Productivity estimation in *Halodule wrightii*: comparison of leaf-clipping and leaf-marking techniques, and the importance of clip height

Joseph L. Kowalski¹, Hudson R. DeYoe¹,*, Terry C. Allison¹, James E. Kaldy²

¹Department of Biology, The University of Texas – Pan American, 1201 West University Dr., Edinburg, Texas 78539, USA
²Texas A&M University, Oceanography Department, College Station, Texas 77843, USA

ABSTRACT: We compared estimates of *Halodule wrightii* leaf growth rates obtained from leaf-clipping and leaf-piercing methods in a south Texas lagoon. Leaf clipping underestimated leaf production from 15 to 37% in winter and 25 to 60% in summer relative to leaf piercing. The underestimation of leaf-clipping derived growth rates were corrected using a linear regression between leaf growth rates determined by leaf-clipping and leaf-piercing methods. To examine the effect of clip height on *H. wrightii* leaf growth rate estimation, leaves were clipped at 0, 2, 4, 6, and 8 cm above the sediment. Leaves clipped at 2 cm exhibited the fastest leaf growth rate (average = 3.66 mm d⁻¹) while leaves clipped at 8 cm had the slowest rate (average = 2.30 mm d⁻¹). Depressed leaf growth rates for 8 cm clip height were likely due to the slowing of growth rate with increasing leaf age. Reduced growth rate for the 0 cm clip height treatment may be attributable to removal of nearly all photosynthetic tissue and limited below-ground resources. In design of leaf-clipping studies, it is suggested that the selection of clip height and the period of growth after clipping be optimized for each season of a study.

KEY WORDS: Leaf clipping · Leaf piercing · Productivity techniques · *Halodule wrightii* · Texas · Seagrass

INTRODUCTION

*Halodule wrightii* (shoal grass) is a narrow-leaved seagrass found in bays and estuaries across the Gulf of Mexico (Dawes 1987). It is the most abundant seagrass in Texas coastal waters (Adair et al. 1994), where it is a key ecological species (Onuf 1994). A widely used method for measuring leaf production in broad-leaved seagrasses, such as *Thalassia testudinum* and *Zostera marina*, involves piercing the leaves in the bundle sheath, just above the basal meristem (a variation of the Zieman 1974 method, Sand-Jensen 1975). The leaves of *H. wrightii* are generally 1 mm or less in width (Phillips & Meñez 1988 pers. obs.) and shoot densities commonly exceed 5000 m⁻² in subtidal populations in Laguna Madre, Texas (Kowalski 1999); thus, piercing the bundle sheath of *H. wrightii* is difficult. Few studies have used leaf piercing for productivity estimation of *H. wrightii* (Powell et al. 1989). Virnstein (1982) devised an in situ method for measuring *H. wrightii* leaf growth, which involved clipping the plants 1 to 2 cm above the sediment and subsequently photographing their growth. This method requires special equipment, relatively clear water, and low to moderate shoot densities. Dunton (1990) modified the protocol of Virnstein (1982) to that of a ‘clip and harvest’ approach (leaf clipping). This technique is simple and inexpensive, and circumvents the problems associated with thin leaves and high shoot densities, but it underestimates productivity by at least 50%, presumably because photosynthetic tissue is removed and clipped leaves are wounded (Tomasko & Dunton...
Although Tomasko & Dunton (1995) found that leaf clipping underestimated production, they concluded that leaf turnover and biomass calculations were appropriate for obtaining accurate production estimates. The need for leaf turnover rates requires estimates of leaf growth rates, and it is this parameter that has proved difficult to assess accurately in *Halodule wrightii*.

Other methods used to assess productivity in *Halodule wrightii* include carbon isotope uptake and oxygen evolution (Morgan & Kitting 1984, Dunton & Tomasko 1994). More recently, pulse amplitude modulated fluorometry has been used with *H. wrightii* to measure the effective quantum yield of photosystem II (Beer & Bjork 2000). These methods are considerably more complex and costly than leaf clipping.

When using leaf-clipping or leaf-piercing techniques to estimate seagrass production, it is useful to understand the leaf growth or elongation pattern for the species of interest and factors influencing leaf growth rate. Virnstein (1982) found that leaves of *Halodule wrightii* initially follow a logistic growth curve, undergo a period of declining growth rate, stop growth, achieve terminal leaf length, and then senesce, as is true of other seagrass species (e.g. *Halodule uninervis*, Brouns 1987, *Syringodium filiforme*, Fry 1983, *T. testudinum*, van Tussenbroek 1995). Studies by Greenway (1974), Virnstein (1982), Wittmann & Ott (1982), Tomasko & Dawes (1989), and Cebrian et al. (1998) have examined the effect of clipping on seagrass leaf growth. Removal of most of the leaf tissue of a shoot causes the subtending rhizome reserves and possibly those of the neighboring shoots and rhizomes to be used (Tomasko & Dawes 1989). Seagrass species that have small rhizomes, such as *H. wrightii*, have little reserve carbon and would be expected to have reduced shoot growth rates, or leaves would fail to achieve average terminal length, if a large proportion of leaf area is removed.

