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Measuring production of *Halodule wrightii*: additional evidence suggests clipping underestimates growth rate

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Abstract

Growth rates of *Halodule wrightii* determined using the traditional method of clipping and re-harvesting were compared to those determined using the hole-punching method in four populations distributed in coastal lagoons of the Yucatan Peninsula, Mexico. For three of the four populations, clipped shoots revealed 38–56% lower growth rates than punched shoots. The decrease in leaf production per shoot and on an areal basis in clipped shoots compared to punched shoots resulted from generally lower new leaf length, and, of equal or greater importance, leaf width of these shoots. Thus, the clip and reharvest method for assessing *H. wrightii* leaf production yielded underestimates. Leaf-punching is a convenient method in populations characterized by relatively broad leaves, such as those in Yucatan lagoons. From these populations, the highest published values for leaf elongation rates of *H. wrightii* to date ($11.7\text{--}20.6\text{ mm shoot}^{-1}\text{ d}^{-1}$) and summer areal production rates of $4.2\text{--}5.8\text{ g dw m}^{-2}\text{ d}^{-1}$. Long-term production data for this species are crucial for managing subtropical coastal environments. Because contrasting methods have been employed to estimate the growth of *H. wrightii*, spatial and temporal comparisons of production from published reports are difficult to make, and a unified robust method for estimating growth rates and areal production of this seagrass is needed. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: *Halodule wrightii*; Seagrass; Production; Growth; Density; Width; Yucatan

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1. Introduction

The relatively narrow-bladed shoal grass, *Halodule wrightii* Ascherson, is commonly distributed in subtropical regions of Caribbean and Gulf of Mexico coasts and contributes to a significant percentage of benthic primary production in these areas (Virnstein, 1982; Morgan and Kitting, 1984; Pulich, 1989; Dunton, 1990, 1994; Gallegos et al., 1994; Lapointe et al., 1994; Czerny and Dunton, 1995; Tomasko and Dunton, 1995). Because *H. wrightii* has relatively little rhizome storage capacity, it may be particularly sensitive to alterations in light, water circulation, temperature, salinity, and nutrients. Reduced water quality caused by a brown tide in the Laguna Madre, TX, resulted in a rapid loss of 2.6 km² of *H. wrightii* cover after only 3.5 years (Onuf, 1996). The opposite, yet rapid, response occurred in Florida Bay where biomass of *H. wrightii* increased by an order of magnitude, shoot production doubled, and areal production increased by three orders of magnitude after 2 years of chronic nutrient addition via bird guano (Powell et al., 1989). *H. wrightii* is thus, a sensitive indicator of alterations in water quality characteristics, and long-term production data for this species are crucial for managing subtropical coastal environments.

Because of the relative ease with which anthropogenic influences on watersheds and adjoining coastal waters negatively impact seagrass systems (Sand-Jensen and Borum, 1991; Duarte, 1995; Short and Burdick, 1996; Short and Wyllie-Echeverria, 1996; Valiela et al., 1997; Hauxwell et al., 2001), it is important to accurately document seagrass production within these systems and monitor for alterations. For wide-bladed (>1 mm) seagrass species (e.g. *Thalassia testudinum* Banks ex König, *Zostera marina* L., *Posidonia oceanica* (L.) Delile) the marking technique of Zieman and Wetzel (1980) or slight modification (punching needle holes at the sheath-blade juncture) has proven to provide the best, least obtrusive method for measuring aboveground seagrass production in situ. However, only two studies have reported estimates of *H. wrightii* production using the marking technique (Morgan and Kitting, 1984; Powell et al., 1989), because *H. wrightii* is very difficult to tag or punch without tearing its leaves. Consequently, production estimates of *H. wrightii* are scarce relative to other seagrass species.

The few estimates of *H. wrightii* production which do exist have been made using a variety of methods, each with its own inherent caveats, making comparisons between techniques questionable. Laboratory measurements of ¹⁴C uptake or oxygen production extrapolated to field shoot densities and light levels obviously incur a variety of problems, including the manipulation and measurement of plant material outside its actual environment. Rates of photosynthesis versus irradiance relationships obtained in the laboratory applied to field irradiance yielded overestimates of production due to higher slopes of the *P* versus *I* curves (Dunton and Tomasko, 1994). Field measurements of ¹⁴C uptake (Dillon, 1971; Brylinsky, 1977; Montcreiff et al., 1992) or O₂ production (Dunton and Tomasko, 1994; Dunton, 1996) incur the problems of excluding concurrent production by epiphytic, water column, or benthic micro-producers during experiments, require significant field equipment and preparation, and provide instantaneous measurements which must be collected throughout all daylight hours to account for changes in irradiance. ¹⁴C uptake also may result in underestimates of production due to significant CO₂ recycling within intercellular gas lacunae (Zieman and Wetzel, 1980).

