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COMPARISON OF LEAF-CLIPPING AND LEAF-PIERCING TECHNIQUES AS APPLIED TO THE SEAGRASS *SYRINGODIUM FILIFORME*

Joseph L. Kowalski, Hudson R. DeYoe,
Christian P. Krull, and Terry C. Allison

ABSTRACT

Leaf elongation rates of the seagrass *Syringodium filiforme* (Kütz., 1860) were assessed at two sites in a subtropical lagoon of Texas on eleven occasions from January 1996 to April 1997 using two methods, clipping and leaf piercing (marking) to estimate leaf growth. Pierced shoots grew at a significantly faster rate than clipped shoots irrespective of site. Clipping underestimated leaf elongation by 30%–38%, although differences at individual sites were as high as 69%–72%. Underestimation of leaf growth rate derived by clipping could be corrected using a site-specific linear regression relationship between leaf growth rates determined by clipping and piercing methods. The percent difference in overall leaf growth rate during the 14-mo study was 55% (4.47 mm d⁻¹ pierced vs 2.44 mm d⁻¹ clipped leaves). Fastest growth occurred during summer with rates of pierced leaves ranging from 8 to 11 mm d⁻¹, which was generally two to three times that of clipped leaves. Highest leaf growth rates for clipped leaves never exceeded 4 mm d⁻¹, regardless of site. We suggest that use of the leaf-clipping method in *S. filiforme* is appropriate when leaf growth rates are to be compared among sites or treatments and when true growth rate values are not critical.

Leaf clipping is a technique commonly utilized by terrestrial ecologists to determine the effects of competition (Wallace, 1990), nutrient availability (Knops and Reinhart, 2000; Sardans et al., 2006; Gao et al., 2008), grazing (Owensby et al., 1974; Leriche et al., 2003), and physiological integration of ramets and genets at the population level (Westoby, 1980). Leaf clipping has been used to study seagrasses since at least 1974 (Greenway, 1974) and is a useful tool for assessment of leaf production ecology (Virnstein, 1982; Morgan and Kitting, 1984; Dunton, 1990; Kowalski et al., 2001; Kaldy, 2006; Shafer et al., 2008). Other seagrass studies have utilized the clipping method to examine the effects of simulated herbivory on faunal components (Cebrián et al., 1998; Kuiper-Linley et al., 2007; Vergés et al., 2008), competition (Williams, 1987), and mobilization of resources along the rhizomes between physiologically integrated short shoots (Dawes and Lawrence, 1979; Dawes and Guiry, 1992; Schwarzschild and Zieman, 2008a).

Studies comparing productivity estimation methods (clipping vs piercing) have focused on *Halodule wrightii* Aschers. (shoal grass), a species that possesses flat, but narrow leaves (ca. 1 mm) (Tomasko and Dunton, 1995; Hauxwell et al., 2001; Kowalski et al., 2001) but the method comparison has also been applied to *Zostera* spp. and *Thalassia* spp. (Zieman, 1974; Sand-Jensen, 1975). These studies demonstrated that leaf production is underestimated by clipping from 15 to 100% (dependent on season and location) compared to pierced leaves (Hauxwell et al., 2001; Kowalski et al., 2001). Reasons for the underestimation may be a result of singular or synergistic effects of wounding, interfering with physiological function, removal of photosynthetic tissue reducing carbon acquisition and/or insufficient stored resources (Tomasko

and Dunton, 1995; Hauxwell et al., 2001; Kowalski et al., 2001). Another method of measuring production is to tag or mark rhizomes (Kenworthy et al., 1989; Short and Duarte, 2001). Rhizome tagging involves finding the rhizome apical meristem and subtending shoots, placing a tag (or mark) on the rhizome and measuring the dry weight increase of rhizome, roots, and associated short shoots and leaves after an appropriate period of time (Kenworthy et al., 1989; Dennison, 1990; Short et al., 1993; Kaldy and Dunton, 2000). Rhizome tagging provides an alternate estimate of shoot or whole plant production, but is dependent on sufficient underwater visibility and fine manipulations of the plant. Lastly, the reconstruction (indirect) technique utilizes detailed knowledge of the growth pattern of a given species (Duarte et al., 1994; Short and Duarte, 2001). This method is suggested as a useful tool to estimate past leaf production and to apply past rates to estimate present rates; however, this method has been criticized as misrepresenting true leaf production history (Jensen et al., 1996; Kaldy et al., 1999).

Despite chronic underestimation, some researchers have resorted to use of the leaf-clipping method for estimates of leaf production rates in *H. wrightii* and *Zostera japonica* Aschers. and Graeb. because narrow leaves combined with sub-optimal water clarity make leaf piercing, reconstruction methods, or rhizome marking untenable (Kowalski et al., 2001; Kaldy et al., 2004; Kaldy, 2006). In addition to *H. wrightii* and *Z. japonica*, which are difficult to mark, there are two species of *Syringodium*, which bear cylindrical, rather than flat leaves common to most seagrasses (Phillips and Meñez, 1988). *Syringodium isoetifolium* (Aschers.) Dandy occurs in Indo-Pacific waters, while *Syringodium filiforme* (Kütz.) (manatee grass) is widely found in tropical and subtropical coastal waters of the North Atlantic Ocean, Gulf of Mexico, and Caribbean Sea (Phillips and Meñez, 1988). The leaves of these plants have been described as “inflexible” and “brittle” (Fry, 1983; Fry and Virnstein, 1988; Aioi and Pollard, 1993; Cruz-Palacios and van Tussenbroek, 2005). The brittleness of these leaves has led to difficulty in accurately estimating shoot production using the leaf-piercing technique. Typically, the mark causes mechanical weakness in the leaf and breakage with loss of leaf material above the mark (Fry, 1983; Aioi and Pollard, 1993).

Clipping and piercing of *S. filiforme* leaves to obtain shoot production estimates was employed by Fry and Virnstein (1988), but no comparison was made between the two methods. Recently, Schwarzschild et al. (2008) developed the emergent leaf (EL) method based on measuring the oldest intact leaf on a short shoot that develops in a known time interval to estimate leaf growth in *S. filiforme*. They compared the EL method to the repeated measures method (Fry, 1983) and the reconstruction method (Short and Duarte, 2001). They found their new method was comparable to the traditional leaf-piercing method used in studies of flat, strap-like leaves (Dennison, 1990; Short et al., 1993; Kaldy and Dunton, 2000; Kowalski et al., 2001). We know of no study that simultaneously examines the efficacy of leaf-clipping and leaf-piercing methods in *S. filiforme*. We conducted a 14-mo study to examine leaf production rates at two sites in the Lower Laguna Madre of Texas (LLM) utilizing leaf-piercing and leaf-clipping methods to determine which method produces higher leaf growth rates and assessed the degree of difference between the two methods at the two sites. This study was made to complement an examination of the autecology of *S. filiforme* from the same sites in LLM (unpubl. data).

