST OF REFERENCES

Andorfer JH 2000. Physiological integration and translocation of Carbon and Nitrogen in the seagrass Thalassia testudinum Banks ex König. Ph. D. Dissertation. University of South Florida, Tampa, Florida.

Andorfer J and Dawes CJ. 2002. Production of rhizome meristems by the tropical seagrass *Thalassia testudinum*: the basis for slow recovery into propeller scars. Journal of Coastal Research. 37:130-142.

Dawes CJ 1998. *Marine Botany*, 2nd Ed. John Wiley and Sons, Inc., New York, New York. 408 pp.

Dawes CJ and Andorfer JH. 2002. Production of Rhizome Meristems by *Thalassia testudinum*. pp. 185-197 in Greening, HS (ed.), Proceedings, Seagrass Management: It's Not Just Nutrients! St. Petersburg, FL. Tampa Bay Estuary Program. 246 pp.

Dawes CJ, Andorfer J, Rose C, Uranowski C, Ehringer N. 1997. Regrowth of the seagrass *Thalassia testudinum* into propeller scars. Aquatic Botany 59:139-155.

Dawes, C.J., R. C. Phillips, and G. Morrison. 2004. Seagrass Communities of the Gulf Coast of Florida: Status and Ecology. Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, and the Tampa Bay Estuary Program. St. Petersburg, FL. iv + 74 pp.

Fonseca MS, Kenworthy WJ, Courtney FX. 1996. Development of planted seagrass beds in Tampa Bay, Florida, USA. I. Plant components. Marine Ecology Progress Series 132:127-139.

Fonseca MS, Kenworthy WJ, Thayer GW. 1998. Guidelines for the Conservation and Restoration of Seagrasses in the United States and Adjacent Waters. NOAA Coastal Ocean Program Decision Analysis Series No. 12. NOAA Coastal Ocean Office, Silver Spring, MD. 222 pp.

Gallegos ME, Marino M, Marba N, Duarte CM. 1993. Biomass and dynamics of *Thalassia testudinum* in the Mexican Caribbean: elucidating rhizome growth. Marine Ecology Progress Series 95:185-192.

Gallegos ME, Marino M, Rodriguez A, Marba N, Duarte CM. 1994. Growth patterns and demography of pioneer seagrasses *Halodule wrightii* and *Syringodium filiforme*. Marine Ecology Progress Series 109:99-104.

Grey WF and Moffler MD. 1978. Flowering of the seagrass *Thalassia testudinum* (Hydrocharitaceae) in the Tampa Bay, Florida Area. Aquatic Botany 5:251-259.

Kaldy KE and Dunton KH. 2000. Above- and below-ground production, biomass and reproductive ecology of *Thalassia testudinum* (turtle grass) in a subtropical coastal lagoon. Marine Ecology Progress Series 193:271-283.

Lewis RR, Durako MD, Moffler MJ, Phillips RC. 1985. Seagrass meadows of Tampa Bay: a review. in Treat SF, Simon JL, Lewis RR, Whitman RL (eds) Proceedings, Tampa Bay Area Scientific Information Symposium (May 1982). Burgess Publishing Co, Minneapolis, p 210-246.

Marbà, N, Hemminga, MA, Mateo, MA, Duarte, CM, Mass, YEM, Terrados, J, Garcia, E. 2002. Carbon and nitrogen translocation between seagrass ramets. Mar. Ecol. Prog. Ser. 226:287-300.

Nielsen SL and Pedersen MF. 2000. Growth, photosynthesis and nutrient content of seedlings and mature plants of Cymodocea nodosa—the importance of clonal integration. Aquatic Botany 68:265-271.

Patriquin D 1973. Estimation of growth rate, production and age of the marine angiosperm *Thalassia testudinum* König. Caribbean Journal of Science 13:(1-2)111-123.

Tanner, WF. 1960. Florida coastal classification. Transactions of the Gulf Coast Association Geological Society 10:259-266.

Tomasko DA, Dawes CJ, Hall MO. 1991. Effects of the number of short shoots and presence of the rhizome apical meristem on the survival and growth of transplanted seagrass *Thalassia testudinum*. Contributions in Marine Science 32:41-48.

Tomasko DA and Dawes CJ. 1989. Evidence for physiological integration between shaded and unshaded short shoots of Thalassia testudinum. Marine Ecology Progress Series 54:299-305.

Tomlinson, PB. 1974. Vegetative morphology and meristem dependence – the foundation of productivity in seagrasses. Aquaculture. 4:107-130.

van Tussenbroek, BI. 2002. Static life-table analysis and demography of the foliar shoots of the tropical seagrass *Thalassia testudinum*. Bulletin of Marine Science 7:1247-1256.

Witz MJA and Dawes CJ. 1995. Flowering and short shoot age in three Thalassia testudinum meadows off west-central Florida. Botanica Marina 38(5):431-436.

Zieman, JC. and Zieman, RT. 1989. The ecology of the seagrass meadows of the west coast of Florida: a community profile. Biological Report 85 (7.25). U. S. Fish and Wildlife Service, Washington, D.C. 155 pp.