In situ clipping of entire shoots near meristems and reharvesting several days later (Virnstein, 1982; Morgan and Kitting, 1984; Pulich, 1989; Dunton, 1990, 1994; Lapointe et al., 1994; Czerny and Dunton, 1995) has become the most widely used method for measuring leaf production of *H. wrightii* due to its relative ease and inexpense and also because of the integrative nature of the approach, where fluctuations in abiotic or biotic influences over several days are incorporated in the measurement. However, it is quite likely that this method alters actual rates of production due to (1) the removal of such a high percentage of photosynthetic material (85%, in this study) and (2) potential mobilization of stored reserves within rhizomes in response to leaf removal (Cebrián et al., 1998). Data from Morgan and Kitting (1984) suggest that tagged blades show higher production values than clipped blades, but because of significant loss of tagged blades and because measurements were not made on a per shoot basis, we are unable to generalize these differences. Only one other study has attempted to assess the validity of the clip and reharvest approach using an independent estimate of aboveground production based on aboveground shoot biomass and turnover (Tomasko and Dunton, 1995). They found that, on an annual basis, the clip and reharvest method provided estimates of $360 \text{ g m}^{-2} \text{ y}^{-1}$ versus the independent estimate of $570 \text{ g m}^{-2} \text{ y}^{-1}$, an approximate 40% discrepancy (data converted from Tomasko and Dunton, 1995: figs. 3 and 5, methods 2 versus 4). Overall, information regarding the validity of the clip and reharvest method is still quite meager.

The goals of our study were (1) to determine whether the clip and reharvest method provided accurate estimates of leaf production rates of *H. wrightii* and (2) to test the applicability of the punching method in populations of *H. wrightii* characterized by relatively broad leaves. A field experiment was conducted during summer 1999 in four *H. wrightii* populations on the Yucatan Peninsula, Mexico, which were comprised of shoots with leaves of sufficient width (1–2 mm) to employ the hole-punching method.

2. Methods

2.1. Study sites

The experiment ran from late August to early September 1999 in populations of *H. wrightii* in coastal lagoons of the Yucatan Peninsula, including one located on the north-eastern coast (Bojorquez: $21^{\circ}07'51.4''\text{N}$, $86^{\circ}45'3.0''\text{W}$), one on the northwestern coast (Punta Cambalám: $20^{\circ}44'50.0''\text{N}$, $90^{\circ}26'3.0''\text{W}$), and two on the north-central coast (Chelem East: $21^{\circ}15'57.0''\text{N}$, $89^{\circ}41'28.0''\text{W}$ and Chelem West: $21^{\circ}16'11.0''\text{N}$, $89^{\circ}41'18.0''\text{W}$). Each site contained monospecific stands of *H. wrightii*, comprised of shoots ranging in height from 10 to 30 cm, in depths of approximately 0.5–1.0 m. Temperatures ranged from 30 to 33°C , and salinities ranged from 34.1 to 36.6‰. Sediments at Bojorquez and Punta Cambalám were sandier (~70% sand, 30% silt or clay) relative to sediments at the Chelem sites (~40% sand, 60% silt or clay). Additional information regarding physical and biological features of these Yucatan lagoons may be found in Herrera-Silveira et al. (1998).

2.2. Description of techniques for measuring leaf production

For the clip and reharvest method, all shoots within a 25-cm² quadrat were clipped with scissors just above the sheath-blade juncture of the oldest leaf on the shoot by SCUBA divers. For the punching method, all shoots within a 25-cm² quadrat placed approximately 1 m from the clipped shoots were carefully punched twice with a 29-gauge (350- μ m diameter) hypodermic insulin needle just above the sheath-blade juncture of the oldest leaf on the shoot by SCUBA divers. Approximately 2 weeks later, all shoots within the quadrats were retrieved and brought to the laboratory where we sorted out all shoots bearing leaves with clearly recognizable sharp cut tips, resulting from clipping, or the two holes we imprinted by punching. Fewer measurable shoots were retrieved than the number initially clipped or marked, since in some shoots, the clear cut edges or punched holes were lost due to intensive grazing or physical breakage. Additionally, due to the high turnover rate of this species, some shoots clipped or punched may have died during the experimental period. This loss of shoots did not, however, affect the significance of the results obtained.

For shoots that were recognizably clipped or punched, number of leaves, sheath length of the oldest leaf, and width and length of each leaf was recorded. For every leaf on each clipped shoot, new leaf length was measured as the difference between total length and length of the oldest sheath. For every leaf on each punched shoot, new leaf length was measured as the distance traveled by the holes, or, in the case of leaves that emerged after punching, the difference between total length and length of the oldest sheath. Estimates of shoot growth rates (cm² shoot⁻¹ d⁻¹) were determined by multiplying new length for each leaf by its width, summing for all leaves within a shoot, and dividing by the number of days in the experimental period. To express growth rates as mg dw shoot⁻¹ d⁻¹ 10 measured shoots from each population were dried overnight at 60°C and weighed to determine an average conversion factor from leaf surface area to biomass. Estimates of areal production (g dw m⁻² d⁻¹) were obtained for each method by multiplying mean growth rates at each population by shoot density measured in triplicate 15-cm diameter cores taken randomly at each study site.

2.3. Statistical analyses

To be certain that results were not influenced by any initial size differences between clipped and punched shoots, shoot biomass versus number of leaves per shoot was first regressed for an independent sample of approximately 100 shoots collected at the beginning of the experiment in each population. Each regression was highly significant ($P < 0.001$), with regression coefficients ranging from 0.38 to 0.65. Number of leaves per shoot was used as a proxy for biomass, and the number of leaves per shoot between the retrieved clipped and punched shoots in each population were compared with a Mann–Whitney *U*-test. A Mann–Whitney *U*-test was also employed to determine whether leaf widths, new lengths, and total new surface area produced differed between clipped versus punched shoots for pooled data from all populations and also within each population. The choice of the non-parametric Mann–Whitney *U* comparisons was due to the marked non-normal distribution of the variables analyzed.