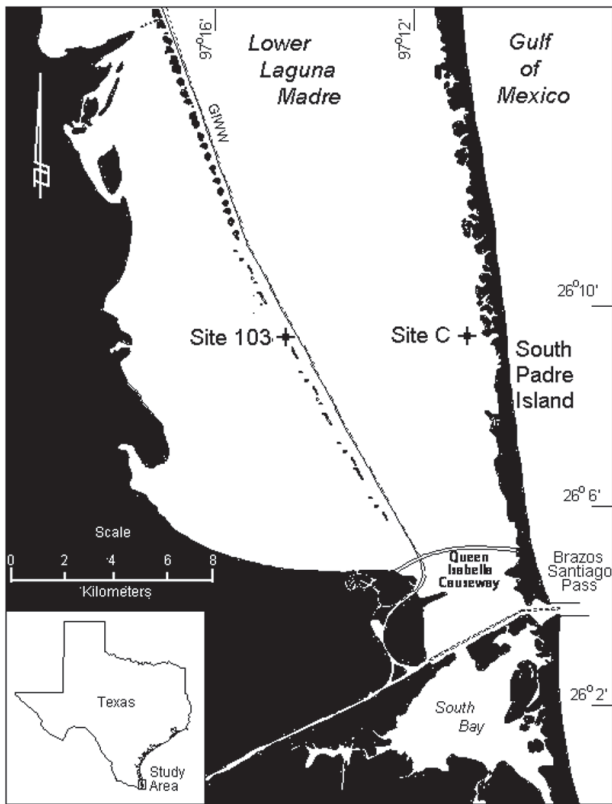


Figure 1. Map of study sites in Lower Laguna Madre, Texas.

METHODS AND MATERIALS

STUDY SITES

The study was carried out in the LLM at two sites (Site 103 and Site C) of comparable depth (ca. 1.2 m) (Fig. 1) and tidal influence, but which differed in fetch, current speed, underwater photosynthetically active radiation (PAR), and proximity to the Gulf Intracoastal Waterway (GIWW), a dredged navigation channel. Site 103 was located approximately 11 km north of the Brazos-Santiago Pass, a modified natural pass connecting the LLM to the Gulf of Mexico, at (26°9.09'N by 97°14.37'W) and was comprised of a monotypic stand of *S. filiforme*, approximately 500 m west of the GIWW. Site 103 was exposed to an 8 km fetch from the southeast (Fig. 1) that occasionally produced high waves and caused water levels to fluctuate between 0.9–1.3 m (mean = 1.2 m) at the study site. Sediments at site 103 were dredged material from the GIWW and estimated to be approximately 25 yrs old. Mean grain size distribution at Site 103 was 8% rubble, 67% sand, 13% silt, and 12% clay. Site C was 11 km north of the Brazos-Santiago Pass at (26°9.11'N by 97°11.72'W) and 6 km east of Site 103 at 1.2 m depth (Fig. 1). Mean grain size distribution at Site C was 11% rubble, 64% sand, 10% silt, and 15% clay. This site has never been dredged. Astronomical tides seldom exceed 20 cm in the LLM (Hedgepeth, 1947). Current speed and direction for the LLM was obtained from channel dredging studies (Militello and Kraus, 1994; Brown and Kraus, 1997). These studies indicate current speed in deep water (1.7 m) in the vicinity of Site 103 was typically < 10 cm s⁻¹ most of the year (84%). Current speed at Site C was not directly monitored, but modeled values indicate a current speed well below 10 cm s⁻¹ during flood tide (Militello and Kraus, 1994; Brown and

Kraus, 1997). Underwater PAR was about 40 moles photons $m^{-2} d^{-1}$ at both sites during summer, but fall and winter values differed (5 moles photons $m^{-2} d^{-1}$ at Site 103 and 7 to 8 moles photons $m^{-2} d^{-1}$ at Site C). Annual PAR from Site 103 was 6300 mol photons $m^{-2} yr^{-1}$ and 7000 mol photons $m^{-2} yr^{-1}$ from Site C. Although wind events can occlude the water column with suspended sediment during the passage of cold fronts (northers), sufficient PAR is typically restored within a few days (Onuf, 1994; Kowalski et al., 2009). Wind velocities for both sites were generally 6–12 $m s^{-1}$ for most of the year (Brown and Kraus, 1997) and surface water temperatures varied seasonally between 8 (for brief periods) and 30 °C while surface salinity varied between 24 and 40.

EXPERIMENTAL DESIGN

Leaf Clipping vs Leaf Piercing.—In eleven trials performed between March 1996 and April 1997, leaf growth rates based on leaf clipping and leaf piercing were compared following the techniques of Kowalski et al. (2001). Trials were conducted in uniformly dense stands of *S. filiforme* at each site. Annual mean shoot density is approximately 2600 shoots m^{-2} for Site 103 and 4600 shoots m^{-2} for Site C. For each trial, shoots were clipped 2 cm above the sediment in one 50 × 50 cm quadrat at each site. The 2 cm clip height was selected because it allows for a longer regrowth period, minimizes the likelihood of diminished growth rates associated with senescing leaves accompanied by longer clip heights (Fry, 1983; Kowalski et al., 2001), and is near the clip height used by Fry and Virnstein (1988). The shoot meristem of *S. filiforme* is typically located at the shoot to leaf transition zone typically at or just below the sediment surface when sediment is stable (pers. obs.). Areas chosen for clipping were selected haphazardly, except for the appearance of uniform grass cover. Just after clipping, one 9 cm diameter core (0.006 m^2 core $^{-1}$; 15 cm in depth) was taken in each quadrat near the periphery for determination of average clip height and clipped shoot stubble was measured to the nearest mm. Post-clip growth periods varied between 14 (summer) and 28 d (winter). After the growth period, four 9 cm diameter cores were harvested from the middle of each quadrat to reduce the likelihood of edge effects. Edge effect here is defined as the ability of neighboring shoots to supplement re-growth of clipped shoots (sensu Schwarzschild and Zieman, 2008,b). The five 9 cm cores taken per quadrat sampled approximately 13% of a quadrat. Subsequent clipping trials were conducted as described above using 50 × 50 cm quadrats at least 2 m from previously clipped areas.

