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FILIFORME IN OUTER FLORIDA BAY, FLORIDA

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ABSTRACT

Leaf growth of the seagrass *Syringodium filiforme* (Kütz., 1860) was determined using a new technique based on the growth of emergent leaves (EL method) and compared to the more labor intensive repeated measurements (RM) and demographic allometric age reconstruction techniques (DA). All three techniques were used to compare leaf growth dynamics of plants with different morphologies at two sites, a shallow water (0.5 m) banktop and an adjacent deeper water (1.5 m) environment in outer Florida Bay, Florida. Leaf formation rates (Leaf Plastochrone Interval or PI) determined using the EL and RM methods were nearly identical, with means of 20 and 21 d leaf^{-1} at both sites, significantly faster than the 30 d leaf^{-1} calculated using the DA method. The EL method produced the highest estimate of leaf growth, 1.8 and 1.9 cm d^{-1} at the 0.5 m and 1.5 m sites, respectively, followed by the RM method (1.3 and 1.3 cm d^{-1}) and the DA method (1.0 and 1.1 cm d^{-1}). None of the methods detected differences in leaf PI, leaf growth or leaf fragmentation rates between sites. However, leaves at the 1.5 m site typically retained intact leaf tips longer than those at the 0.5 m site, and total leaf lifespan was longer at the 1.5 m site. Based on these results and the amount of field and laboratory work required by each of the methods, the new EL method is the preferred technique for monitoring leaf growth in *S. filiforme*.

Syringodium filiforme (Kütz., 1860) (Fig. 1), an abundant and widely distributed seagrass species in the Caribbean region (Zieman, 1982; Dawes, 1998; Green and Short, 2003), grows in a variety of habitats ranging from shallow bank tops, to deeper open water environments in both oligotrophic and mesotrophic waters (Iverson and Bittaker, 1986; Kenworthy and Fonseca, 1996; Kenworthy and Schwarzschild, 1998; Fourqurean et al., 2001). Often observed growing in mixed species assemblages with *Thalassia testudinum* (Banks ex. König, 1805) or *Halodule wrightii* (Aschers, 1864) (Williams, 1990; Kenworthy and Fonseca, 1996), *S. filiforme* also grows in nearly monospecific beds (Zieman, 1982; Kenworthy and Schwarzschild, 1998; Fourqurean et al., 2001). In the Florida Keys, monospecific stands of *S. filiforme* can reach leaf biomass values of 100–500 g dw m^{-2} and canopy heights of 0.5–1.0 m, comparable to *T. testudinum* (Short et al., 1993; Gallegos et al., 1994; Kenworthy and Schwarzschild, 1998; Rose et al., 1999) with leaves serving as important sources of organic matter and physical structure (Zieman et al., 1979; Mortimer, 1981; Tribble, 1981; Zieman, 1982; Brown-Peterson et al., 1993; Rose et al., 1999; Lefebvre et al., 2000). *Syringodium filiforme* leaves are highly buoyant and are easily transported long distances following senescence and fragmentation. As a result, *S. filiforme* meadows do not generate dense leaf litter layers, but instead, export significant amounts of biomass and nutrients to adjacent systems (Zieman et al., 1979; Zieman, 1982; Fry and Virnstein, 1988). Based on the regional abundance, wide distribution and valuable ecological services that *S. filiforme* provides, it is important to quantify the leaf growth dynamics of this species in order to better understand its ecological role in coastal ecosystems.

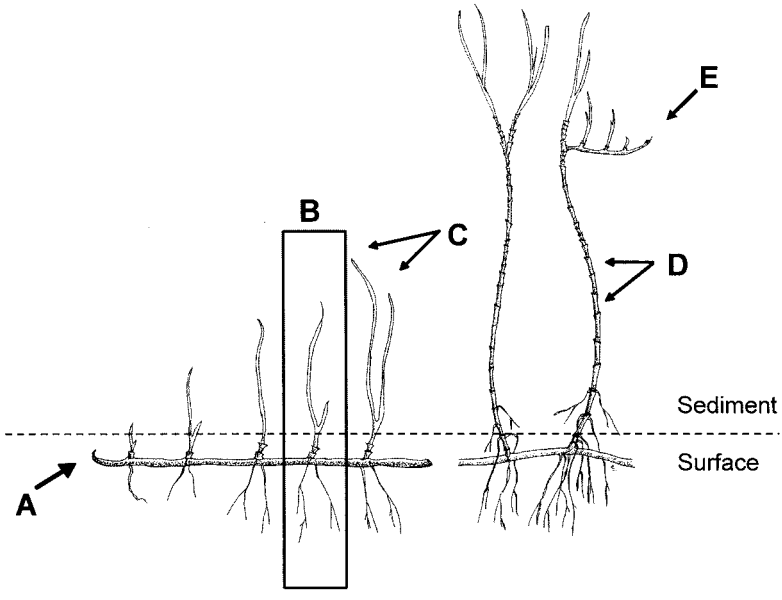

Figure 1. Illustration of sections from a *Syringodium filiforme* plant. Dashed line indicates the location of the sediment water interface. (A) indicates the location of the rhizome apical meristem. (B) indicates an individual ramet which is defined as a short shoot attached to the parent plant and includes leaves, stem (vertical rhizome), a section of rhizome and roots. (C) indicates leaves attached to a young ramet. (D) indicates leaf scars on the stem of an older ramet. (E) indicates a rhizome branch with a new rhizome apical meristem produced in the water column. Branches can also be generated below the sediment surface.