2.4. Compilation of published reports of leaf production, shoot density, and leaf widths

We searched the peer-reviewed published literature through 1999 to compile reports of leaf production, shoot density, and leaf widths for *H. wrightii*. These compilations allowed comparisons of these measurements with several other sites, allowed comparison of methods used to measure production, and provided important information regarding the applicability of leaf punching, which is dependent on leaf width, for other populations.

3. Results

There was no significant difference in number of leaves per shoot between clipped and punched shoots in any of the populations ($P > 0.1$ for all cases). Due to the significant regressions between number of leaves per shoot versus shoot biomass, the initial sizes of clipped shoots were not different from those of punched shoots. Therefore, differences in the following measured parameters between the two methods may only be a result of the differential effects of clipping versus punching, and not of shoot size.

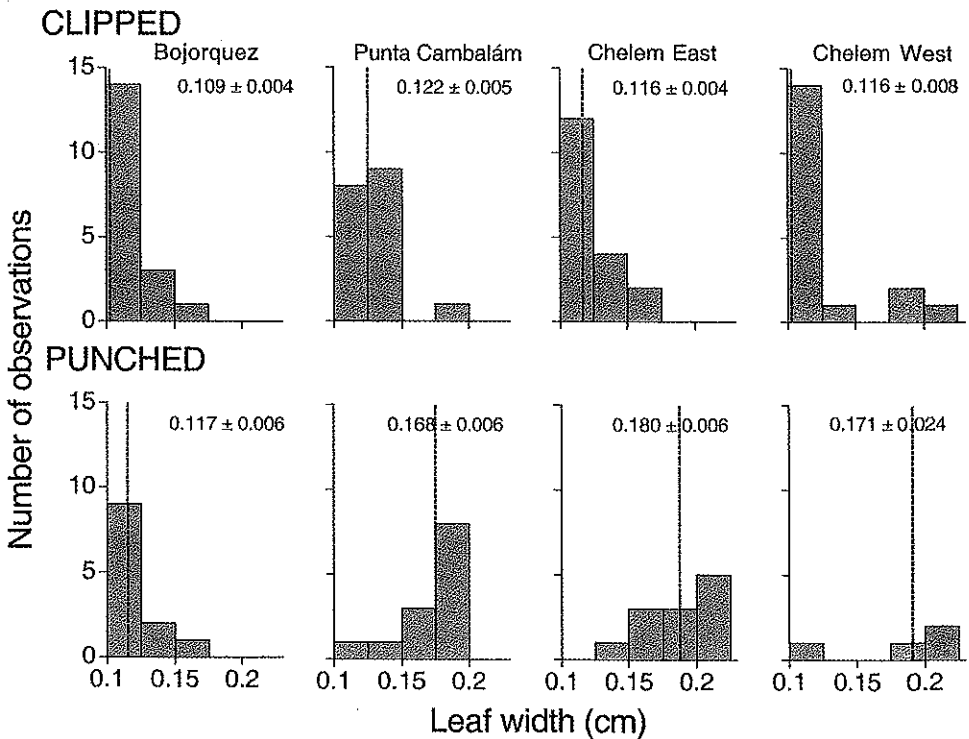


Fig. 1. Frequency distributions of mean leaf width for both clipped and punched shoots in four populations of *H. wrightii* on the Yucatan Peninsula, Mexico, during late summer 1999. Mean leaf widths per shoot \pm S.E. are provided, and dashed lines indicate medians.

Clipped shoots exhibited narrower leaves than punched shoots when all populations were considered together, and also within each population considered individually except Bojorquez (Fig. 1 and Table 1). Similarly, clipped shoots produced shorter leaf lengths than punched shoots when all populations were considered together, and specifically, within populations at Punta Cambalám and Chelem West (Fig. 2; Tables 1 and 2). The contribution of leaf width to differences in leaf growth rates were, for each population, similar to or greater than the contribution of new leaf length. When comparing averages between methods for shoots from Punta Cambalám, leaf widths were 27% lower in clipped than in punched shoots compared to a 24% difference in new leaf lengths. Widths of leaves from Chelem East were 36% lower in clipped than in punched shoots compared to only a 4% difference in new leaf lengths. For shoots from Chelem West, widths were 32% lower in clipped than in punched shoots compared to a 31% difference in new leaf lengths.

Because leaf widths and new lengths were, in most cases, significantly lower in clipped than in punched shoots (Figs. 1 and 2; Table 1), clipped shoots produced less total leaf surface area than punched shoots overall, and in three of the four populations (44% lower in Punta Cambalám, 38% in Chelem East, and 56% in Chelem West) (Fig. 3 and Table 1). These differences were also present when leaf production was expressed on an areal basis (Fig. 4 and Table 2) after applying the conversion factor from surface area to biomass ($1 \text{ cm}^2 = 0.0039 \text{ g}$) and extrapolating to actual shoot densities (Table 3).