For the leaf-piercing technique, the bundle sheaths of 30 haphazardly chosen shoots were selected at least 2 m outside of the clipped quadrat and pierced just below the bundle sheath, above the basal meristem, with a 28-gauge hypodermic needle (< 0.5 mm diameter). The base of each pierced shoot was tagged with a ring of tubing to relocate pierced shoots. On the same day clipped plots were harvested, pierced shoots were harvested by severing shoots at rhizome level, sealed in plastic bags and refrigerated until analysis. In the laboratory, the needle mark on the leaf sheath was located and the shoot cut at that location. Leaves from the shoot were separated by age and the growth interval or distance between the cut surface and the needle mark, or in absence of a mark the leaf tip was measured to the nearest mm. Unmarked leaves developed sometime in the period between marking and harvesting. Leaf growth rates, expressed as $mm d^{-1}$, were calculated by dividing the increase in leaf lengths by the number of days of growth. At the end of the growth period, four 9 cm cores were taken outside the clipped areas at least 2 m from the quadrats and at least 1 m from each other for estimation of shoot density and mean untreated leaf length. Untreated leaf lengths were used as a comparative maximum reference to ensure that re-grown clipped leaves did not reach leaf lengths accompanied by senescence. Samples were washed of sediment through a 1 mm sieve immediately upon return to the lab and stored in sealed plastic bags and refrigerated until processing. In the laboratory, the total number of shoots per core were counted and all leaves from 30 shoots per core were measured to the nearest mm.

STATISTICAL DESIGN

For each trial, one 50 cm × 50 cm clipped experimental plot was used and four cores taken from each plot. The statistical unit was the core. The data were not normally distributed so non-parametric statistical analysis was used. Mean leaf growth rates of the two leaf production techniques (pierced and clipped) were compared using Friedman's method of randomized blocks with growth rate blocked against sampling date and site. The Friedman's method of randomized blocks is an alternative to the repeated measures analysis of variance and allows the use of sampling date (time) as a block with time as a substitution for replication (Sokal and Rohlf, 1995). To compare pooled leaf elongation means at each site a Mann-Whitney rank sum test was used to determine the differences between the two methods with the eleven different trials at each site treated as replicates (Sokal and Rohlf, 1995). Kruskal-Wallis one-way analysis of variance on ranks was used to block sampling date against treatment (leaf piercing and clipping) at each site (SPSS, Inc., 1993). Regression analysis was performed using a least-squares fit between average growth rates determined by leaf-clipping and leaf-piercing methods. An alpha level of 0.05 was used for all statistical tests.

RESULTS

There was a clear difference between leaf elongation rates by treatment, site, and sampling date (Table 1). Pierced shoots grew at a significantly faster rate than clipped shoots irrespective of site or date (Table 1). The mean growth rate for all pierced leaves averaged over all eleven trials and both sites during the 14-mo study was 4.47 mm d⁻¹ (SE = 0.191) compared to clipped plants with an overall mean of 2.44 mm d⁻¹ (SE = 0.159), a 55% difference (Mann-Whitney U = 12456.0; P = < 0.001). Fastest growth occurred during the months of June and September 1996 at Site 103 (> 10 mm d⁻¹) which was generally two to three times that of clipped leaves (Fig. 2). Spring and summer elongations rates of both methods were significantly faster than those of fall and winter. Mean seawater temperatures from May to September 1996 ranged from 22.3 (SE = 0.03) to 30 °C (SE = 0.01) and 25.4 (SE = 0.02) to 13.67 °C (SE = 0.06), during October 1996 through April 1997. Salinity varied from 28.88 (SE = 0.04) to 37.53 (SE = 0.02) during the entire study (data not shown). Fastest leaf growth rates for clipped leaves were never more than 4 mm d⁻¹, regardless of site, except for July 1996 at Site 103, while slowest clipped leaf growth rates of < 1 mm d⁻¹ occurred during February 1997 and were coincident with lowest seawater temperature (17 °C; SE = 0.03) (Fig. 2).

Comparison of the leaf-pierce method averaged across all sampling dates by site revealed that elongation rates of 4.96 mm d⁻¹ (SE = 2.62) at Site 103 were nearly 30% greater than those of Site C, 3.86 mm d⁻¹ (SE = 2.60) (H = 10.393; 1 df; P = 0.001).

The leaf-clip method average at Site 103 was 4.07 mm d⁻¹ (SE = 2.60) including all sampling dates, while that for Site C was 3.54 mm d⁻¹ (SE = 2.74), a 13% difference (H = 4.066; 1 df; P = 0.044). Analysis of both sites and both treatments by date showed a strong seasonal interaction (Table 2). Estimates of leaf growth rates for clipped and

Table 1. Results of Friedman method for randomized blocks testing variations in leaf growth rate by treatment (pierced and clipped leaves) in *Syringodium filiforme* against sample date at Site 103 and Site C in Lower Laguna Madre, Texas.

Treatment	Chi-square	df	N	Mean rank treatment	Mean rank site	Mean rank month	Significance (P)
Leaf-pierce	239.835	2	291	2.24	1.28	2.48	< 0.001
Clip	60.022	2	92	1.95	1.47	2.59	< 0.001