Currently the most widely used methods for measuring leaf productivity in a large number of seagrass species are modifications of the hole-punch technique (Zieman, 1984; Barber and Behrens, 1985; Dennison, 1990; Kenworthy, 1991; Short and Duarte, 2001). Originally based on a leaf marking method developed by Zieman (1974) for monitoring *T. testudinum* leaf growth, these methods are not effective for the narrow, cylindrical leaves of *S. filiforme* which fragment easily when manipulated or damaged, resulting in the loss of marked leaves and an underestimate of growth and productivity. Canopy clipping followed by harvesting new leaves has been used to generate estimates of seagrass growth potential, but typically underestimates natural growth rates due to the loss of photosynthetic material and physiological stress (Cebrian et al., 1998; Hauxwell et al., 2001; Kowalski et al., 2001; Alcoverro and Mariani, 2005). Estimates of *S. filiforme* leaf growth have also been derived by monitoring plants grown in mesocosms, but this is time consuming, expensive, and requires the assumptions that growth in mesocosm conditions accurately reflects those in the field (Short et al., 1993).

Fry (1983) utilized repeated measurements of tagged plants to monitor *S. filiforme* leaf growth and fragmentation rates in the Indian River Lagoon, FL. While labor intensive, repeated measurements (RM) provide accurate estimates of leaf growth. Along with leaf growth rates, Fry (1983) reported several important ways in which *S. filiforme* leaf growth differs from that of *T. testudinum* and *H. wrightii*. He observed that, unlike *T. testudinum* and *H. wrightii,* where all but the oldest leaves are growing at different rates, virtually all *S. filiforme* leaf growth occurs on just the youngest emergent leaf (Fig. 1). Furthermore, leaf growth in *T. testudinum* and *H. wrightii*

steadily decline as leaves age, whereas *S. filiforme* leaves grow at a nearly constant rate until maturity when growth stops with the simultaneous emergence of a new leaf. Further analysis of Fry's data also allows for the estimation of leaf lifespan and the leaf formation rate. Based on these characteristics, it should be possible to improve the Fry (1983) method and estimate *S. filiforme* leaf productivity with a "one time" marking of old leaf tips followed by harvesting marked shoots and measuring growth of new leaves only, eliminating the need to conduct expensive and time consuming repeated field measurements. This method would not be as labor intensive as Fry's method and would allow for estimates of productivity across wider spatial and temporal scales. It would also improve on demographic and allometric techniques because it does not depend on reconstructing growth over entire seasons or the life span of the plants (Duarte et al., 1994; Kenworthy and Schwarzschild, 1998; Short and Duarte, 2001) and would be more comparable to data generated using the hole-punch technique.

The main objective of this research was to compare leaf growth dynamics of *S. filiforme* plants with different leaf morphologies in a range of environmental conditions typical for this species. These environmental conditions include relatively high energy, shallow water banktops and lower energy, deeper water sites. Additionally, we sought to develop and validate a time and cost effective method for monitoring *S. filiforme* leaf dynamics which could be utilized across this range of environmental conditions.

Materials and Methods

Study Site.—Two sampling sites were established in nearly monospecific *S. filiforme* meadows at Sprigger Bank on the western margin of Florida Bay. A shallow water bank top site (0.5 m) (24°54.777′N, 080°56.273′W) was located on the northeast end of Sprigger Bank, where water depth ranged tidally from approximately 0.5–1.0 m. A deeper water site (1.5 m) (24°54.731′N, 080°56.193′W) was located approximately 300 m to the east of the bank, where water depths ranged tidally from 1.5–2.0 m. Approximately 85% of surface irradiance reaches the top of the leaf canopy at the 0.5 m site, with 70% of surface irradiance penetrating to the bottom. At the 1.5 m site, only 40% of surface irradiance reaches the top of the canopy with 20% penetrating to the bottom (Schwarzschild, 2004). Tidal velocities and wave energy are both higher at the 0.5 m site compared to the 1.5 m site (Schwarzschild, 2004).

Leaf Growth Measurements.—*Syringodium filiforme* leaf growth and leaf formation rates, defined as the time interval between the emergence of two successive leaves and commonly referred to as the Leaf Plastochrone Interval (PI) (Brouns, 1985), were determined using three independent methods: (1) the Repeated Measurements (RM) method, a modification of the technique used by Fry (1983); (2) the Demographic-Allometric (DA) method (Duarte, 1991), and (3) the Emergent Leaf (EL) method, a new technique based on Fry's (1983) observations and a modification of methods used by Kenworthy and Schwarzschild (1998). The RM method also provided estimates of the leaf fragmentation rates and total leaf lifespan (defined as the time interval between the emergence and loss of a leaf). The DA method also provided estimates of leaf lifespan, the length of intact mature leaves [defined as leaves with intact leaf tips that were not the youngest leaf on a ramet (short shoot)] and ramet population structure. The data generated by all three methods were used to compare the leaf dynamics of *S. filiforme* plants with different morphologies growing at the two study sites. Additionally, the results of the three methods were compared to examine the relative magnitude and differences of each growth estimate and to determine the cost effectiveness (least labor- and time- intensive way of determining *S. filiforme* leaf growth) of each method.