Table 1

Results of Mann–Whitney *U*-tests comparing distributions of mean leaf width, new leaf length, and total new leaf surface area in clipped versus punched shoots for pooled data from all populations and within each individual population of *H. wrightii* on the Yucatan Peninsula, Mexico

Shoot characteristic	Population	<i>N</i> ^a	<i>U</i> ^b	<i>P</i> ^c
Width	Pooled data	113	562	<0.001
	Bojorquez	30	84	0.25
	Punta Cambalám	30	17	<0.001
	Chelem East	31	5	<0.001
	Chelem West	22	14	<0.05
New length	Pooled data	113	957	<0.01
	Bojorquez	30	110	0.95
	Punta Cambalám	30	45	<0.01
	Chelem East	31	125	0.75
	Chelem West	22	9	<0.05
Total new surface area	Pooled data	113	580	<0.001
	Bojorquez	30	102	0.80
	Punta Cambalám	30	25	<0.001
	Chelem East	31	33	<0.001
	Chelem West	22	9	<0.05

^a Sample size.

^b Mann–Whitney *U*-test statistic.

^c Probability.

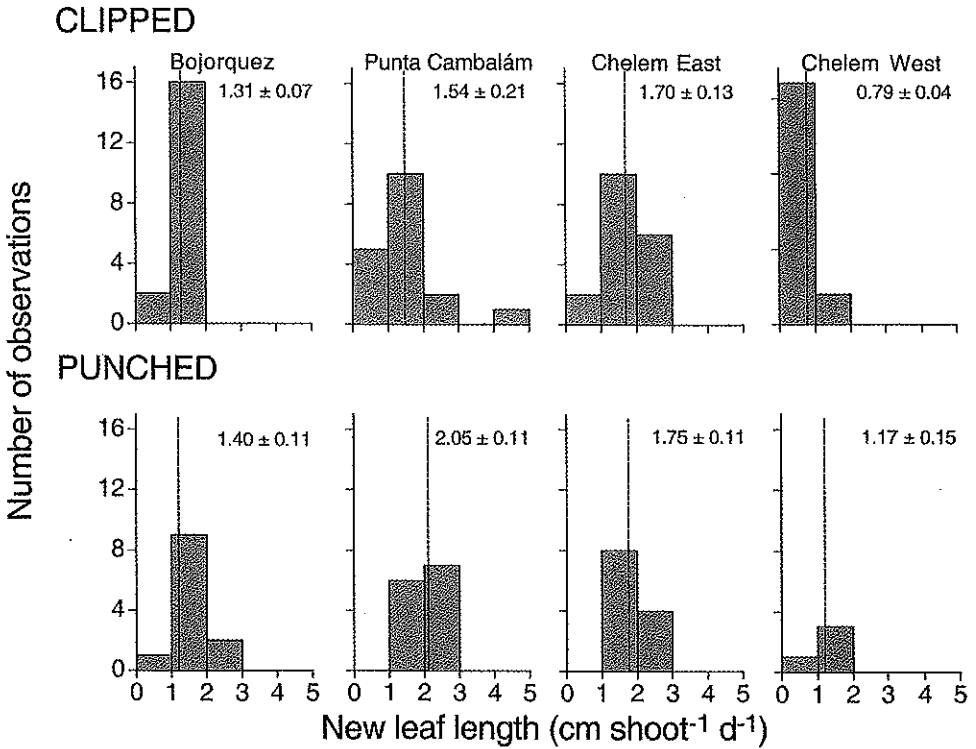


Fig. 2. Frequency distributions of new leaf length produced for both clipped and punched shoots in four populations of *H. wrightii* on the Yucatan Peninsula, Mexico, during late summer 1999. Mean ± S.E. are provided, and dashed lines indicate medians.

Review of the literature revealed 20 estimates of *H. wrightii* production (Table 2), density measurements within 17 populations (Table 3), and leaf width information for 26 populations (Table 4), in addition to the Yucatan populations reported here. Table 2 illustrates (1) that spatial and temporal gaps in production estimates and inconsistent methodologies make comparisons difficult; (2) disparities in leaf and areal production estimates based on clipping versus punching within the Yucatan populations were consistent with differences in other populations; and (3) Yucatan populations were highly productive relative to other populations. Shoot density for the Yucatan populations (Table 3; our study, up to 15,545 shoots m⁻²; Gallegos et al., 1994, 14,872 shoots m⁻²) were relatively high compared to Florida, Mississippi, and the San Antonio Bay, TX, populations and were similar to densities in populations from Laguna Madre and Corpus Christi, TX, and Brazil. From these Yucatan populations, the widest leaves, up to 2.00 mm, have been recorded (Table 4). The overall range of previously reported leaf widths was 0.42–1.27 mm.

Table 2
Compilation of published leaf production values (elongation per shoot and on an areal basis; ranges or mean \pm S.E., unless noted) measured for *H. wrightii* using several methods and results from this study^a

