

OBSERVATIONS ON THE REPRODUCTIVE ECOLOGY OF *THALASSIA TESTUDINUM* (HYDROCHARITACEAE)

II. LEAF WIDTH AS A SECONDARY SEX CHARACTER

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ABSTRACT

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Short-shoots of *Thalassia testudinum* Banks ex König were collected from 5 sites in Florida. A total of 284, 625-cm² quadrat samples, containing 6182 short-shoots was analyzed for leaf width, inflorescence number and sex (the latter if determinable). Although leaf widths and reproductive densities differed at the 5 sites, leaf width was consistently greater when reproductive structures were present, and when the number of inflorescences increased. The mean number of inflorescences per short-shoot was significantly higher for shoots bearing male inflorescences compared to female shoots. Female inflorescences were normally solitary; male short-shoots usually had 2 or 3 inflorescences. Sex ratios were male-biased for 4 of the 5 sites. Comparisons of leaf widths between the sexes indicated that leaf width constituted a secondary sex character for this species. Female short-shoots tend to have narrower leaves than male short-shoots. This relationship should also be considered when evaluating the significance of morphogeographic and stress-related variation in leaf width for this species.

INTRODUCTION

Flowering plants can generally be divided into 2 groups with respect to sexual reproduction. The majority of species are sexually monomorphic (i.e., hermaphroditic), producing flowers which contain both androecia and gynoecia. A second, smaller group of species are sexually dimorphic. In this group, a given flower has either androecia or gynoecia. Sexually dimorphic plants may be either monoecious or dioecious, but only 3 or 4% of all flowering plants are dioecious (Pettitt et al., 1981). In contrast, 9 of the 12 genera of seagrasses exhibit dioecy (Pettitt et al., 1981). The seagrass *Thalassia testudinum* Banks ex König (turtle grass) is sexually dimorphic, producing androecia and gynoecia on separate, imperfect uniflorous inflorescences. Tomlinson (1969) suggested that *T. testudinum* was also appar-

ently dioecious based on his analyses of individual short-shoots (ramets). Grey and Moffler (1978) noted that rhizome sections with more than one reproductive short-shoot had either all male or all female short-shoots, reinforcing the suggestion that *T. testudinum* plants are dioecious.

In many dioecious species, male plants bear greater numbers of inflorescences than female plants (Bawa and Opler, 1975; Lloyd and Webb, 1977; Opler and Bawa, 1978; Barrett and Helenrum, 1981; Bawa et al., 1982). *Thalassia testudinum* exhibits a similar pattern. Male short-shoots may have between 2 and 5 flowers while female flowers are usually solitary (Tomlinson, 1969; Grey and Moffler, 1978). Sexual differences in structures other than reproductive organs, termed secondary sex characters, have also been observed for a number of terrestrial dioecious plants (Lloyd and Webb, 1977), but the presence of secondary sex characters in seagrasses has not previously been documented.

Moffler et al. (1981) recently reported an apparent relationship between leaf width and the number of reproductive buds per short-shoot in *T. testudinum*. Based on these preliminary observations, a quantitative sampling program was initiated in order to further investigate this relationship and other aspects of the reproductive ecology of *T. testudinum*. In the present study, we examined the relationship between leaf width and number of inflorescences per short-shoot for several populations in Florida. We present evidence that leaf width may constitute a secondary sex character for this species.

MATERIALS AND METHODS

Collections of *T. testudinum* short-shoots were obtained from 3 locations in Tampa Bay, Florida and 2 sites in the Florida Keys. The primary study site was at Lassing Park (27°45' N, 82°38' W) in Tampa Bay. This area has previously been described in detail (see Beach Drive SE, Phillips, 1960). Thirty-four collections were made at Lassing Park from 15 February 1979 to 15 October 1980. Six randomly located 625-cm² quadrats were sampled per collection. Previous statistical analyses using Stein's 2-stage procedure (see Moffler et al., 1981) had indicated that this was an adequate sample size for reproductive short-shoot densities and developmental-stage analysis. Additional Tampa Bay collections were made during 1980 at Egmont Key (27°36' N, 82°45' W) and Cockroach Bay (27°41' N, 82°32' W). Two collections, the first on 8 May 1979 and the second on 6 May 1980, were made in the Florida Keys at Big Coppit Key (24°36' N, 81°32' W) and No Name Key (24°42' N, 81°19' W).