Repeated Measurements Method (RM).—On July 29, 2001, 50 ramets from each site were haphazardly selected and tagged with numbered cable ties. A thin strip (~ 2 mm wide) of foil tape was wrapped around the stem, below the leaf mersitem, of each tagged ramet to serve as a base-mark for leaf measurements. Every 2–7 d, leaves on tagged ramets were measured from the base-mark to the leaf tip, and leaf condition was recorded as either a new leaf, a mature leaf with intact leaf tip, or a fragmenting leaf without a leaf tip (Fig. 1) through September 3, 2001. Leaf growth curves (Fig. 2) for the tagged ramets were generated and analyzed to determine leaf PI and the initiation of fragmentation (# days between leaf emergence and loss of the leaf tip). The curves were decomposed into three sections; (1) growth phase, (2) zero net growth phase (when leaf growth had stopped or was balanced by fragmentation), and (3) fragmentation phase. The slopes of these curve sections were used to calculate leaf growth and fragmentation rates. Only curve sections containing a minimum of three valid data points with an r^2 value of > 0.85 were included in the analyses. This ensures that the curve segments used in the analysis were collected from the period of constant leaf growth, as opposed to during the transition periods of leaf initiation or senescence. A *t* test was used to compare the mean leaf PI, and growth and fragmentation rates at each of the study sites.

Demographic-allometric Method (DA).—In August 2001, three replicate 0.25 m^2 sod samples were collected at each of the sites for demographic and allometric analyses by haphazardly tossing a 50 cm × 50 cm PVC quadrat from a boat, cutting the rhizomes around the perimeter of the quadrat and extracting the entire sod from the sediment. In the lab, sods were rinsed free of sediments and the number of standing leaves, leaf scars, ramet age (calculated as the sum of the leaf scars (nodes), and the standing leaves on a ramet) were recorded for all ramets connected to rhizomes (Duarte et al., 1994; Kenworthy and Schwarzschild, 1998). All intact, mature leaves (leaves with identifiable leaf tips, which were not the youngest leaf on a ramet) were measured. The mean number of leaves per ramet and mean leaf length for each sod were calculated.

Age-frequency histograms were generated to compare the population age structure of the sites and analyzed for the existence of cohort peaks (see Kenworthy and Schwarzschild 1998). The number of PI between peaks is an indication of the number of leaves formed in a year. Therefore, the distance between cohort peaks provides an estimate of the average leaf PI over the course of one year (Durako, 1994; Durako and Duarte, 1997; Kenworthy and Schwarzschild, 1998). A second estimate of leaf PI was derived by observing sequences of short and long leaf scar internodes on the oldest ramets collected (Duarte et al., 1994; Gallegos et al., 1994; Kenworthy and Schwarzschild, 1998). This estimate averages leaf PI over the lifespan of the ramets measured. These average leaf PI values which are in units of d $leaf^{-1}$ can then be used to estimate the lifespan of leaves or the age (in days) of individual ramets.

The DA data were used to estimate leaf growth rate and lifespan at each site using the following equations:

$$Leaf\ growth\ rate\ (cm/d) = \frac{Mean\ mature\ leaf\ length\ (cm)}{Leaf\ PI\ (d)} \quad (1)$$

$$Leaf\ lifespan\ (d) = Mean\ number\ of\ standing\ leaves\ on\ a\ ramet - Leaf\ PI\ (d) \quad (2)$$

and the mean values for each site were compared using a *t* test.

Emergent Leaf Method (EL).—Leaf PI and leaf growth rates at each site were measured four times during July and August 2001. Six replicate 10 cm × 20 cm wire quadrats were deployed at each site and the leaves on ramets within the quadrats were clipped no more than 1 cm below the leaf tip, leaving identifiable scars. After 10–20 d the ramets were harvested and transported to the lab where the numbers of marked ramets in each quadrat were counted; unmarked ramets were discarded. Marked ramets were observed for the presence of new leaves, recognized as young leaves with intact tips, and the leaf PI for each quadrat was calculated as:

$$Leaf\ PI\ (d) = \left(\frac{Growth\ period\ (d) * Number\ of\ marked\ ramets}{Number\ of\ ramets\ with\ new\ leaves} \right) \quad (3)$$

The mean leaf PI at each site during each monitoring period were computed and used to calculate the mean leaf PI for the growing season. Leaf growth rates were derived by measuring the longest new leaves from each of the EL sampling quadrats and dividing by the number of days between marking and harvesting. This calculation assumed that the longest new leaves had formed shortly after the ramets were marked.