In terrestrial plants, differences in leaf growth rates resulting from different clip heights have long been observed in grasses (Albertson et al. 1953, Cook et al. 1958). More recently, Moreno et al. (1999) and Paige (1999) found that terrestrial grass shoots clipped closer to the meristem had slower leaf growth rates than those clipped at higher levels (McNaughton et al. 1983). The effect of clipping on final leaf biomass varies in terrestrial plants, with undercompensation (Sharma et al. 1998), exact compensation (Raillard & Svaboda 1999), and overcompensation (Moreno et al. 1999, Paige 1999) having been observed. Explanations for varying results have been attributed to life history differences (Sharma et al. 1998, for wetland annual compared with perennial), sediment and plant nutrient status (Raillard & Svaboda 1999, for an arctic sedge; Moreno et al. 1999 for *Amaranthus* sp.) and sexual reproduction differences (Mulder & Ruess 1998, for arrow grass).

To investigate the above issues, we clipped *Halodule wrightii* shoots at 0, 2, 4, 6, and 8 cm above the sediment to determine which clip height results in the highest leaf growth rate. In addition, we compared *H. wrightii* leaf elongation rates based on leaf-piercing and leaf-clipping methods to determine which method produces the highest leaf growth rates.

### MATERIALS AND METHODS

**Study site.** The studies were conducted in Lower Laguna Madre, Texas, approximately 10 km north of Brazos-Santiago Pass in a monotypic stand of *Halodule wrightii*, approximately 500 m west of the Gulf Inland coastal Waterway (26° 9.0952′ N, 97° 14.432′ W) (Fig. 1). Sediment at the study site is dredged material and is approximately 15 yr old. Sediment grain size distribution was 9% shell rubble (grain size > 0 phi, Folk 1964), 62% sand, 13% silt, and 16% clay (Kowalski 1999). Astronomical tides seldom exceed 20 cm in Laguna Madre (Hedgepeth 1947). Wind velocities are generally 6 to 12 m s⁻¹ for most of the year (Brown & Kraus 1997) and with an 8 km fetch from the southeast that during the study occasionally produced high waves and caused water levels to fluctuate between 0.9 and 1.3 m (average = 1.2 m) at the study site. During the study, surface water temperatures varied seasonally ranging between 8 and 31°C while surface salinity varied between 24 and 40‰.

**Experimental design.** *Leaf-clipping versus leaf-piercing experiment:* In 9 experiments performed between July 1995 and January 1997, leaf growth rates based on leaf-clipping and leaf-piercing techniques were compared. Experiments were conducted in dense (>5000 shoots m⁻²) and uniform stands of *Halodule wrightii*. For each experiment, shoots were clipped 2 cm above the sediment in one 50 × 50 cm quadrat (no replicate quadrats). Leaves were clipped with garden shears with clip height measured using a 50 × 50 cm (0.25 m²) adjustable PVC pipe and wire frame. Just after clipping, 1 core was taken in each quadrat near the periphery for determination of average clip height. Post-clip growth periods varied between 7 and 14 d. After the growth period, 4 cores were harvested from the middle of each quadrat to reduce the likelihood of edge effects. Also at the end of the growth period, 4
unclipped cores were taken outside the clipped areas about 2 m from the quadrats for estimation of average terminal leaf length. The 5 cores (9 cm diameter, 0.006 m² core⁻¹, 15 cm in depth) taken from each quadrat sampled approximately 13% of a quadrat. For the leaf-piercing technique, the bundle sheaths of 20 to 30 shoots were pierced just above the basal meristem with a 28 gauge hypodermic needle (<0.5 mm diameter). Piercing was done coincident with clipping at least 2 m from clipped quadrats. The base of each pierced shoot was tagged with a ring of tubing to facilitate relocation of marked shoots. On the same day as clipped plots were harvested, pierced shoots were harvested by severing shoots at rhizome level, sealing them in plastic bags, and refrigerating them until analysis. In the laboratory, the needle mark on the oldest leaf sheath was located and the shoot was cut at that location. Leaves from the shoot were separated by age and the distance between the cut surface and the needle mark, or in the absence of a mark, the leaf tip was measured (mm). Leaf growth rates expressed as mm d⁻¹ were calculated by dividing the average leaf growth interval by the number of days of growth.