Source	Study location	Time period	Method	Leaf production (mm shoot ⁻¹ d ⁻¹)	Areal production (g dw m ⁻² d ⁻¹)
Dillon (1971)	North Carolina	April 1980	¹⁴ C uptake		~1.25–5.0
Vinstein (1982)	Indian River, FL	Annual average	Clip and reharvest	3.7–8.5	1.3–3.0
Morgan and Kitting (1984)	Corpus Christi, TX	July–August 1980	(1) ¹⁴ C uptake in field incubations extrapolated to actual biomass		~1.4–1.9
		July–August 1980	(2) Clip and reharvest	~3.3	
		October 1986	(3) Tag and reharvest	~5.7	
Powell et al. (1989)	Florida Bay		Punched holes using number 00 stainless steel insect pins		
	Control site			~3.1	0.013 \pm 0.013
	Nutrient-enriched site			~6.9	16.4 \pm 5.7
Pulich (1989)	Redfish Bay, TX	May 1985	Clip and reharvest		0.58 \pm 0.05 (S.D.)
		September 1985			2.12 \pm 0.09 (S.D.)
Montcreiff et al. (1992)	Horn Island, Mississippi Sound	Annual average	¹⁴ C uptake in field incubations extrapolated to actual biomass		~1.75
Dunton (1990)	Corpus Christi, TX	Annual range	Clip and reharvest	~0.2–6.0	
Dunton (1994)	Laguna Madre, Corpus Christi, San Antonio Bay, TX	Annual range (5 years)	Clip and reharvest	~0.8–7.0	
Lapointe et al. (1994)	Florida Keys, FL	Summer 1991–Winter 1992	Clip and reharvest		0.7–1.0
Gallegos et al. (1994)	East Yucatan Peninsula, Mexican Caribbean	Annual average	Reconstructive techniques		12.7
Czerny and Dunton (1995)	Corpus Christi Bay, TX	October 1992	Clip and reharvest	1.7 \pm 1.2	
		November 1992		2.2 \pm 1.9	
		January 1993		1.2 \pm 0.6	
		February 1993		1.2 \pm 0.6	
		Annual range	(1) Clip and reharvest		0.1–1.7
Tomasko and Dunton (1995)	Laguna Madre, TX		(2) Estimate based on above ground shoot biomass and turnover		0.1–4.3
		August–September 1999	(1) Clip and reharvest	7.9–17.0	2.4–3.3
			(2) Punch and reharvest	11.7–20.6	4.2–5.8

^a ~ indicates our approximation or conversion from actual data sets; 40% carbon content was assumed to convert from units carbon to g dw.

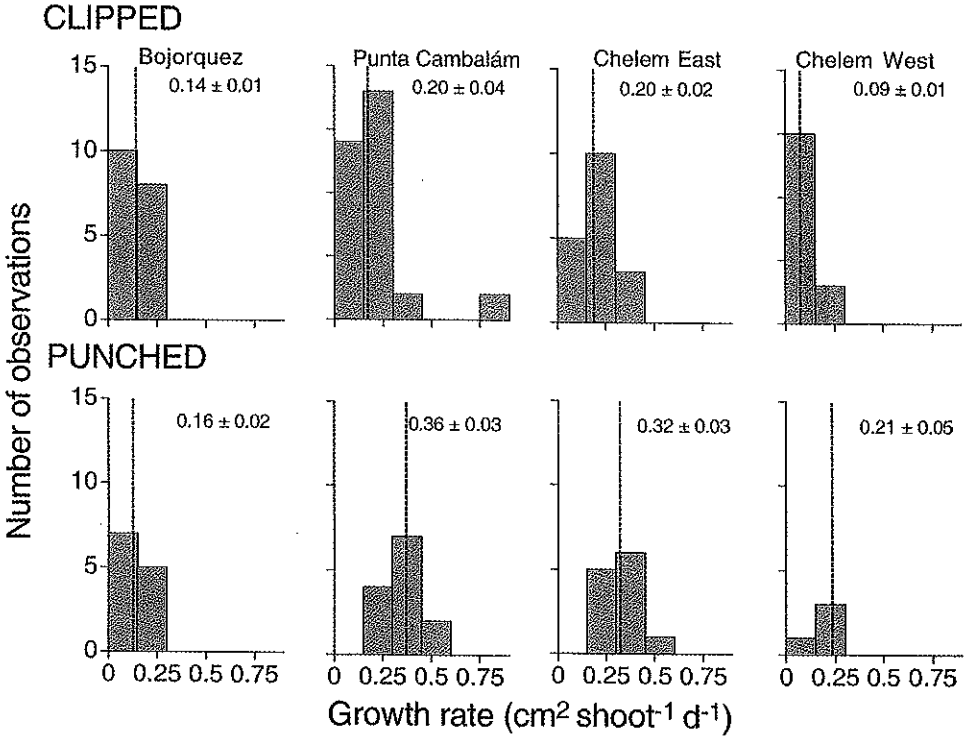


Fig. 3. Frequency distribution of leaf surface area produced for both clipped and punched shoots in four populations of *H. wrightii* on the Yucatan Peninsula, Mexico, during late summer 1999. Mean \pm S.E. are provided, and dashed lines indicate medians.

Table 3

Density of shoots in populations of *H. wrightii* on the Yucatan Peninsula, Mexico, and compilation of other published reports of shoot densities

Source	Study location	Shoot density (shoots m ⁻²)	
		Range	Mean (\pm S.E.)
This study	Bojorquez, Yucatan, Mexico	1698–15545	6710 \pm 4431
	Punta Cambalám, Yucatan, Mexico	3339–4923	4225 \pm 467
	Chelem East, Yucatan, Mexico	5602–6451	6149 \pm 274
	Chelem West, Yucatan, Mexico	4980–6508	5923 \pm 476
Gallegos et al. (1994)	East Yucatan Peninsula, Mexico		14872 \pm 2444
Pangallo and Bell (1988)	Tampa Bay, FL	1250–5000	
Lapointe et al. (1994)	Florida Keys, FL	1500–3250	
Eleuterius (1987)	North of Horn and Petit Bois Islands, MS	300–3200	2400
Dunton (1996)	Gulf of Mexico, East Texas coast	1200–12500	
	Laguna Madre		6351 \pm 412
	Corpus Christi		5551 \pm 411
	San Antonio Bay		3201 \pm 514
Creed (1999)	10 Populations of Rio de Janeiro, Brazil	2000–12000	