Selecting an appropriate time interval between marking and harvesting of the ramets is critical to the successful use of the RM method. If the time interval is too short there is the possibility that none of the marked ramets will generate new leaves before they are harvested, making it impossible to calculate leaf PI. Additionally, shorter time periods result in fewer new leaves and a smaller sample size for the determination of leaf growth. If, however, the time interval between marking and harvesting is too long, some ramets may generate multiple new leaves making it difficult or impossible to differentiate between marked and unmarked ramets. In areas subject to significant grazing pressure, loss of leaf tips and marked leaves due to grazing may also confound results. From previous studies in the area, we have determined that the optimum time interval is between ½–¾ of the leaf PI (Schwarzschild, 2004) which we estimated at 20–30 d and used to set the 10–20 d period between marking and harvesting. The mean Leaf PI and growth rates calculated for each site were compared using a *t* test.

Methods Comparisons.—The data for each method were pooled across sites and the pooled datasets used to compare leaf growth rates and leaf PIs determined by each method. Observations on the minimum amount of field and laboratory time needed to generate the data was also recorded.

Results

Repeated Measurements Method (RM).—The mean leaf growth rate at both sites was 1.3 cm d^{-1} (Table 1). The mean fragmentation rate at the 0.5 m site was 1.5 cm d^{-1} and was not significantly different from the mean of 1.6 cm d^{-1} determined for the 1.5 m site. Leaf growth was not significantly different from fragmentation at either site, indicating that the leaf canopy was in steady state, with leaf growth balancing leaf fragmentation. The leaf PIs determined from the growth curves (Fig. 2, Table 1) for the 0.5 m and 1.5 m sites were 20 and 21 d respectively, and were not significantly different, yielding a mean PI of 20 d $leaf^{-1}$. Significant differences were, however, detected in both the onset of leaf fragmentation and the lengths of the zero net growth period observed at the two sites. New leaves at the 0.5 m site lost their leaf tips and began to fragment after 18 d, a significantly shorter period than the 29 d observed for leaves at the 1.5 m site. The mean zero net growth period of 8 d determined for the 0.5 m site was significantly shorter than the mean of 13 d observed at the 1.5 m site. This indicates that while the leaf formation, growth and fragmentation rates are the same at both sites; the total leaf lifespan is significantly shorter at the 0.5 m site compared to the 1.5 m site.

Demographic-allometric Method (DA).—The age structures of the ramets sampled at both sites were similar (Table 2), and the data were pooled to generate a single age frequency histogram (Fig. 3). The main cohort peak (new recruits) observed on the pooled age frequency histogram is located at 4 PI (Fig. 3) with a much smaller cohort peak (recruitment peak after 1 yr) at 12 PI, indicating that ramets produce 12 leaves annually (Kenworthy and Schwarzschild, 1998), equivalent to an annual mean PI of 30 d.

From the 1869 ramets aged, the 12 oldest, unbranched ramets were sub-sampled for analysis of cyclical patterns in leaf scar internode length sequences (Fig. 4). The mean number of nodes between peaks of maximum node length was 12, indicating that ramets produced 12 leaves per year, with an annual mean leaf PI of 30 d (Table 3). This is the same as the annual mean leaf PI determined from the cohort analysis.

Table 1. Mean *Syringodium filiforme* leaf growth parameters and standard errors (SE) for the two study sites determined using the RM method. Fifty ramets were tagged at each site, but ramets were lost throughout the monitoring period. For growth, fragmentation and zero-growth parameters, n indicates the number of leaf curve sections used in the calculations. For initiation of fragmentation and plastochrone interval, n indicates the number of ramet growth curves that captured these events. Significant differences between sites were identified with a *t* test ($\alpha = 0.05$).

	0.5-m site			1.5-m site			
Leaf growth parameter	Mean	SE	n	Mean	SE	n	P
Growth (cm d^{-1})	1.3	0.07	43	1.3	0.07	30	0.95
Fragmentation (cm d^{-1})	1.5	0.19	16	1.6	0.13	23	0.66
Initiation of frag. (d)	18	2.40	8	29	1.80	6	0.00
Zero-growth period (d)	8	1.00	18	13	0.70	30	0.00
Plastochrone interval (d)	20	1.30	9	21	0.80	4	0.35

The mean length of mature leaves with intact leaf tips at the 0.5 m site was 29.8 cm, and was not significantly different from the mean of 33.5 cm determined for the 1.5 m site (Table 3). Since only one leaf of a ramet was observed growing at a time, the leaf growth rate can be estimated by dividing the mean mature leaf length by the leaf PI. This yields growth rates of 1.0 and 1.1 cm d^{-1} at the 0.5 and 1.5 m sites, respectively. Ramets at the 0.5 m site had fewer leaves than those at the 1.5 m site, 2.1 vs 2.4, respectively (Table 3), indicating that the leaf lifespan at the 0.5 m site was 63 d, which is 9 d shorter than the lifespan of 72 d determined for leaves at the 1.5 m site (Table 3).

Emergent Leaf Method (EL).—The mean leaf PI values at the two sites (Tables 4 and 5) were not significantly different, and when pooled yielded a mean Leaf PI of 21 d $leaf^{-1}$. The leaf growth rates at the 0.5 and 1.5 m sites determined from the EL method were 1.8 and 1.9 cm d^{-1}, respectively, and were not significantly different (Table 5).