For these experiments, mean leaf growth rates of the 2 techniques were compared using an independent samples t-test. In the t-test, the 9 different experiments were treated as replicates. A generalised linear model procedure (Sigma Plot 4.0 for Windows, SPSS Inc, Chicago, IL) was used for regression analysis using a least-squares fit between average growth rates determined by leaf-clipping and leaf-marking methods. An alpha level of 0.05 was used for statistical tests.

**Variable clip height experiment:** Five clip height experiments were performed between June and July 1995. There were 5 clip height treatments, i.e. seagrasses were clipped 0, 2, 4, 6, and 8 cm above the sediment, in separate quadrats at least 2 m apart. In each experiment, clip treatments were not replicated but new quadrats were established for each experiment.

Leaves were clipped using garden shears with clip heights measured using a 50 × 50 cm (0.25 m²) adjustable PVC pipe and wire frame. Just after clipping, 1 core was taken in each quadrat near the periphery for determination of average clip height. Post-clip growth periods varied between 7 and 14 d. After the growth period, 4 cores were harvested from the middle of each quadrat. Also at the end of the growth period, 4 unclipped cores were taken from outside the clipped areas about 2 m from the quadrats for estimation of average terminal leaf length.

All cores were gently cleaned of sediment and the plant material was sealed in plastic bags and refrigerated until processed. In the laboratory, leaves were separated from the shoots and leaf lengths were measured from the leaf tip to where the leaf changed from green (photosynthetic tissue) to white (non-photosynthetic tissue). All leaves of a shoot were measured and 10 to 20 leaves were measured to the nearest mm from each core. Leaves less than the clip height were not included in the data set. The initial average clip height was subtracted from the average final leaf length of clipped shoots of each treatment and divided by the growth period to obtain leaf growth rate in mm d⁻¹.

The variable clip height experiment was repeated 5 times over a 2 mo period. Each experiment used the same clip height treatments. For statistical analysis, the 5 experiments were considered blocks in a 1-way ANOVA with the dependent variable being leaf growth rate and the independent variable being leaf clip height. The sampling unit was the leaves of a shoot. When differences in means were found, Tukey's multiple comparison test was used to detect which clip treatments were different. Post-hoc testing determined that the data were normally distributed. An alpha level of 0.05 was used for statistical tests.
RESULTS AND DISCUSSION

Leaf-clipping versus leaf-piercing experiment

Pierced leaves were clearly discernible as complete holes (scars) or nicked edges. In general, the holes created by piercing were approximately half the width of the blade, but piercing generally did not damage leaves to the point that leaf tissue above the scar died. All leaf portions, whether clipped or pierced, appeared as green as control leaves. The mean growth rate for pierced leaves during June and July 1995 was 6.8 mm d$^{-1}$ and was 2 to 3 times the rate of the 2 cm clipped plants ($p < 0.001$, Fig. 2). Clipping underestimated leaf growth by more than 60% during the peak growth season in late spring to early summer, and by 15% in winter (December to February). Estimates of leaf growth rates for clipped and pierced plants were consistently different and well correlated over the 20 mo study period ($p = 0.0018$, $r^2 = 0.77$). The relation is described by $y = 2.18x - 0.34$, where $y$ is clip method growth rate and $x$ is the pierce method growth rate (Fig. 3).

Although leaf piercing damages the leaf, piercing appears to be less damaging than clipping as the leaf-piercing technique consistently gives higher leaf growth rates in *Halodule wrightii* than the leaf-clipping method. The linear relation between leaf growth rates of clipped and of pierced plants can be used to correct for underestimation inherent in the leaf-clipping technique. Because of site-specific differences that commonly exist among natural seagrass populations (Dunton 1996), our quantitative relation is likely not appropriate for *H. wrightii* populations outside the Lower Laguna Madre or at depths and shoot densities not encountered in this study. We recommend that investigators develop a regression equation appropriate for their particular region and use this relation with appropriate caution.

Adequate water transparency is necessary to pierce leaves little more than 1 mm wide. When water transparency is poor, the leaf-clipping technique is invaluable because leaves can be clipped almost entirely by feel. Exclusive use of the leaf-clipping technique regardless of clip height (see below) would significantly underestimate leaf elongation rates. The leaf-clipping method is appropriate for site or treatment comparison studies without correction and for determining leaf production in *Halodule wrightii* when used with a correction based on the leaf-piercing technique.

