

## COMPARISON OF LEAF MORPHOLOGY AMONG SUBMERSED SPECIES OF *MYRIOPHYLLUM* (HALORAGACEAE) FROM DIFFERENT HABITATS AND GEOGRAPHICAL DISTRIBUTIONS<sup>1</sup>

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An image analysis system was used to calculate the surface area and volume of the submerged leaves of seven species of *Myriophyllum*. We separated the species into two categories based on their distribution and habitat differences and compared their surface area, volume, dry mass, specific leaf area, and surface to volume ratios. The geographic location and habitat of the species explained the greatest percentage of total variance for these variables when compared by analysis of variance. A leaf shape analysis was performed by regressing surface area with volume for each species. Interspecific comparisons of leaf shape were made using analysis of covariance. Fundamental interspecific differences in submerged leaf shape, associated with differences in their geographic distributions and habitats, are explained as adaptations for different nutrient uptake regimes.

Because leaf shape plays a fundamental role in the photosynthetic energy-biomass relationship (Solbrig et al., 1979), changes in foliar morphology can confer important adaptations to species. While the adaptiveness of a particular leaf shape is not easily ascertained, modifications in leaf morphology and other photosynthetic adaptations are constrained by energetic compromises or "costs" of resource capture (Mooney and Gulmon, 1979). Specific examples are water loss relative to carbon gain, and energetic costs associated with tissue construction/maintenance that accompany increased leaf photosynthetic capacity (Givnish, 1986).

Photosynthetic adaptations of leaves are variously manifest. It is evident that increases in leaf surface area alone may enhance photosynthesis (Aerts, De Caluwe, and Konings, 1992), lead to increased leaf nitrogen content (Field and Mooney, 1986; Aerts, De Caluwe, and Konings, 1992), and promote accelerated relative growth rates (Poorter and Remkes, 1990). Understandably, adaptive changes in leaf morphology should be closely associated with habitat features, particularly with respect to nutrient availability. When resources are not limiting, plants will attain maximum growth rates by maximizing resource allocation to leaves (Tillman, 1988) within the permissible range governed by associated energetic costs (Givnish, 1986). The study variation in leaf morphology, its physiological consequences, and the associated habitat

characteristics of plant species can potentially provide important insight into the adaptive evolutionary process.

We are interested in morphological adaptations of aquatic plant leaves. Aquatic plants provide an appropriate model for studying foliar adaptations because submerged leaves are typically characterized by morphology that is peculiar relative to that of emergent or terrestrial foliage. The unusual morphology of hydrophytes is correlated with their atypical environment. Notably, the highly dissected form of many underwater leaves has been implicated as adaptive to the particular conditions of light and nutrient availability, water current stresses, etc. that characterize the aquatic habitat (Sculthorpe, 1967; Wetzel, 1983).

The convergent origin of submerged leaf dissection among many unrelated aquatic angiosperms (e.g., *Armoracia* [Brassicaceae], *Ceratophyllum* [Ceratophyllaceae], *Megalodonta* [Asteraceae], *Myriophyllum* [Haloragaceae], and *Ranunculus* [Ranunculaceae]) argues strongly for an adaptive function; however, specific functional relationships of leaf dissection in hydrophytes remain to be investigated thoroughly or demonstrated convincingly.

The genus *Myriophyllum* (Haloragaceae) is well suited for studying such structure/function relationships because considerable leaf variation and habitat diversity exist among species. Leaf variation in all *Myriophyllum* species centers around modifications of a pinnately dissected prototype. It is advantageous to study leaf variation among congeners of a monophyletic genus such as *Myriophyllum* because the dissected condition is likely to have arisen from a common ancestor. Consequently, particular modifications of leaf dissection among species are likely to disclose adaptive features, particularly if correlated with specific habitat differences. This type of comparison would be more difficult to evaluate in a study that simply compared patterns of leaf dissection among unrelated taxa.

In this study, we investigate interspecific leaf variation among seven North American *Myriophyllum* species and relate various components of structural variation to phys-

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iological factors associated with distinct habitat characteristics of the species. We have employed computerized image analysis techniques to facilitate the morphological analysis of the complex dissected leaves found within this genus.

## MATERIALS AND METHODS

**Collection and measurement**—Five plants each of *Myriophyllum alterniflorum*, *M. exalbescens*, *M. farwellii*, *M. heterophyllum*, *M. spicatum*, *M. tenellum*, and *M. verticillatum* were collected in August/September 1992 from a total of 34 different lakes and one bay (five plants/species from each of five different sites/species) in Wisconsin and the Upper Peninsula of Michigan.

We separated these seven species into two groups based on their geographical distribution and habitats (see discussion): 1) a northern group included *Myriophyllum alterniflorum*, *M. farwellii*, and *M. tenellum*; 2) a widespread group included *M. exalbescens*, *M. heterophyllum*, *M. spicatum*, and *M. verticillatum*. All plants were collected in <2 m of water. For six of the seven species (excluding *M. tenellum*), three leaves were removed from each individual plant. One healthy green leaf was removed from each of three nodes located 5 cm, 15 cm, and 25 cm below the nonflowering shoot apex of each plant. A total of 75 leaves/species was thus removed, placed in water, and stored at 10 C. The shoots of *M. tenellum* were too short to sample leaves from nodes at the above intervals. Because the minute leaves of *M. tenellum* play little if any role in photosynthesis, the entire shoot was used in the shape analysis described below.