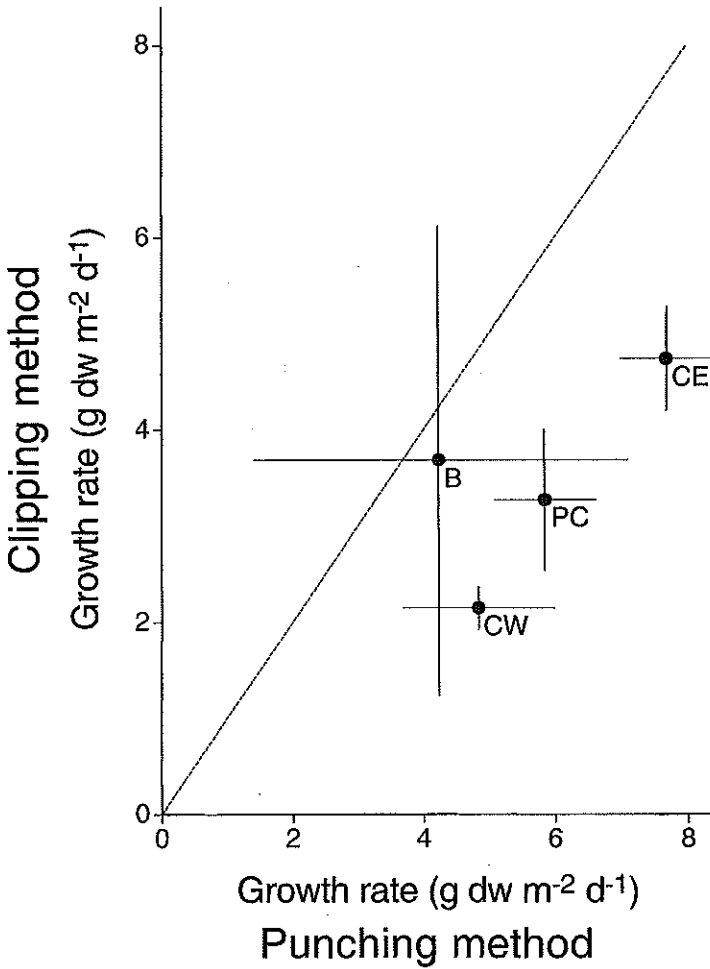


Fig. 4. Areal leaf production rates of clipped versus punched shoots (mean \pm S.E.) in four populations of *H. wrightii* on the Yucatan Peninsula, Mexico, during late summer 1999 (B, Bojorquez; PC, Punta Cambalám; CE, Chelem East and CW, Chelem West). The 1:1 line is also shown.

4. Discussion

Due to the combined effect of generally narrower widths and shorter new lengths, the clip and reharvest method provided underestimates of leaf growth compared to the punching method overall, and specifically, in three of our four populations of *H. wrightii*. These results demonstrate (1) that clipping alters the morphology of the replacing leaf material by decreasing width and (2) that elongation rates of new leaf material are also decreased relative to unclipped leaves.

Table 4

Compilation of published ranges of leaf widths of *H. wrightii* and results from this study^a

Source	Study location	Leaf widths (mm)
den Hartog (1970)		0.33–1.00
Phillips (1960)	Boca Ciega Bay, FL	
	Plants exposed at low tides	0.38–0.50
	Plants exposed at spring low tides	1.00–1.00
	Plants always submerged	1.50–1.50
Pangallo and Bell (1988)	Tampa Bay, FL	0.50–1.00
McMillan (1978)	Matagordo Bay, TX	0.50–0.56
	Redfish Bay, TX	0.45–0.75
	Upper Laguna Madre, TX	0.42–0.47
	Port Isabel, TX	0.61–0.70
	Marathon, FL	0.95–1.07
	Key West, FL	1.09–1.27
	La Pesca, Tamaulipas, Mexico	0.76–0.87
	Tamiahua, Veracruz, Mexico	1.00–1.12
	Isla de Enmedio, Veracruz, Mexico	1.03–1.09
	Tampamachaco, Veracruz, Mexico	0.86–1.07
	Montego Bay, Jamaica	0.52–0.80
	San Pedro Island, Belize	0.60–0.82
	St. Croix, US Virgin Islands	0.42–0.58
Hauxwell pers. obs.	Glovers Atoll, Belize	0.75–1.20
Magalhaes et al. (1997)	Pernambuco, Brazil	0.60–0.79
Creed (1997)	Nine populations of Rio de Janeiro, Brazil	0.22–0.92
This study	Four populations of Yucatan, Mexico	1.00–2.00

^a Data from McMillan (1978) were ranges from experiments designed to determine phenotypic plasticity of *H. wrightii* from different locations. The samples from Hauxwell (personal observation) were ranges from 100 measured leaves collected in December 1999.