A total of 284 quadrats, containing 6182 short-shoots, was analyzed for leaf width, inflorescence number and sex (the latter when determinable). Leaf width was measured for the widest leaf blade of a short-shoot just above the sheath. Leaf widths were measured to the nearest 0.1 mm using a dissecting microscope equipped with an ocular micrometer. Leaves

were then peeled away from the short-shoot until the shoot apex was visible. The occurrence and number of sexual reproductive structures (buds, flowers or peduncles, depending on the time of year) were then recorded. Short-shoots were classified as vegetative if no visible (≥ 1.0 mm) reproductive structures were present. Reproductive short-shoots were separated into male, female or unknown classes. Sex determinations were based on floral morphology; the unknown class represented short-shoots that had flower buds that were too small to reliably classify ($< 3-4$ mm) or post-anthesis short-shoots which had only peduncles present.

Statistical analysis began by testing for normal distribution of the leaf-width data by calculating a *D* statistic (D'Agostino, 1971). A 1-way analysis of variance (ANOVA) was then performed to determine if mean leaf widths for the 5 sites were significantly different. Subsequent statistical analyses were performed on each site. Regression analyses and ANOVA were performed to determine if variations in leaf widths (dependent variable) were attributable to either inflorescence number or sex (independent variables). Analysis of covariance was used to test for differences in slopes of the resulting least squares lines for the 5 sites. Mean leaf widths were compared with respect to site and inflorescence number using Duncan's multiple range tests. The mean number of inflorescences for male and female short-shoots was compared at each site using *t*-tests (except Cockroach Bay, at which only male short-shoots were identified). Calculations were performed utilizing Statistical Analysis System programs (Helwig and Council, 1979).

RESULTS

Mean leaf blade widths for the 5 sites exhibited significant differences (Table I), although no obvious pattern was evident. No pattern was evident for mean reproductive short-shoot densities with values ranging from 3.6% for the No Name Key population to 34.9% for the Cockroach Bay popu-

TABLE I

Mean leaf widths (and standard deviations) for *Thalassia testudinum* short-shoots from sites in Florida

Site	N	Mean leaf width (mm)	Grouping ^a
Lassing Park	4471	6.72 (1.40)	C
Egmont Key	923	7.52 (1.60)	B
Cockroach Bay	170	7.87 (1.34)	A
Big Coppit Key	279	6.18 (1.40)	D
No Name Key	339	7.49 (2.04)	B

^aThe significance of the means ($P < 0.05$) was evaluated using Duncan's multiple range test. Means with the same letter grouping are not significantly different. Means with different grouping letters are significantly different.

TABLE II

Mean leaf widths for vegetative and reproductive *Thalassia testudinum* short-shoots

Site	Number of inflorescences/short-shoot									
	0	1	2	3	4	5				
	N	MLW/SD ^a	N	MLW/SD	N	MLW/SD	N	MLW/SD	N	MLW/SD
Laasing Park	3821	6.58 ±1.46	633	6.77 ±1.04	301	7.37 ±1.13	2.16	7.76 ±0.99	—	—
Cockroach Bay	106	7.67 ±1.45	40	8.05 ±1.00	19	8.29 ±1.10	5	9.16 ±1.31	—	—
Egmont Key	681	7.29 ±1.68	130	8.13 ±1.11	48	8.37 ±1.46	50	7.74 ±0.77	8	8.38 ±1.02
Big Coppitt Key	208	5.29 ±1.46	54	6.73 ±0.95	5	7.72 ±0.55	10	6.69 ±0.94	2	7.55 ±1.20
No Name Key	327	7.40 ±2.00	4	9.22 ±1.13	5	9.96 ±1.04	3	11.30 ±0.75	—	—

^aMean leaf width/± one standard deviation.