Combined Data and Methods Comparison.—None of the three methods detected differences in leaf growth or leaf formation rates (Leaf PI) between sites (Tables 1, 3, 4, and 5). As a result, site data were pooled and the pooled data were used to compare the three methods (Fig. 5A). The highest leaf growth rate was determined with the EL method and the lowest

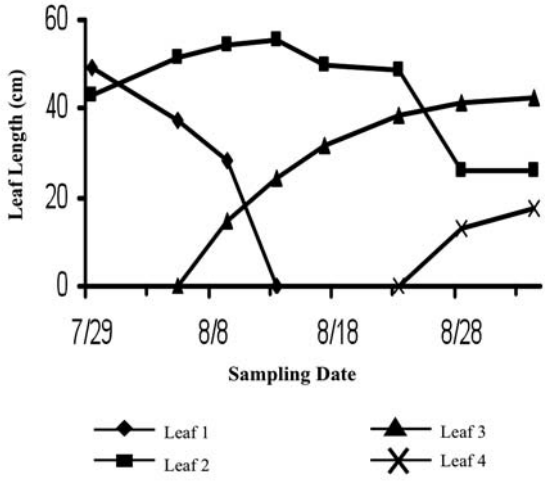

Figure 2. *Syringodium filiforme* sample leaf growth plot for one of the monitored ramets. Each line represents the growth of an individual leaf. Leaf 1, the oldest leaf on the ramet at the start of the monitoring, did not have an intact leaf tip and continued to fragment throughout the monitoring period. Leaf 2 lost its leaf tip on 8/6, but growth and fragmentation were roughly in balance until 8/13. Leaves 3 and 4 are new leaves formed during the monitoring period. The time interval between the formation of leaves 3 and 4 is equivalent to the leaf PI.

Table 2. *Syringodium filiforme* ramet age (in Leaf PI) structure from sod samples collected at both sites for the DA method. Rep indicates the replicate sod sample analyzed. n = the number of intact ramets that were aged.

Site	Rep	n	Mean	Median	Mode	Max
0.5 m	1	385	7.0	6	4	19
	2	219	8.5	7	4	26
	3	192	7.3	7	4	22
	Mean		**7.6**	**6.7**	**4.0**	
1.5 m	1	416	7.2	6	4	18
	2	260	8.0	7	5	17
	3	397	7.3	6	5	29
	Mean		**7.5**	**6.3**	**4.7**	
Pooled			7.4	6.3	4	

with the DA method. Leaf PI determined by the RM and EL methods were similar but shorter than the estimate determined by the DA method (Fig. 5B).

An idealized plot of *S. filiforme* leaf dynamics for both of the study sites (Fig. 6) was generated utilizing data from the three monitoring methods. The leaf growth rates determined from the RM and EL methods were averaged yielding mean rates of 1.55 and 1.60 cm d^{-1} for the 0.5 and 1.5 m sites respectively. Maximum leaf lengths for each site were determined from the DA data (Table 3), while the length of the zero net growth period and leaf fragmentation rates were derived from the RM data (Table 1). Leaf lifespans of 43 and 49 d for the 0.5 and 1.5 m sites respectively were calculated (Equation 2) by multiplying the number of standing leaves $ramet^{-1}$ determined with the DA method (Table 3) by the mean leaf PI determined from the RM and EL methods (Tables 1 and 4). The leaf PIs from the RM and EL methods were used in place of those derived using the DA method since it was determined that these values more accurately reflected the summer time growth. Results indicate a constant growth rate until leaves reach maturity when growth rate rapidly declines to zero. Maximum leaf height, and the length of the zero net growth period are slightly greater at the 1.5 m site compared to the 0.5 m site. Leaf fragmentation begins earlier at the 0.5 m site. Once fragmentation has been initiated, however, leaf fragmentation rates are similar between sites and remain constant until the entire leaf has been lost.

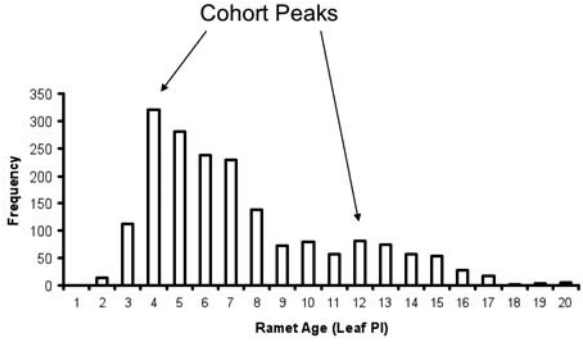

Figure 3. Pooled age frequency histogram for all of the *Syringodium filiforme* ramets collected and aged at the 0.5 and 1.5 m study sites using the DA method. Ramets were aged by counting the numbers of standing leaves and leaf scars on individual ramets. The main cohort peak observed at 4 PI and the secondary peak at 12 PI are identified with arrows.