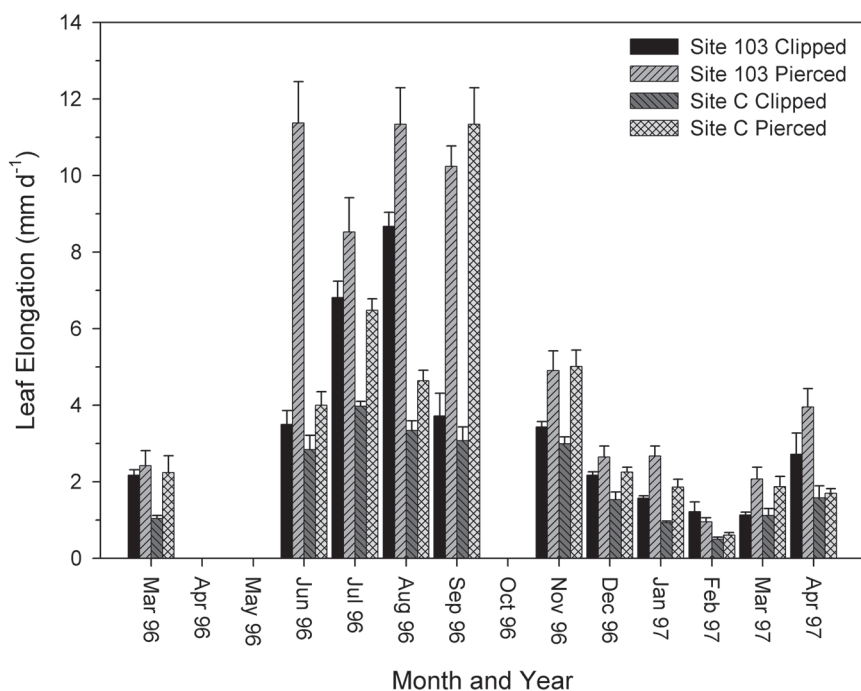


Figure 2. Seasonal comparison of leaf elongation rates in *Syringodium filiforme* from 11 experiments from January 1996 to April 1997, Lower Laguna Madre, Texas. Error bars represent one standard error of the mean.

pierced shoots from both sites were consistently different, but well correlated over the study period. Site C showed a stronger relationship between methods than Site 103 (Fig. 3). Untreated leaf lengths at Site 103 were longest from March to November (190–220 mm) and shortest during January and February (60–80 mm). Mean untreated leaf lengths at Site C were never more than 100 mm during any month and were lowest during May and June 1996. Overall means for untreated leaf lengths at Site 103 were 125.11 mm (SE = 2.58) and 76.85 mm (SE = 2.62) at Site C. In no instance at either site did the lengths of re-grown clipped leaves equal or surpass the lengths of untreated leaves (data not shown).

DISCUSSION

Leaf clipping has been used for more than 30 yrs by seagrass ecologists (Greenway, 1974) to study leaf production (Virnstein, 1982; Morgan and Kitting, 1984; Fry and Virnstein, 1988; Dunton, 1990; Kowalski et al., 2001; Kaldy, 2006; Shafer et al., 2008), herbivory (Cebrián et al., 1998; Kuiper-Linley et al., 2007; Verges et al., 2008), com-

Table 2. Results of Kruskal-Wallis analysis of variance on ranks testing variations in leaf growth rate by treatment (pierced and clipped leaves) in *Syringodium filiforme* blocked against sample date in Lower Laguna Madre, Texas.

	Pierced leaves	df	Significance (P)	Clipped leaves	df	Significance (P)
Site 103	H = 80.160	9	< 0.001	H = 32.444	9	< 0.001
Site C	H = 123.852	8	< 0.001	H = 36.980	8	< 0.001

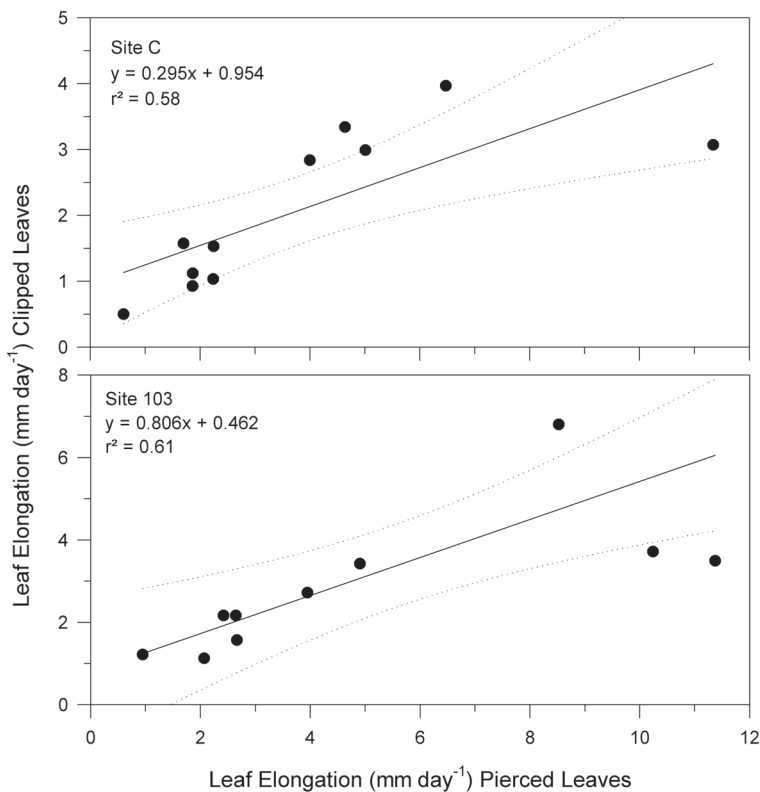


Figure 3. Regression relationships between leaf production methods in *Syringodium filiforme* from 11 experiments at two sites, January 1996 to April 1997, Lower Laguna Madre, Texas.

petition (Williams, 1987), and resource mobilization (Dawes and Lawrence, 1979; Dawes and Guiry, 1992; Schwarzschild and Zieman, 2008a). Leaf clipping, as used to estimate leaf production rates, has the inherent detrimental artifact of moderate to severe underestimation of true production rates. This has been consistently demonstrated for seagrasses other than *Syringodium* in studies published since 1995 (Tomasko and Dunton, 1995; Hauxwell et al., 2001; Kowalski et al., 2001).