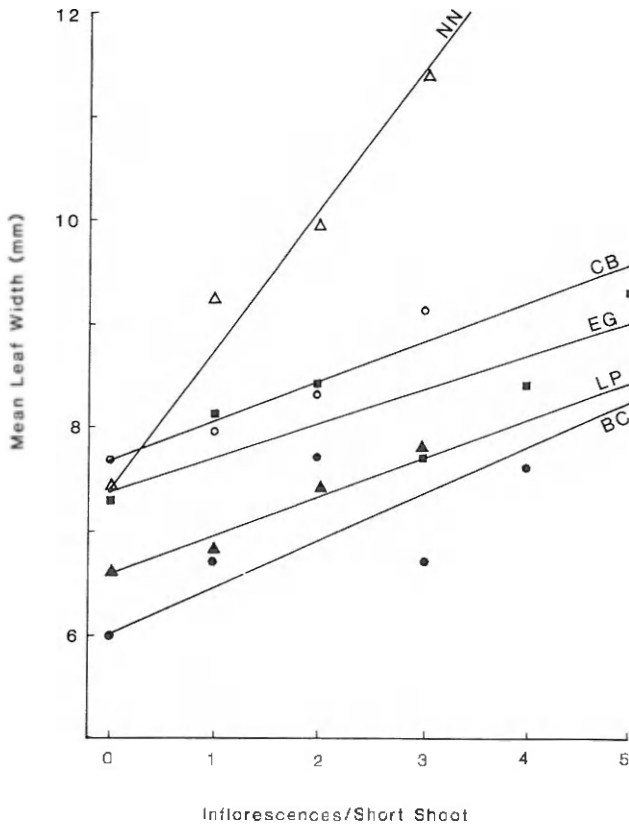


Fig. 1. Mean leaf widths for vegetative and reproductive *Thalassia testudinum* short-shoots from Florida. NN (open triangles) = No Name Key, CB (open circles) = Cockroach Bay, EG (closed squares) = Egmont Key, LP (closed triangles) = Lassing Park, BC (closed circles) = Big Coppit Key.

lation. For all short-shoots sampled, leaf widths were significantly ($P < 0.05$) greater when reproductive structures were present (Table II). There was also a positive relationship between leaf width and inflorescence number ($P < 0.1$) for reproductive short-shoots at all 5 sites (Fig. 1). The relationship between leaf width and inflorescence number (Fig. 1) had a significantly greater slope (1.33 mm increase/inflorescence) in the No Name Key population than in all other sites (0.33–0.45 mm increase per inflorescence). Narrowest mean leaf widths and greatest variation were characteristic of the vegetative short-shoots. This was expected because this group included new, immature short-shoots.

Frequency distributions of Lassing Park short-shoots with 1, 2 or 3 inflorescences over 7 leaf-width classes (Fig. 2a) illustrated a dichotomy. Short-shoots with one inflorescence had a size distribution that was distinct from those with 2 or 3 inflorescences, with the former tending to have narrower leaves than the latter.

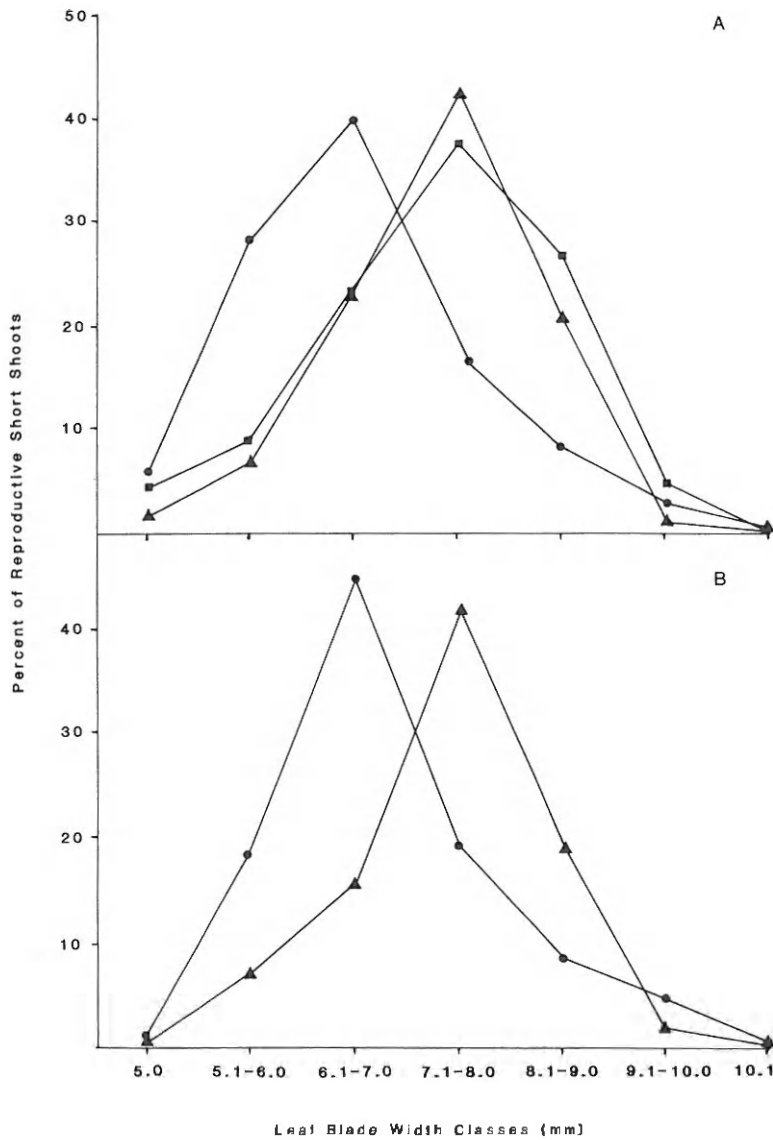


Fig. 2. Frequency distribution of reproductive *Thalassia testudinum* short-shoots from Lassing Park with respect to : (A) number of inflorescences (circles = 1; triangles = 2; squares = 3) per short-shoot; (B) sex (circles = female; triangles = male) of short-shoots.