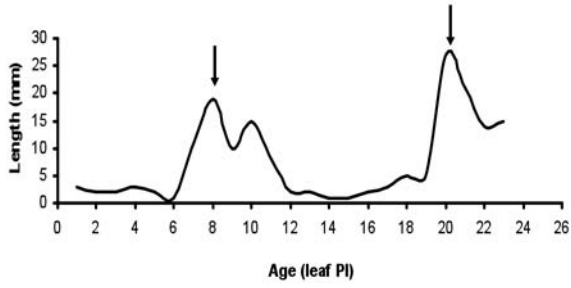

Figure 4. Sample plot depicting the variation in leaf scar internode lengths for one of the 12 oldest *Syringodium filiforme* ramets collected. Peaks (identified with arrows) in leaf scar internode length correspond to annual growth maximum. The number of internodes between the peaks indicates the number of leaves produced in a year.

Discussion

Syringodium filiforme Leaf Growth Dynamics.—The results of this study show that *S. filiforme* leaf formation (PI) and growth rates are similar for plants growing on a bank top in 0.5 m of water and plants growing adjacent to the bank in 1.5 m of water. At both sites, leaf growth was balanced by leaf fragmentation, suggesting that leaf standing crop remained fairly constant during this portion of the growing season. However, the total leaf lifespan was longer at the 1.5 m site, indicating that leaf turnover occurred more rapidly on the bank top. Leaf fragmentation was initiated earlier, and the zero net growth period was significantly shorter at the 0.5 site compared to the 1.5 m site. We observed that the bank top leaves lost their tips while still actively growing. In contrast, leaves at the 1.5 m site generally maintained intact leaf tips until reaching maximum length, and fragmentation did not begin until after an extended period of no growth. These differences in leaf fragmentation patterns and lifespan are most likely the result of exposure to higher tidal current velocities, increased wave turbulence, longer periods of low tide exposure and/or variations in grazing pressure at the shallower 0.5 m site compared to the 1.5 m site (Schwarzschild, 2004).

Table 3. Mean *Syringodium filiforme* leaf growth parameters with standard errors in brackets for the two study sites determined using the DA method. The mean leaf lengths and number of leaves ramet^{-1} for each of the three replicate sods collected at both sites were calculated and compared using a t-test of the means. Leaf PI was derived from the 12 oldest ramets collected at the two study sites resulting in a single Leaf PI estimate for both sites, and was the same as that derived from the analysis of the pooled age frequency histogram. Leaf growth rate = Leaf length / Leaf PI. Leaf lifespan = Number of leaves ramet^{-1} * Leaf PI.

	0.5-m site		1.5-m site		P
Leaf growth parameter	Mean	n	Mean	n	
Leaf length (cm)	29.8 (1.65)	3	33.6 (1.86)	3	0.13
Number of leaves ramet^{-1}	2.1 (0.02)	3	2.4 (0.03)	3	0.01
Leaf PI (d leaf^{-1})		30 (0.55)	n = 12		
Leaf growth rate (cm d^{-1})	1.0		1.1		
Leaf lifespan (d)	63		72		

Table 4. Time interval between the initiation of successive *Syringodium filiforme* leaves (Leaf PI) calculated by the EL Method. The mean leaf PIs calculated from the six replicate quadrats for each site during each of the four marking periods are presented, along with the seasonal site means. Standard errors are in brackets. Sampling period PIs were compared using a *t* test, and the P-values are presented in the table. A *t* test was used to compare the mean values for the entire sampling season. While there was a significant difference observed between the sites during the third monitoring period, no significant differences were observed during the other periods, or in the seasonal means as indicated by $P > 0.05$.

Marking period	Date marked	Date harvested	No. days in marking period	0.5-m site PI (d)	1.5-m site PI (d)	*t* test P
1	7/6/01	7/19/01	13	19.9 (0.6)	20.4 (1.1)	0.76
2	7/19/01	7/28/01	9	23.0 (2.4)	19.4 (1.6)	0.24
3	7/29/01	8/8/01	10	19.4 (0.9)	22.4 (0.6)	0.02
4	8/12/01	9/23/01	10	19.8 (0.6)	20.5 (0.4)	0.38
Mean				20.5 (0.8)	20.6 (0.6)	0.94

The longer leaf lifespan observed at the 1.5 m site may be an important factor that enables plants to grow in deeper water, where light availability is reduced. Since the carbon and nitrogen content of leaves at the adjacent 0.5 and 1.5 m sites are similar (Schwarzschild, 2004), increasing the leaf lifespan results in a reduction in the construction costs associated with leaf formation (Spencer et al., 1997). Leaf construction cost can be viewed as the ratio of physiological energy expanded in the formation and growth of leaf tissue to the net photosynthetic output of the tissue (Chiariello et al., 1989; Griffin, 1994). If their physiological state allows them to continue to actively photosynthesize up to and after reaching maximum length (zero net growth phase), leaves with a longer lifespan have higher net photosynthetic output than shorter-lived leaves, resulting in a larger energetic gain for the plant (Chiariello et al., 1989; Griffin, 1994; Spencer et al., 1997). Increased energetic gain from longer lived leaves may support the increased vertical growth of photosynthetic and non-photosynthetic tissue allowing the plants to extend vertically upward to minimize self shading, colonize deeper water environments, propagate vegetatively and outcompete other species (Kenworthy and Schwarzschild, 1998).