Our results demonstrate that use of the clipping method in *S. filiforme* significantly underestimated leaf production rates from 7 to 72%, depending on season and site in LLM, compared to the pierce method. Similar results were found for *H. wrightii* from Texas (USA) and Mexico (Hauxwell et al., 2001; Kowalski et al., 2001). Mean leaf elongation rates of *S. filiforme* from the Indian River Lagoon clipped near the basal meristem varied from 2 mm d⁻¹ during November and December to 12 mm d⁻¹ during July (Fry and Virnstein, 1988). These values are considerably greater than those found in this study. Fastest leaf elongation rate of clipped *S. filiforme* at Site 103 was 8.3 mm d⁻¹ (SE = 0.36) in August 1996 while slowest rates occurred in February 1996 and March 1997 (1.1 mm d⁻¹; SE = 0.07 and 0.03, respectively). We found mean leaf growth rates of pierced plants from Site 103 in this study ranged from 8.5 (SE = 0.89) to 10.2 (SE = 0.53) mm d⁻¹, considerably less than those from Florida Bay (18–19 mm d⁻¹; SE = 0.6 and 1.0, respectively) for the same time of year estimated by the emergent leaf (EL) method (July and August) (Schwarzschild et al., 2008) as well as

for *S. filiforme* from the Indian River Lagoon using the repeated measures method (21.1 mm d^{-1} ; May and June; Fry, 1983). *Syringodium filiforme* growth rate differences between this study and Florida studies may be attributable to physiochemical differences (Koch, 2001) and/or genetic differences (Kaldy et al., 2004). Seagrass growth in LLM was demonstrated to be nitrogen-limited at a *Thalassia testudinum* Banks ex König site (Lee and Dunton, 1999), while phosphorus limitation has been postulated for a *H. wrightii* site (Kowalski et al., 2009).

There was a statistically significant linear relationship between leaf piercing and leaf clipping at both sites that allows for a determination of a correction factor for the degree of underestimation of the leaf-clipping method at each site. To correct for the extent of underestimation inherent with the clip method, it would be necessary to make simultaneous leaf-piercing and leaf-clipping assessments but once over one seasonal cycle at each site under consideration, as done with this study (Kowalski et al., 2001). Given the presumed linear relationship between both leaf-pierce and leaf-clip methods, annual differences in one should correspond directly to differences in the other. Thus, after establishment of a seasonally-based site-specific correction factor using both methods, subsequent use of the clip method alone should account for annual variations (Kowalski et al., 2001, 2009). Utilization of these regression equations could then be employed in seagrass monitoring programs (sensu Herbert and Fourqurean, 2009).

We lack a clear understanding of the physiological responses clipping evokes in seagrasses. In some terrestrial plants, clipping has resulted in slower growth rates on shoots clipped closer to the basal meristem (Albertson et al., 1953; Cook et al., 1958; McNaughton et al., 1983; Moreno et al., 1999; Paige, 1999). Kowalski et al. (2001) clipped *H. wrightii* at five clip heights using 2 cm intervals and found fastest growth rates on the shortest clipped shoots (0–2 cm). How species respond to clipping is variable and the degree of variation has been attributed to differences in life history (annual and perennial) (Sharma et al., 1998), sediment and plant nutrient status (Moreno et al., 1999; Raillard and Svaboda, 1999), and sexual reproduction differences (Mulder and Ruess, 1998). Obviously, the removal of photosynthetic tissue places a burden on the stored reserves and nutrients of the clipped plant (Dawes and Lawrence, 1979; Dawes and Guiry, 1992; Stapel and Hemminga, 1997), or on neighboring shoots to supplement regrowth of lost leaf tissue (Schwarzschild and Zieman, 2008a,b). This would produce an increase in respiratory costs to the clipped shoot at and near the tissues of the basal meristem to re-grow lost tissue (Griffin, 1994). Clipping also wounds the plant (Tomasko and Dunton, 1995; Kowalski et al., 2001) and may allow pressure from the surrounding water column to force salty water into the leaf's lacunae and generate osmotic stress on the nearby meristematic tissues. This maybe true for the pierce method also, but perhaps not the same extent. The bundle sheath has been suggested to act as a buffer to reduce osmotic pressure near the basal meristem (Tyerman et al., 1984). Kuo and den Hartog (2006) state that some seagrass species possess septa within the aerenchyma system as a possible barrier to flooding of tissue. It is unknown whether *S. filiforme* possesses such adaptation, as this study did not explore leaf growth rates of varying clip heights as was done in Kowalski et al. (2001) with *H. wrightii*. Any of these factors, acting alone, or in concert, are sufficient to explain the slower growth rates associated with leaf-clipping compared to the leaf-piercing method.

We clipped *S. filiforme* at 2 cm above the sediment as this clip height allowed for a sufficient re-growth period and avoided the slowest leaf growth rates associated with leaf senescence (*sensu* Fry, 1983). Fry and Virnstein (1988) clipped *S. filiforme* “near the basal meristem” in the Indian River Lagoon. Although we did not test the effects of variable clip height on leaf elongation rates in *S. filiforme*, it could be that the 2 cm clip height is more detrimental than longer clip heights. A study evaluating variable clip heights would be useful to determine the optimal height.

The depressed leaf production, exhibited by clipped *S. filiforme* in this study appears to simulate the response of some seagrasses, including *S. filiforme*, to grazing adjacent to coral reef communities by fish and sea urchins (Rose et al., 1999; Armitage and Fourqurean, 2006; Valentine and Duffy, 2006; Heck et al., 2008). These “grazing halo” areas can be grazed to the point that shoot density and canopy height are minimized or are rendered barren (Sweatman and Robertson, 1994; Alevizon, 2002). The impairment of leaf regrowth caused by clipping and how the clipping method may parallel seagrass growth response associated with coral reef communities deserves consideration.

We pierced 20–30 *S. filiforme* leaf shoots during each experiment and generally recovered at least 60% of these shoots with pierced leaves intact. The leaves of the genus *Syringodium* have been described as “brittle” and “inflexible” (Fry, 1983; Fry and Virnstein, 1988; Aioi and Pollard, 1993; Cruz-Palacios and van Tussenbroek, 2005). The resultant hole likely causes mechanical weakness in the leaf and subsequent tendency to detach with loss of leaf material above the mark (Fry, 1983; Aioi and Pollard, 1993; Schwarzschild et al., 2008). If this method is to be used, the number of shoots marked should take into account the low recovery rate of marked shoots.

Variation of clip and pierce method results between LLM sites is likely a result of environmental differences. Although Site 103 lies adjacent to the GIWW, it receives about 9% less PAR than Site C (6300 vs 7000 mol photons $\text{m}^{-2} \text{yr}^{-1}$). Shoot density at Site 103 is less than one-half that of Site C (2235 vs 5128 shoots m^{-2} , respectively) and mean leaf length is more than three times greater at Site 103 compared to Site C (133 vs 39 mm, respectively). Greater leaf length at Site 103 suggests that the plants may be compensating for the lower light. Shoot density and leaf length of seagrasses have been demonstrated to be affected by lower light conditions (Backman and Barilotti, 1976; Lee and Dunton, 1997). It is unknown if there are sediment geochemical differences between the sites. These kinds of dynamic physical site differences are commonly found in other seagrass studies involving site differences and exert a strong influence on biological and ecological plant performance (Koch, 1994, 2001).