Analysis using chi-square indicated that sex ratios departed significantly from unity for 3 of the 4 sites which had both sexes present (Table III); ratios were male biased, except for the Big Coppit Key population. Comparisons between the number of inflorescences per short-shoot for females and males (Tables III) indicated a high degree of uniformity in females

TABLE III

Sex ratios and mean inflorescence number per male and female short-shoot for *Thalassia testudinum* in Florida

Site	Number of male short-shoots	Number of female short shoots	Male/female	χ^2	Inflorescences (number/short-shoot \pm SD)	
					Male	Female
Lassing Park	414	295	1.40	19.97**	1.49 \pm 1.18	1.05 \pm 0.23*
Egmont Key	164	96	1.71	17.78**	1.52 \pm 1.54	1.14 \pm 0.40*
Big Coppit Key	15	47	0.32	16.52**	2.80 \pm 0.77	1.04 \pm 0.20*
No Name Key	8	4	2.00	1.33	2.38 \pm 0.52	1.00 \pm 0.00*

* $P \leq 0.05$.** $P \leq 0.01$.

and substantial variation in males (compare standard deviations in Table III). Female flowers were usually solitary (range 1–3); male short-shoots usually had 2 or 3 flowers (range 1–5). The mean number of inflorescences per short-shoot was significantly higher ($P < 0.05$) for males at these 4 sites. Sex-related differences in leaf width become evident when the frequency distribution of male and female short-shoots is plotted against the 7 leaf-width classes (Fig. 2b). Female short-shoots tend to have narrower leaves than male short-shoots. This difference was highly significant for the Lassing Park population (which represents the largest data set). The same difference was evident, but not statistically significant for the other 3 sites, which may be reflective of the low number of short-shoots which were reproductive or could be positively sexed.

DISCUSSION

Leaf width for *Thalassia testudinum* from Tampa Bay and the Florida Keys, did not show morphological variation correlative to latitudinal or any obvious environmental stress gradients. However, it should be pointed out that these factors were not specifically investigated. The data indicated significant variation in leaf width within populations which correlated with presence and number of inflorescences. Leaf width in *T. testudinum* has been previously correlated with a number of parameters. Narrow leaf widths have been attributed to low salinity (Phillips, 1960), reduced plant vigor (Tomlinson, 1969), and low-light conditions, due to either depth or turbidity (McMillan and Phillips, 1979; Phillips and Lewis, 1983). Clonal leaf-width patterns in *T. testudinum* seedling growth have also been observed; leaf widths are characteristically narrower in northern populations and increase with decreasing latitude (McMillan, 1978; Durako and Moffler, 1981).

Previous studies have utilized small or undefined sample sizes and correlations between narrow leaf widths and environmental stress have been

based on the assumption that seagrass leaves within a locale do not vary greatly (Phillips and Lewis, 1983). We utilized statistical procedures to determine adequate sample size for our collections (Moffler et al., 1981). The range of leaf widths (approximately 3–11 mm) at each site was relatively large and encompassed the range of values reported for morpho-geographic and environmental stress induced variation. Mean reproductive short-shoot densities at the 5 sites were also quite variable. They were greater, with the exception of the No Name Key population, than the 1–15% range estimated for easily observable late reproductive structures in south and central Florida populations (Phillips, 1960; Orput and Boral, 1964; Zieman, 1975; Thorhaug and Roessler, 1977; Grey and Moffler, 1978), but below the value of 44% which was reported from a preliminary survey of early reproductive buds at Lassing Park (Moffler et al., 1981). Highest reproductive densities in Tampa Bay were observed during late winter–early spring collections; they generally decreased through the summer as the inflorescences developed and matured, and reached zero by September. This supports an earlier suggestion that factors, such as microbial infection or natural abortion of early buds, may reduce visible flower expression in this species (Moffler et al., 1981).