Based on these results, as well as those of Morgan and Kitting (1984) and Tomasko and Dunton (1995), it is likely that *H. wrightii* summer leaf production has been repeatedly underestimated when the clip and reharvest method has been employed (Virstein, 1982; Morgan and Kitting, 1984; Pulich, 1989; Dunton, 1990, 1994; Lapointe et al., 1994; Czerny and Dunton, 1995). For this one summer sampling interval, values of leaf production were 1.6–2.2 times higher using the punching method compared to the clipping method. These values, along with an underestimation factor of 1.7 found by Morgan and Kitting (1984) and the range of underestimation factors of 1.0–2.5 found by Tomasko and Dunton (1995) (calculated from Table 1), suggest that estimates of summer leaf production of *H. wrightii* obtained with the clipping technique lie within 40–100% of the real value. However, because the response of shoots to clipping is variable and dependent on many factors (i.e. seasonal fluctuations in resource availability, rhizome energy reserves, intensity of grazing or leaf breakage) (Cebrián et al., 1998) it is not valid to use our results to generalize discrepancies on an annual basis. Future research, comparing year-round measurements of growth rates using both methods, would be necessary to determine the extent to which annual measurements of leaf production in the literature may be offset.

Application of the punching method showed that the Yucatan populations of *H. wrightii* are highly productive when compared to published values from regions at higher latitudes.

For the Yucatan populations in this study, elongation rates per shoot ranged from 11.7 to 20.6 mm d⁻¹; the maximum from any other population was 8.5 in the Indian River, FL (Virnstein, 1982). This difference in productivity is even magnified when expressed on an areal basis due also to the high density of shoots in the Yucatan populations compared to published estimates from other populations in Florida, Mississippi, and San Antonio Bay, TX. However, because of temporal and spatial gaps in published accessible reports and the variety of methods employed, it is not yet possible to evaluate whether there is a latitudinal gradient in *H. wrightii* production associated with greater light availability. Because of the phenotypic variation in leaf widths within several populations (potential twofold differences), width information, provided when “elongation rate” is the reported unit of production, would be useful; this information would allow estimates of total new surface area or biomass produced and make production comparisons among systems more accessible.

As with each other method of estimating *H. wrightii* production, the punching method has inherent advantages and disadvantages. Most limiting is the fact that it may be possible to employ only in certain populations characterized by shoots bearing relatively wide-bladed leaves (>1 mm). After compiling literature reports of widths, it appears that the punching method may be feasible for 34% of the populations compiled. For these populations, use of this method would also be beneficial in that it would allow simple comparisons of leaf production to be made with other seagrass species for which this method has also been employed. Second, field and in laboratory processing may be time-consuming, although this method is much more efficient than methods using PI curves or ¹⁴C incubations. Third, unless field incubation periods are relatively short (i.e. less than 1 week) growth rates of small one-leafed shoots are neglected (in our study, the plastochrone interval of *H. wrightii* was approximately 8 days). However, compared with instantaneous results obtained with other methods, punching provides, at the least, week-long integrative measurements representative of in situ conditions. Last, as with clipping, it provides only estimates of leaf production. Tomasko and Dunton (1995) showed that methods that include whole plant production may yield rates from one to ten times greater than those using only aboveground estimates.

The compilation of production measurements illustrates not only the spatial and temporal gaps in published *H. wrightii* production data, but also that spatial and temporal comparisons are not valid to make based even on existing data due to the inconsistent methodologies employed. A unified, robust, long-term method for estimating growth rates and areal production of this seagrass is much needed. This report, along with others (Morgan and Kitting, 1984; Tomasko and Dunton, 1995), indicates that the clip and reharvest method does not provide accurate measurements of leaf production. For Yucatan and other populations characterized by wide-bladed phenotypes (Table 4), the punching method provides reliable measurements of short-term leaf production. Development of an underwater non-destructive “marker”, such as a non-water soluble marker pen (Penol 700; Sand-Jensen, 1975) may be useful for all populations. Non-destructive marking or the punching method, combined with reconstruction techniques (Gallegos et al., 1994), where information gathered on a single visit may allow researchers to elucidate temporal patterns of above- and belowground seagrass production, may provide the most comprehensive approach by which to monitor *H. wrightii* production within these less-studied Gulf of Mexico and Caribbean habitats, as well as others.