Clipping may be the most practical method under conditions of low visibility or when excessive water motion precludes the use of making fine determinations of measurement or position on a leaf. The adverse effects of leaf clipping found in this study may have been accentuated by clipping an excessively large area that could not be supported by neighboring shoots. We harvested four cores near the center of the clipped quadrat. The clipped shoots which possessed green tissue could at least partially supplement their re-growth through photosynthesis and using stored reserves from the rhizome system (Dawes and Lawrence, 1979; Dawes and Guiry, 1992; Rey and Stephens, 1996; Schwarzschild and Zieman, 2008a). The growth rate as determined by clipping may be a function of the size of the clip area and the connections with neighboring ramets. A large clip area with shoot sampling from the center of the clipped area may result in lower growth rates due to a reduced neighbor

effect. Small clip areas, consisting of a few shoots, may result in higher growth rates due to a greater neighbor effect i.e., resource translocation from adjacent unclipped shoots (sensu Dawes and Lawrence, 1979; Dawes and Guiry, 1992; Rey and Stephens, 1996; Stapel and Hemminga, 1997; Schwarzschild and Zieman, 2008a). Since ramet growth is typically a “group” activity (Schwarzschild and Zieman, 2008a,b), more ecologically significant growth rates might be best estimated using small clip areas.

The leaf-clipping protocol does not require good water clarity, can be done by feel, using the back of the hand as a guide, and laboratory processing of shoots only involves the measurement of the leaf fraction of the plant. The EL method of Schwarzschild et al. (2008) utilizes the youngest emergent *S. filiforme* leaf on a short shoot to assess leaf growth. This protocol also involves clipping leaves, approximately one cm from the tip. Leaves with intact tips are measured only if attached to a shoot with clipped leaves. Their findings show this new method is comparable to the traditional leaf-piercing method (Dennison, 1990; Short et al., 1993; Kaldy and Dunton, 2000; Kowalski et al., 2001) but without inflicting damage to the leaves being measured. Caveats associated with the EL method include determination of an appropriate time window to harvest marked shoots and potential loss of marked and younger unmarked leaf tips related to grazing (Schwarzschild et al., 2008). This method is yet to be widely used under various water conditions. If the EL method is found to be amenable to low visibility, high energy conditions, the accompanying more realistic leaf growth rates would be preferable to the leaf-piercing and clipping methods discussed here. The leaf-clipping method may still be useful when making comparisons among sites, or treatments where accurate assessment of leaf production per se is not required.

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LITERATURE CITED

- Alevizon, W. 2002. Enhanced seagrass growth and fish aggregations around Bahamian patch reefs: [The case for a functional connection. Bull. Mar. Sci. 70: 957–966.](#)
- Armitage, A. R. and J. W. Fourqurean. 2006. [The short-term influence of herbivory near patch reefs varies between seagrass species. J. Exp. Mar. Biol. Ecol. 339: 65–74.](#)
- Aioi, K. and P. C. Pollard. 1993. Biomass, leaf production and loss rate of the seagrass *Syringodium isoetifolium* on Dravuni Island, Fiji. *Aquat. Bot.* 46: 283–292.
- Albertson, F. W., A. Riegel, and J. L. Launchbaugh, Jr. 1953. Effects of different intensities of clipping on short grasses in west-central Kansas. *Ecology* 34: 1–20.
- Backman, T. W. and D. C. Barilotti. 1976. [Irradiance reduction: Effects on standing crops of the eelgrass *Zostera marina* in a coastal lagoon. Mar. Biol. 34: 33–40.](#)

- Brown, C. A. and N. C. Kraus. 1997. Environmental monitoring of dredging and processes in Lower Laguna Madre, Texas. Texas A&M University-Corpus Christi/Conrad Blucher Institute Tech. Rep. TAMU-CC-CBI-96-01.
- Cebrián, J., C. M. Duarte, N. S. R. Agawin, and M. Merino. 1998. Leaf growth response to simulated herbivory: a comparison among seagrass species. *J. Exp. Mar. Biol. Ecol.* 220: 67–81.
- Cook, C. W., L. A. Stoddart, and F. E. Kinsinger. 1958. Responses of crested wheatgrass to various clipping treatments. *Ecol. Monogr.* 28: 237–272.
- Cruz-Palacios, V. and B. I. van Tussenbroek. 2005. Simulation of hurricane-like disturbances on a Caribbean seagrass beds. *J. Exp. Mar. Biol. Ecol.* 324: 144–60.
- Dawes, C. J. and J. M. Lawrence. 1979. Effects of blade removal on the proximate composition of the rhizome of the seagrass *Thalassia testudinum* Banks ex König. *Aquat. Bot.* 7: 255–266.
- _____ and M. D. Guiry. 1992. Proximate constituents in the seagrasses *Zostera marina* and *Z. noltii* in Ireland: Seasonal changes and the effect of blade removal. *Mar. Ecol.* 13: 307–315.
- Dennison, W. C. 1990. Leaf production. Pages 77–78 in R. C. Phillips and C. P. McRoy, eds. *Seagrass research methods*. UNESCO, Paris.
- Duarte, C. M., N. Marba, N. Agawin, J. Cebrián, S. Enriquez, M. D. Fortes, M. E. Gallegos, M. Merino, B. Olesen, K. Sand-Jensen, et al. 1994. Reconstruction of seagrass dynamics: age determinations and associated tools for the seagrass ecologist. *Mar. Ecol. Prog. Ser.* 107: 195–209.
- Dunton, K. H. 1990. Production ecology of *Ruppia maritima* L. s.l. and *Halodule wrightii* Aschers. in two subtropical estuaries. *J. Exp. Mar. Biol. Ecol.* 143: 147–164.
- Fry, B. 1983. Leaf growth in the seagrass *Syringodium filiforme* Kütz. *Aquat. Bot.* 16: 361–368.
- _____ and R. W. Virnstein. 1988. Leaf production and export of the seagrass *Syringodium filiforme* Kütz. in Indian River Lagoon, Florida. *Aquat. Bot.* 30: 261–266.
- Gao, Y., D. Wang, L. Ba, Y. Bai, and B. Liu. 2008. Interactions between herbivory and resource availability on grazing tolerance of *Leymus chinensis*. *Environ. Exp. Bot.* 63: 113–122.
- Greenway, M. 1974. The effects of cropping on the growth of the *Thalassia testudinum* (König) in Jamaica. *Aquaculture* 4: 199–206.
- Griffin, K. L. 1994. Calorimetric estimates of construction costs and their use in ecological studies. *Funct. Ecol.* 8: 551–562.
- Hauxwell, J., J. Cebrián, J. A. Herrera-Silveira, J. Ramirez R., A. Zaldivar J., N. Gomez, and N. Aranda-Cirerol. 2001. Measuring production of *Halodule wrightii*: additional evidence suggests clipping underestimates growth rate. *Aquat. Bot.* 69: 41–54.
- Heck, K. L., Jr., T. J. B. Carruthers, C. M. Duarte, A. R. Hughes, G. Kendrick, R. J. Orth, and S. Williams. 2008. Trophic transfers from seagrass meadows subsidize diverse marine and terrestrial consumers. *Ecosystems* 11: 1198–1210.
- Hedgepeth, J. 1947. The Laguna Madre of Texas. Pages 364–380 in *Trans. 12th N. Am. Wildl. Conf.*
- Herbert, D. A. and J. W. Fourqurean. 2009. Phosphorus availability and salinity control productivity and demography of the seagrass *Thalassia testudinum* in Florida Bay. *Estuar. Coast.* 32: 188–201.
- Jensen, S. L., B. D. Robbins, and S. S. Bell. 1996. Predicting population decline: seagrass demographics and the reconstructive technique. *Mar. Ecol. Prog. Ser.* 136: 267–276.
- Kaldy, J. E. 2006. Production ecology of the non-indigenous seagrass, dwarf eelgrass, (*Zostera japonica* Aschers. and Graeb.) in a Pacific Northwest Estuary, USA. *Hydrobiologia* 553: 201–217.
- _____ and K. H. Dunton. 2000. Above- and below-ground production, biomass and reproductive ecology of *Thalassia testudinum* (turtle grass) in a subtropical coastal lagoon. *Mar. Ecol. Prog. Ser.* 193: 271–283.