Methods Evaluation and Comparison.—Accurate and cost effective techniques for estimating leaf growth are important tools needed for conducting eco-

Table 5. *Syringodium filiforme* leaf growth rates calculated by the EL method. The mean leaf growth rates calculated from the six replicate quadrats for each site during each of the four marking periods are presented, along with the seasonal site means. Standard errors are shown in brackets. Sampling period growth rates were compared using a standard *t* test, and a *t* test of means was used to compare the seasonal means. While there was a significant difference observed between the sites during the second monitoring period, no significant differences were observed during the other periods, or in the seasonal means as indicated by $P > 0.05$.

	0.5-m site		1.5-m site		
Marking period	Number of leaves measured	Leaf growth (cm d^{-1})	Number of leaves measured	Leaf growth (cm d^{-1})	*t* test P
1	28	1.7 (0.04)	54	1.7 (0.03)	0.28
2	11	1.9 (0.03)	28	2.1 (0.06)	0.00
3	20	1.8 (0.05)	22	1.9 (0.06)	0.30
4	14	1.7 (0.07)	24	1.8 (0.04)	0.67
Mean		1.8 (0.06)		1.9 (0.09)	0.10

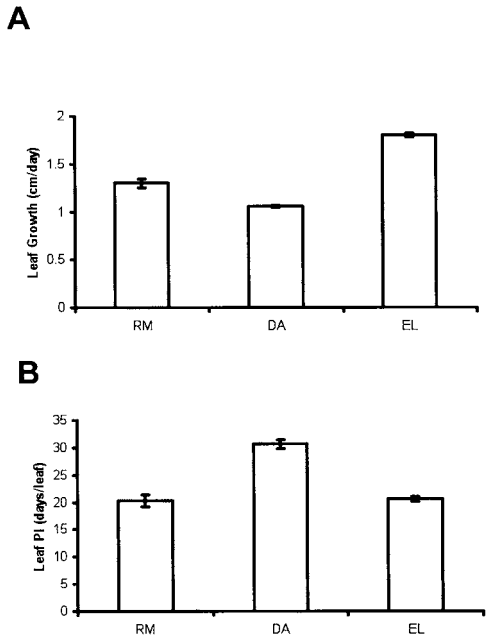

Figure 5. (A) Mean *Syringodium filiforme* leaf growth rates pooled across sites for each of the 3 methods. (B) Mean *S. filiforme* leaf PI values pooled across sites for each of the three methods.

logical studies in seagrass systems; including studies of net productivity, nutrient cycling, food web dynamics and seagrass response to environmental stressors. *Syringodium filiforme* is an important component of seagrass ecosystems throughout the western Atlantic, Caribbean, and Gulf of Mexico and in some locations it can dominate the biomass of seagrasses while either permanently or temporarily substituting for *T. testudinum* and *H. wrightii* (Williams, 1987, 1990; Kenworthy and Schwarzschild, 1998; Fourqurean et al., 2001). However, few studies have measured *S. filiforme* leaf growth and productivity because it is extremely tedious and difficult. We evaluated three methods for monitoring and measuring leaf growth of this tropical seagrass under different environmental conditions related to water depth. The methods included two previously utilized techniques [repeated measurements of tagged plants (RM) and demographic and allometric reconstructive aging (DA)] and a new method, based on the emergence and growth of new leaves (EL) (Fry, 1983;

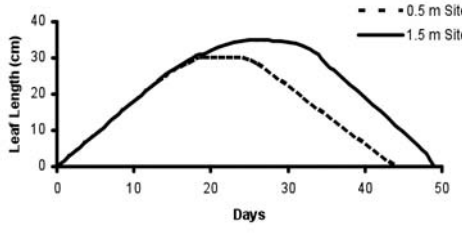

Figure 6. Schematic representation of the generalized leaf growth patterns for *Syringodium filiforme* at the 0.5 and 1.5 m study sites generated using data from the three monitoring methods. Leaf growth and fragmentation rates were similar at both sites. However, leaf fragmentation begins earlier at the 0.5 m site, and the length of the zero net growth period is longer at the 1.5 m site. Therefore, maximum leaf length and leaf lifespan are greater at the 1.5 m site.

Duarte et al., 1994; Kenworthy and Schwarzschild, 1998; Short and Duarte, 2001). The results exposed biases and potential errors associated with the three methods but confirmed that the EL technique requires the least time and effort for obtaining leaf PI and growth estimates.

Leaf growth rate estimates obtained using the EL method were higher than either the RM or DA estimates, suggesting that marking the plants by clipping just the tips of the standing leaves did not have a negative impact on new leaf growth unlike what has been observed following grazing or removal of larger amounts of leaf tissues (Cebrian et al., 1998; Hauxwell et al., 2001; Kowalski et al., 2001; Alcoverro and Mariani, 2005). Differences in the estimates provided by these three techniques can be explained by examining the assumptions behind each method and the specific metrics used in the calculation steps. A comprehensive evaluation of each method should also take into account the logistics and costs associated with data collection to determine if the increased expenses are justified by greater accuracy or precision of the results obtained.