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References

- Brylinsky, M., 1977. Release of dissolved organic matter by some marine macrophytes. *Mar. Biol.* 39, 213–220.
- Cebrián, J., Duarte, C.M., Agawin, N.S.R., Merino, M., 1998. Leaf growth response to simulated herbivory: a comparison among seagrass species. *J. Exp. Mar. Biol. Ecol.* 220, 67–81.
- Creed, J.C., 1997. Morphological variation in the seagrass *Halodule wrightii* near its southern distributional limit. *Aquat. Bot.* 59, 163–172.
- Creed, J.C., 1999. Distribution, seasonal abundance and shoot size of the seagrass *Halodule wrightii* near its southern limit at Rio de Janeiro state, Brazil. *Aquat. Bot.* 65, 47–58.
- Czerny, A.B., Dunton, K.H., 1995. The effects of in situ light reduction on the growth of two subtropical seagrasses, *Thalassia testudinum* and *Halodule wrightii*. *Estuaries* 18, 418–427.
- den Hartog, C., 1970. *The Sea-grasses of the World*. North-Holland, Amsterdam, pp. 1–275.
- Dillon, C.R., 1971. A comparative study of the primary productivity of estuarine phytoplankton and macrobenthic plants. Ph.D. Dissertation, University of North Carolina, Chapel Hill, NC, 112 pp.
- Duarte, C., 1995. Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia* 41, 87–112.
- 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.
- Dunton, K.H., 1994. Seasonal growth and biomass of the subtropical seagrass *Halodule wrightii* in relation to continuous measurements of underwater irradiance. *Mar. Biol.* 120, 479–489.
- Dunton, K.H., 1996. Photosynthetic production and biomass of the subtropical seagrass *Halodule wrightii* along an estuarine gradient. *Estuaries* 19, 436–447.
- Dunton, K.H., Tomasko, D.A., 1994. In situ photosynthesis in the seagrass *Halodule wrightii* in a hypersaline subtropical lagoon. *Mar. Ecol. Prog. Ser.* 107, 281–293.
- Eleuterius, L.N., 1987. Seagrass ecology along the coasts of Alabama, Louisiana, and Mississippi. In: Durako, M.J., Phillips, R.C., Lewis III, R.R. (Eds.), *Proceedings of the Symposium on Subtropical–Tropical Seagrasses in the Southeastern United States*. Fla. Mar. Res. Publ. 42, pp. 11–20.
- Gallegos, M.E., Merino, M., Rodriguez, A., Marbà, N., Duarte, C.M., 1994. Growth patterns and demography of pioneer Caribbean seagrasses *Halodule wrightii* and *Syringodium filiforme*. *Mar. Ecol. Prog. Ser.* 109, 99–104.
- Hauxwell, J., Cebrián, J., Furlong, C., Valiela, I., 2001. Macroalgal canopies contribute to eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. *Ecology*, in press.
- Herrera-Silveira, J.A., Ramírez, R.J., Zaldivar, J.A., 1998. Overview and characterization of the hydrology and primary producer communities of selected coastal lagoons of Yucatán México. *Aquat. Ecosyst. Health Manage.* 1, 353–372.
- Lapointe, B.E., Tomasko, D.A., Matzie, W.R., 1994. Eutrophication and trophic state classification of seagrass communities in the Florida Keys. *Bull. Mar. Sci.* 54, 696–717.
- Magalhaes, K.M., Eskinazi-Leca, E., Moura Jr., A.M., 1997. Morphometry and biomass of the seagrass *Halodule wrightii* Ascherson in North Littoral of Pernambuco. *Trab. Oceanogr. Univ. Fed. Pernambuco* 25, 83–92.
- McMillan, C., 1978. Morphogeographic variation under controlled conditions in five seagrasses, *Thalassia testudinum*, *Halodule wrightii*, *Syringodium filiforme*, *Halophila engelmannii*, and *Zostera marina*. *Aquat. Bot.* 4, 169–189.

- Montcreiff, C.A., Sullivan, M.J., Daehnick, A.E., 1992. Primary production dynamics in seagrass beds of Mississippi Sound: the contributions of seagrass, epiphytic algae, sand microflora, and phytoplankton. *Mar. Ecol. Prog. Ser.* 87, 161–171.
- Morgan, M.D., Kitting, C.L., 1984. Productivity and utilization of the seagrass *Halodule wrightii* and its attached epiphytes. *Limnol. Oceanogr.* 29, 1066–1076.
- Onuf, C.P., 1996. Seagrass responses to long-term light reduction by brown tide in upper Laguna Madre, Texas: distribution and biomass patterns. *Mar. Ecol. Prog. Ser.* 138, 219–231.
- Pangallo, R.A., Bell, S.S., 1988. Dynamics of the aboveground and belowground structure of the seagrass *Halodule wrightii*. *Mar. Ecol. Prog. Ser.* 43, 297–301.
- Phillips, R.C., 1960. Environmental effect on leaves of *Diplanthera* Du Petit-Thouars. *Bull. Mar. Sci. Gulf Carib.* 10, 346–353.
- Powell, G.V.N., Kenworthy, W.J., Fourqurean, J.W., 1989. Experimental evidence for nutrient limitation of seagrass growth in a tropical estuary with restricted circulation. *Bull. Mar. Sci.* 44, 324–340.
- Pulich Jr., W.M., 1989. Effects of rhizosphere macronutrients and sulfide levels on the growth physiology of *Halodule wrightii* Aschers. and *Ruppia maritima* L. s.l. *J. Exp. Mar. Biol. Ecol.* 127, 69–80.
- Sand-Jensen, K., 1975. Biomass, net production and growth dynamics in an eelgrass (*Zostera marina* L.) population in Vellerup Vig, Denmark. *Ophelia* 14, 185–201.
- Sand-Jensen, K., Borum, J., 1991. Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. *Aquat. Bot.* 41, 137–175.
- Short, F.T., Burdick, D.M., 1996. Quantifying eelgrass habitat loss in relation to housing development and nitrogen loading in Waquoit Bay, MA. *Estuaries* 19, 730–739.
- Short, F.T., Wyllie-Echeverria, S., 1996. Natural and human-induced disturbance of seagrasses. *Environ. Conserv.* 23, 17–27.
- Tomasko, D.A., Dunton, K.H., 1995. Primary productivity in *Halodule wrightii*: a comparison of techniques based on daily carbon budgets. *Estuaries* 18, 271–278.
- Valiela, I., McClelland, J., Hauxwell, J., Behr, P.J., Hersh, D., Foreman, K., 1997. Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. *Limnol. Oceanogr.* 42, 1105–1118.
- Virnstein, R.W., 1982. Leaf growth rate of the seagrass *Halodule wrightii* photographically measured in situ. *Aquat. Bot.* 12, 209–218.
- Zieman, J.C., Wetzel, R.G., 1980. Productivity in seagrasses: methods and rates. In: Phillips, R.C., McRoy, C.P. (Eds.), *Handbook of Seagrass Biology: An Ecosystem Perspective*. Garland STPM Press, New York, pp. 87–116.