Our estimates of male-biased sex ratios for *T. testudinum* were comparable to those of Tomlinson (1969), but the inverse of those reported by Grey and Moffler (1978). Male-biased sex ratios have also been reported for *T. hemprichii* (Ehrenb.) Aschers. (Pascasio and Santos, 1930). In *T. testudinum*, as in most plants, sex can only be determined visually from inflorescences. Therefore, the term sex ratio, as it is used here, refers to the sex ratio of flowering short-shoots and does not imply intrinsic sex ratio. Grey and Moffler (1978) suggested that their female-biased sex ratios may have been due to the time of year when their observations were made (i.e., during peak anthesis of female short-shoots). Male inflorescences develop, mature and deteriorate more rapidly than female inflorescences in *T. testudinum* (M.D. Moffler and M.J. Durako, in preparation) and they are not recognizable after anthesis (Grey and Moffler, 1978). The majority of our collections were prior to anthesis and should, therefore, more accurately portray actual sex ratios over a reproductive event. The numerical predominance of male short-shoots may be due to their propensity to flower more frequently (although it is not known if *T. testudinum* short-shoots flower more than once), from differential mortality or abortion of inflorescences, especially if the energy required for reproduction is greater for females (Lloyd and Webb, 1977; Bawa et al., 1982; Lovett Doust and Lovett Doust, 1983).

The greater mean number of inflorescences per short-shoot for males (1.5–2.8) than females (approximately 1) agrees with previous reports for this species (Tomlinson, 1969; Grey and Moffler, 1978), and is characteristic for most dioecious species (Lloyd and Webb, 1977). The numerical predominance of male inflorescences has been attributed to sex re-

lated differences in the energetics of reproduction and sexual selection (Bawa and Opler, 1975). In fact, males often exceed females in general vegetative vigor and size, reflecting the generally lower costs of being male (Lloyd and Webb, 1977; Lovett Doust and Lovett Doust, 1983). *Thalassia testudinum* seems to follow the above patterns, with widest leaf widths characteristic of male short-shoots or at least short-shoots with more than one inflorescence. The significance of this relationship cannot presently be clearly defined. However, we can offer some plausible possibilities.

Relative leaf size is a reflection of plant development or age in most monocotyledons (Tomlinson, 1970). Leaf width polymorphism in the seagrass *Enhalus acoroides* (L.f.) Royle is a function of plant development and is not caused by environmental factors (Johnstone, 1979). Tomlinson and Vargo (1966) reported that the size of a *T. testudinum* short-shoot was proportional to its age, and Zieman (1975) observed that leaf width also increases with age. This suggests that male short-shoots may generally be older (i.e., mature later) than female short-shoots. However, the leaf size of a short-shoot may also vary in relation to the vigor of the plant, which is environmentally influenced (Tomlinson, 1972). For example, leaf width in *Zostera marina* L. exhibits a linear increase with sediment nitrogen (Orth, 1977; Short, 1983). There are also numerous examples of the environmental modification of sex expression in dioecious plants (see Heslop-Harrison, 1957, 1972). Factors affecting sex expression are day-length, light intensity, temperature and mineral nutrition. The patchiness of sexual reproduction in *T. testudinum* reported by several investigators (Phillips, 1960; Marmelstein et al., 1968; Grey and Moffler, 1978), may reflect environmental patchiness. Charnov and Bull (1977) suggested that a labile sexual phenotype could be a strategy of plants inhabiting patchy environments; we believe this line of investigation certainly deserves attention.

Patchiness in sexual reproduction may also be due to clonal variation. The majority of plants growing in aquatic environments display some form of clonal growth and proliferation (Cook, 1983). McMillan (1978) hypothesized that seagrasses contain populations with distinct genetic limits of ecoplasticity, and that within a population, individual clones may have distinct limits of endogenous plasticity. Plasticity of response would be expected in species inhabiting a range of conditions or experiencing variable levels of environmental stress (Soule and Werner, 1981). Inter- and intra-population differences in leaf width were evident for *T. testudinum* in Florida. The inter-population differences could not be correlated to latitudinally-influenced stress gradients, but may suggest either ecotypic or clonal variation (note similarity of standard deviations in Table I). Intra-population differences in leaf width were significantly related to inflorescence presence and number, and to sex at the primary study site. This secondary sex character may be an incidental consequence of development or genetic sex determination and may or may not be adaptive (Lloyd and

Webb, 1977). Our conclusions are based on measurements of ramets whose clonal connection and genetic identity were unknown. Therefore, the ecological significance of the sex ratios and differences found between the sexes in *T. testudinum* may depend on the number of clones present in a population and the degree to which they are physiologically linked (Cook, 1983). Knowledge of the clonal structure of seagrass populations can greatly alter interpretation of data based on ramets alone. The number and distribution of genets in seagrass populations should be identified to more accurately evaluate genetic, physiological and ecological processes occurring in these communities.

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