Methods Assumptions and Weaknesses.—The DA method is based on reconstructing the growth cycle for one year, or over the entire lifespan of the oldest ramets in a population. Therefore, the leaf PI and growth estimates generated using this method are either annual or lifetime averages. This explains why the estimate of the annual mean leaf PI derived from the DA data in this study (30 d) was considerably higher than the estimates of leaf PI generated by the RM (20.5 d) and EL (20.5 d) methods during the summer growth peak. The higher leaf PI values (longer time interval between the emergence of two successive leaves) suggest that leaf PI varies seasonally, with leaves formed more quickly in the summer than in the winter, thus leading to the higher annual estimate but underestimating summer growth.

In the DA method leaf growth rates are calculated by dividing mean leaf length by the leaf PI. Thus, overestimating leaf PI (30 d vs 20 d) will result in an underestimate of leaf growth. Additionally, leaf lengths may vary seasonally, further affecting the results. For these reasons, DA analysis will tend to underestimate growth during the summer months and overestimate growth in the winter. Therefore, estimates of leaf growth generated exclusively by DA analysis will not be as accurate and are not directly comparable to those derived from shorter-term, season-specific measurements of leaf growth parameters, like those obtained by the RM and EL methods.

Since the RM analysis derives growth rates from sections of leaf growth curves, it will underestimate the mean leaf growth rate if the curve segments used contain data points near the end of the active growth period, when leaf growth is slowing. This can be minimized by carefully screening the curve segments used in the analysis. In contrast, by using only the longest new leaves from the samples collected, the EL method is biased towards the maximum growth rate, especially if several individuals in the population have above average growth rates. However, including shorter leaves would potentially underestimate leaf growth, as it is probable that the shorter leaves were formed several days after the ramets were marked. Despite these confounding factors, the leaf growth rates generated during this study from all three techniques are within the range of leaf growth rates reported in the literature for *S. filiforme* in the Indian River Lagoon, FL, and St. Croix, US Virgin Islands (Fry, 1983; Williams, 1987; Fry and Virnstein, 1988).

Comparison of Field and Laboratory Time Requirements.—The RM method required monitoring the tagged ramets every 2–7 d in the field. Under ideal

weather and water clarity conditions it took approximately 3 hrs to establish each monitoring plot and 1 hr to measure and record leaf lengths at each sampling site on the subsequent visits. Poor water quality greatly increased the time needed to monitor the tagged ramets, and inclement weather occasionally made monitoring impossible, creating unwanted gaps that impacted data acquisition and analysis. Entry and analysis of the data collected using the RM method was also time consuming, requiring the generation and visual inspection of individual growth plots for each monitored shoot after which selected growth curves were decomposed and the resulting curve segments tested to meet the criteria described above.

In comparison with the RM method, six replicate EL monitoring quadrats could be deployed and marked at a site in approximately 0.5 hrs under a wider range of water quality conditions with no further monitoring required until retrieval, which also took approximately 0.5 hr per site, and if needed could be conducted under conditions of near zero visibility. In the lab, all 12 replicate quadrats collected at the two sites for the EL method could be processed by a single technician and the average leaf PI and growth rates determined in less than 4 hrs.

Unlike the RM and EL methods, estimates of leaf productivity can be derived using the DA method with a single sampling event (Duarte et al., 1994). Sample sizes needed for this analysis are relatively large to ensure that a sufficient number of ramets are collected and processed to accurately describe population morphology and age cohort structure. In this study, three replicate 0.25 m^2 samples were collected at each study site with over 1500 ramets aged and measured. The reconstructive aging technique requires measurements of the oldest ramets in a population, typically very low in abundance, further necessitating the collection and processing of large numbers of ramets. While these samples can be obtained during a single field event, the sods are large and heavy, making it logistically difficult to collect and transport samples from numerous sites. In the lab, individual ramets need to be carefully teased out of the sod matrix of intertwined plant material in order to be counted, aged, and measured, requiring considerable processing time. A trained technician was capable of processing one 0.25 m^2 sod sample in an 8-hr day. Sods in poor condition or with high ramet densities could take significantly more time to process. Due to the volume of numbers generated, data entry and analysis also require substantial amounts of time. Based on these considerations, we conclude that the EL method is the most cost- and time-effective method, and is therefore likely to be more widely utilized.

The development of a rapid and reliable method for determining leaf growth characteristics of *S. filiforme* makes it more feasible to study productivity, export of organic matter, and the effects of environmental conditions on plant growth. Following an approach similar to that used in the DA method, the EL method can also be used to calculate estimates of leaf lifespan if the number of standing leaves on the ramets collected are counted. Analysis of the carbon, nitrogen, and phosphorous content of new leaves from the EL samples combined with the growth rate determinations and site-specific ramet density data can be used to generate leaf nutrient assimilation rates. Since *S. filiforme* leaves are buoyant and are typically transported away from the seagrass meadows after being shed, determination of the nutrient content of senescent leaves together with leaf fragmentation rates could be used to estimate nutrient export rates from *S. filiforme* meadows. By coupling these nutrient assimilation and loss rates a nutrient budget for *S. filiforme* leaves can be generated and scaled to small patches, large meadows or entire seagrass ecosystems. Leaf growth is only

one component of total plant production. To generate a complete nutrient budget for *S. filiforme* data on the growth and nutrient contents of all plant tissues including leaves, leaf sheaths, stems, rhizomes and roots are needed. The development of the EL method should prove useful in acquiring these important data.

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