- _____, N. Fowler, and K. H. Dunton. 1999. Critical assessment of *Thalassia testudinum* (turtle grass) aging techniques: implications for demographic inferences. *Mar. Ecol. Prog. Ser.* 181: 279–288.
- _____, K. H. Dunton, J. L. Kowalski, and K. S. Lee. 2004. Evaluation of environmental factors controlling the success of seagrass revegetation onto dredged material deposits: a case study in Lower Laguna Madre, Texas. *J. Coast. Res.* 20: 292–300.
- Kenworthy, W. J., C. A. Currin, M. S. Fonseca, and G. Smith. 1989. Production, decomposition, and heterotrophic utilization of the seagrass *Halophila decipiens* in a submarine canyon. *Mar. Ecol. Prog. Ser.* 51: 277–290.
- Knops, J. M. H. and K. Reinhart. 2000. Leaf area along a nitrogen fertilization gradient. *Am. Mid. Nat.* 144: 265–272.
- Koch, E. W. 1994. Hydrodynamics, diffusion-boundary layers and photosynthesis of the seagrasses *Thalassia testudinum* and *Cymodocea nodosa*. *Mar. Biol.* 118: 767–776.
- _____. 2001. Beyond light: physical, geological, and geochemical parameters as possible submersed aquatic vegetation habitat requirements. *Estuaries* 24: 1–17.
- Kowalski, J. L., H. R. DeYoe, and T. C. Allison. 2009. Seasonal production and biomass of the seagrass, *Halodule wrightii* Aschers. (Shoal Grass), in a subtropical Texas lagoon. *Estuar. Coast.* 32: 467–482.
- _____, H. R. DeYoe, T. C. Allison, and J. E. Kaldy. 2001. Productivity estimation in *Halodule wrightii*: comparison of leaf-clipping and leaf-marking techniques, and the importance of clip height. *Mar. Ecol. Prog. Ser.* 220: 131–136.
- Kuo, J. and C. den Hartog. 2006. Seagrass morphology, structure, and ultrastructure. Pages 51–87 in A. W. D. Larkum, R. J. Orth, and C. M. Duarte, eds. *Seagrasses: biology, ecology and conservation*. Springer, The Netherlands.
- Kuiper-Linley, M., C. R. Johnson, and J. M. Lanyon. 2007. Effects of simulated green turtle regrazing on seagrass abundance, growth and nutritional status in Moreton Bay, south-east Queensland, Australia. *Mar. Freshw. Res.* 58: 492–503.
- Lee, Kun-S. and K. H. Dunton. 1997. Effects of *in situ* light reduction on the maintenance, growth and partitioning of carbon resources in *Thalassia testudinum* Banks ex König. *J. Exp. Mar. Biol. Ecol.* 210: 53–73.
- _____, and _____. 1999. Influence of sediment nitrogen-availability on carbon and nitrogen dynamics of *Thalassia testudinum*. *Mar. Biol.* 134: 217–226.
- Leriche, H., X. Le Roux, F. Desnoyers, D. Benest, G. Simioni, and L. Abbadie. 2003. Grass response to clipping in an African savanna: testing the grazing optimization hypothesis. *Ecol. Appl.* 13: 1346–1354.
- McNaughton, S. J., L. L. Wallace, and M. B. Coughenour. 1983. Plant adaptation in an ecosystem context: effects of defoliation, nitrogen, and water on growth of an African C_4 sedge. *Ecology* 64: 307–318.
- Militello, A. and N. C. Kraus. 1994. Reconnaissance investigation of the current and sediment movement in the Lower Laguna Madre between Port Isabel and Port Mansfield, Texas. Conrad Blucher Institute for Surveying and Science, Corpus Christi.
- Moreno, D. M., J. Núñez-Farfán, T. Terrazas, P. L. del Mar Ruiz, T. L. C. Trinidad-Santos, and A. Larque-Saavedra. 1999. Plastic responses to clipping in two species of *Amaranthus* from the Sierra de Pueblo, Mexico. *Gen. Resources Crop Evol.* 46: 225–234.
- Morgan, M. D. and C. L. Kitting. 1984. Productivity and utilization of the seagrass *Halodule wrightii* and its attached epiphytes. *Limnol. Oceanogr.* 29: 1066–1076.
- Mulder, C. P. H. and R. W. Ruess. 1998. Relationships between size, biomass allocation, reproduction, and survival in *Triglochin palustris*: implications for the effects of goose herbivory. *Can. J. Bot.* 76: 2164–2176.
- Onuf, C. P. 1994. Seagrasses, dredging and light in Laguna Madre, Texas, U.S.A. *Estuar. Coast. Shelf Sci.* 39: 75–91.
- Owensby, C. E., J. R. Rains, and J. D. McKendrick. 1974. Effects of one year intensive clipping on Big Bluestem. *J. Range Management* 27: 341–343.