Variable clip height experiment

Mean leaf growth rate was greatest for the 2 cm clip height (3.66 mm d$^{-1}$) and least for the 8 cm treatment (2.30 mm d$^{-1}$) (Table 1). The rates for the 0, 4, and 6 cm clip heights were intermediate and varied between 3.35 (0 cm treatment) and 3.45 (6 cm treatment) mm d$^{-1}$. ANOVA determined that there were significant differences in mean leaf growth rates among the clip treatments ($p = 0.005$). According to Tukey’s multiple comparison test, there were no significant differences among the 0, 2, 4, and 6 cm treatments while each dif-
Table 1. Variable clip height experiment: results of Tukey’s multiple comparison test based on *Halodule wrightii* mean leaf growth rates (mm d−1). Values with the same letter are not significantly different

<table>
<thead>
<tr>
<th>Clip height (cm)</th>
<th>Mean ± SE</th>
<th>Tukey</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3.66 ± 0.30</td>
<td>A</td>
</tr>
<tr>
<td>4</td>
<td>3.55 ± 0.30</td>
<td>A</td>
</tr>
<tr>
<td>6</td>
<td>3.45 ± 0.27</td>
<td>A, B</td>
</tr>
<tr>
<td>0</td>
<td>3.35 ± 0.41</td>
<td>A, B</td>
</tr>
<tr>
<td>8</td>
<td>2.30 ± 0.35</td>
<td>B</td>
</tr>
</tbody>
</table>

fered from the 8 cm treatment, and in the secondary comparison the 0, 6, and 8 cm treatments differed from the 2 and 4 cm treatments (Table 1).

On the basis of the primary Tukey comparison, the 0 to 6 cm clip height leaf growth rates did not differ, while all differed from the 8 cm treatment. These results contradict our original hypothesis that the higher the proportion of leaf tissue removed the slower the leaf growth rate. On the basis of our results, it appears that the plants had adequate stored reserves (from subtending rhizomes or neighboring ramets) or enough production from the remaining photosynthetic tissue to maintain the same growth rate regardless of the amount of leaf material removed. The low mean growth rate for the 8 cm clip was due to the clipped leaves being relatively old. It is presumed that their rate of elongation slowed with increasing age as seen in *Zostera marina* (Jacobs 1979) and *Thalassia testudinum* (Patriquin 1973, van Tussenbroek 1995). The secondary Tukey comparison indicated no difference among the 0, 6, and 8 cm clip treatments, which differed from the 2 and 4 cm treatments. The low rate for the 0 cm clip suggests that there was a negative effect on growth rate of removing nearly all the photosynthetic tissue from a shoot. The depressed rate for the 6 cm clip suggests that leaves able to be clipped at 6 cm may be old enough to show depressed growth rates as with 8 cm clipped leaves.

In our study, clipping effects on *Halodule wrightii* leaves showed exact compensation of biomass in contrast to undercompensation reported by Morgan & Kitting (1984) for the same species. As with other seagrasses, the roots and rhizomes of *H. wrightii* act as a soluble carbohydrate reservoir for growth, having lowest reserves in spring and highest in the fall (Dawes & Lawrence 1980, Kowalski 1999). Morgan & Kitting (1984) did not explain their findings. Complete biomass compensation in cropped shoots has been shown for other tropical and subtropical seagrass species (Cebrian et al. 1998).

Our results indicate that the selection of clip height in a leaf-clipping study can affect the degree of under-estimation of leaf growth rate values. Besides the obvious effect of very long clip heights, underestimation would likely be amplified when lower clip heights are used where the plants have low resource reserves during springtime, when plants are under environmental stress or when shoot density is low. Under these conditions, actual growth rates (determined by leaf piercing) and leaf clip-determined growth rates may increasingly differ the greater the proportion of each leaf removed and the more leaves that are removed. The more photosynthetic material removed the greater the draw on plant reserves (immediate or distant). When reserves become limiting, growth would become more a function of leaf photosynthetic production rate, resulting in slower leaf growth rates or failure of leaves to achieve the pre-clip terminal height (Morgan & Kitting 1984). It is partly for this reason that the same plots should not clipped repeatedly to measure leaf growth rates in seagrass research.

There is perhaps another reason to avoid the use of short clip heights. In *Thalassia testudinum*, Patriquin (1973) found that a small amount of leaf elongation occurred at leaf marks 5 cm above the sediment. This effect would likely have been more pronounced if marks lower than 5 cm had been used. If post-meristematic region growth occurs with *Halodule wrightii*, it would be of interest to determine how much this type of growth contributes to total leaf growth.

Besides clip height, the choice of the length of the growth period between clipping or piercing and harvest should be evaluated when measuring leaf growth rates. During warmer months, *Halodule wrightii* leaf growth rates and terminal leaf lengths are greater (Kowalski 1999) and likely plastochrone intervals (Erickson & Michellini 1957) shorter than during colder months. The majority of leaves clipped or pierced on a shoot should be harvested before their growth rate starts to slow with age, i.e. before they near their terminal length. On the other hand, the growth period needs to be long enough to achieve an increment of growth that would show significant differences from the initial clip height. Because of these factors, it is suggested that preliminary measurements be performed during each season of a study to determine appropriate intervals between clipping or piercing and harvest.

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