- Paige, K. N. 1999. Re-growth following ungulate herbivory in *Ipomopsis aggregata*: geographic evidence for overcompensation. *Oecologia* 118: 316–323.
- Phillips, R. C. and E. G. Menez. 1988. Seagrasses. *Smithsonian contributions to the marine sciences*. *Smithson. Contrib. Mar. Sci.* 34: 1–104.
- Raillard, M. C. and J. Svaboda. 1999. Exact growth and increased nitrogen compensation by the Arctic sedge *Carex aquatilis* var. *stans* after simulated grazing. *Arc., Antarc. Alpine Res.* 31: 21–26.
- Rey, J. K. and F. C. Stephens. 1996. Effects of shading and rhizome isolation on soluble carbohydrate levels in blades and rhizomes of the seagrass *Syringodium filiforme*. *Gulf Mex. Sci.* 14: 47–54.
- Rose, C. D., W. S. Sharp, W. J. Kenworthy, J. H. Hunt, W. G. Lyons, E. J. Prager, J. F. Valentine, M. O. Hall, P. Whitfield, and J. W. Fourqurean. 1999. Sea urchin overgrazing of a large seagrass bed in outer Florida Bay. *Mar. Ecol. Prog. Ser.* 190: 211–222.
- Sand-Jensen, K. 1975. Biomass, net production and growth dynamics in an eelgrass (*Zostera marina* L.) population in Vellerup Vig, Denmark. *Freshwater Biological Laboratory* 14: 185–201.
- Sardans, J., J. Peñuelas, and F. Rodà. 2006. The effects of nutrient availability and removal of competing vegetation on resprouter capacity and nutrient accumulation in the shrub *Erica multiflora*. *Acta Oecol.* 29: 221–232.
- Schwarzschild, A. C. and J. C. Zieman. 2008a. Effects of physiological integration on the survival and growth of ramets and clonal fragments in the seagrass *Syringodium filiforme*. *Mar. Ecol. Prog. Ser.* 372: 97–104.
- _____ and _____. 2008b. Apical dominance and the importance of clonal integration to apical growth in the seagrass *Syringodium filiforme*. *Mar. Ecol. Prog. Ser.* 360: 37–46.
- _____, W. J. Kenworthy, and J. C. Zieman. 2008. Leaf growth of the seagrass *Syringodium filiforme* Kütz. in the Florida Keys, Florida. *Bull. Mar. Sci.* 83: 571–585.
- Shafer, D. J., S. Wyllie-Echeverria, and T. D. Sherman. 2008. The potential role of climate in the distribution and growth of the introduced seagrass *Zostera japonica* in North America. *Aquat. Bot.* 89: 297–302.
- Sharma, K. P., S. P. S. Kushwaha, and B. Gopal. 1998. A comparative study of stand structure and standing crops of two wetland species, *Arundo donax* and *Phragmites karka*, and primary production in *Arundo donax* with observations on the effect of clipping. *Trop. Ecol.* 39: 3–14.
- Stapel, J. and M. A. Hemminga. 1997. Nutrient resorption from seagrass leaves. *Mar. Biol.* 128: 197–206.
- Short, F. T. and C. M. Duarte. 2001. Methods for the measurement of seagrass growth and production. Pages 155–182 in F. T. Short and R. G. Coles, eds. *Global seagrass research methods*. Elsevier, Amsterdam.
- _____, J. Montgomery, C. F. Zimmermann, and C. A. Short. 1993. Production and nutrient dynamics of a *Syringodium filiforme* Kütz. seagrass bed in Indian River lagoon, Florida. *Estuaries* 16: 323–334.
- Sokal, R. R. and F. J. Rohlf. 1995. *Biometry*, Third Edition. W. H. Freeman and Company, New York.
- SPSS, Inc. 1993. *SPSS for windows base system user's guide release 6.0*. SPSS, Inc., Chicago.
- Sweatman, H. and D. R. Robertson. 1994. Grazing halos and predation on juvenile Caribbean surgeonfishes. *Mar. Ecol. Prog. Ser.* 111: 1–6.
- Tomasko, D. A. and K. H. Dunton. 1995. Primary productivity in *Halodule wrightii*: a comparison of techniques based on daily carbon budgets. *Estuaries* 18: 271–278.
- Tyerman, S. D., A. I. Hatcher, R. J. West, and A. W. D. Larkum. 1984. *Posidonia australis* growing in altered salinities leaf growth regulation of turgor and the development of osmotic gradient. *Aust. J. Plant Physiol.* 11: 35–48.

- Valentine, J. F. and J. E. Duffy. 2006. The central role of grazing in seagrass ecosystems. Pages 463–500 in A. W. D. Larkum, R. J. Orth, and C. M. Duarte, eds. *Seagrasses: biology, ecology and conservation*. Springer, The Netherlands.
- Vergés, A., M. Pérez, T. Alcoverro, and J. Romero. 2008. Compensation and resistance to herbivory in seagrasses: induced responses to simulated consumption by fish. *Oecologia* 155: 751–760.
- Virnstein, R. W. 1982. Leaf growth of the seagrass *Halodule wrightii* photographed in situ. *Aquat. Bot.* 12: 209–218.
- Wallace, L. L. 1990. Comparative responses of big bluestem to clipping versus grazing. *J. Range Management* 43: 58–61.
- Westoby, M. 1980. Relations between genet and tiller population dynamics: survival of *Phalaris tuberosa* tillers after clipping. *J. Ecol.* 68: 863–869.
- Williams, S. L. 1987. Competition between the seagrasses *Thalassia testudinum* and *Syringodium filiforme* in a Caribbean lagoon. *Mar. Ecol. Prog. Ser.* 35: 91–98.
- Zieman, J. C. 1974. Methods for the study of the growth and production of the turtle grass, *Thalassia testudinum* König. *Aquaculture* 4: 139–143.

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