Each leaf and a cylindrical wire calibration standard were photographed with a 35 mm camera using a fiber-optic backlighting source and Tmax 100 film. A developed negative of the leaf and calibration standard image was placed under a camera with an AF Micro Nikkor 55 mm lens, and the image was captured, using a PC-Visions AFG Frame Grabber Board, on a video monitor using OPTIMAS software (Bioscan, Inc., Edmonds, WA). Each image was used to calculate foliar surface area and volume (unpublished data). A total of 450 leaves was processed. Seventy-five shoots of *M. tenellum* were also processed as above. After being photographed, each leaf and each *M. tenellum* shoot was dried at 90 C for 24 hr, and dry mass was measured. After drying, we found marl deposits on 15 leaves of *Myriophyllum spicatum* collected from one lake in Wisconsin, and we removed these leaves from all analyses ( $N = 435$ ).

For each of the seven species, ten shoots (cut at the sediment/water interface) were cleaned and then stripped of foliage. The leaves and stems were dried at 90 C for 48 hr. Total leaf and total shoot dry mass measurements were made.

**Analysis**—All variables were ln-transformed to better approximate normal distributions (Sokal and Rohlf, 1981). A one-way analysis of variance and Tukey HSD posthoc tests were used to make multiple comparisons among the ln-transformed variables mass, volume, and surface area for the leaves of six species, excluding *Myriophyllum tenellum*. The species were also compared with respect to two derived variables: specific leaf area (surface area/dry

leaf mass) and surface area/volume. These five variables were also determined separately for the shoots of *M. tenellum*.

An analysis of variance (ANOVA) with both nested and crossed effects (Littell, Freund, and Spector, 1991) was performed using SAS (SAS, 1985). The 30 collection sites were nested within the six species (excluding the five lakes for *M. tenellum*), and the species were nested within two geographic/habitat units (see above). The three positions on the stem from which the leaves were removed was the same for all six species and was treated as a crossed effect. A species by position interaction was calculated to determine if the effect of position varied significantly among species.

We performed an allometric analysis to explore interspecific differences in leaf shape and growth pattern using the allometric formula  $y = bx^a$  where  $b = y$ -intercept and  $a =$  slope of the relationship (Hammond, 1941; McMahon and Bonner, 1983). Shape was defined as the relationship between two measured variables (Somers, 1986). We used bivariate plots of surface area to volume to compare interspecific differences in leaf shape and growth pattern. We calculated regression lines from these bivariate plots and tested the species for homogeneity of slopes (Sokal and Rohlf, 1981). The intercepts of regression lines with equal slopes were compared using an analysis of covariance.

We compared the percentage of dry leaf mass to dry shoot mass of all seven species to determine the relative amount of shoot mass apportioned to leaf material. A one-way analysis of variance and Tukey HSD posthoc test were used to make multiple comparisons among the species for percent dry leaf mass/total dry shoot mass.

## RESULTS

Pairwise comparisons of means for surface area were significantly different ( $P < 0.05$ ) except between *M. exalbescens* and *M. spicatum* and between *M. exalbescens* and *M. verticillatum* (Fig. 1). Pairwise comparisons of volume means were significantly different ( $P < 0.05$ ) except for *M. exalbescens* and *M. spicatum* (Fig. 1). Pairwise comparisons of dry mass means were significant ( $P < 0.05$ ) except between *M. exalbescens* and *M. spicatum* and between *M. alterniflorum* and *M. farwellii* (Fig. 1). An experimentwise alpha level of 0.05, using a Bonferroni adjustment ( $[0.05]/3$ ), was used to determine significant interspecific differences for the two derived variables. Specific leaf area means differed significantly ( $P < 0.05$ ) among all species comparisons except between *M. alterniflorum* and *M. verticillatum*, between *M. exalbescens* and *M. spicatum*, and between *M. heterophyllum* and *M. verticillatum* (Fig. 2). Surface area/volume ratio means were significantly different ( $P < 0.05$ ) among all species except *M. spicatum* and *M. exalbescens* (Fig. 3). For comparison, the ln-transformed means  $\pm$  SE of similar variables for the shoots of *M. tenellum* were: surface area =  $4.78 \text{ mm}^2 \pm 0.06$ , volume =  $3.41 \text{ mm}^3 \pm 0.08$ , and mass =  $0.34 \text{ mg} \pm 0.08$ . The derived variable means  $\pm$  SE were specific leaf area =  $90.00 \text{ mm}^2/\text{mg} \pm 3.42$  and surface area/volume ratio =  $4.04 \text{ mm}^{-1} \pm 0.11$ .

ANOVA indicated that species from the northern geographic/habitat unit had significantly smaller ( $P < 0.05$ )

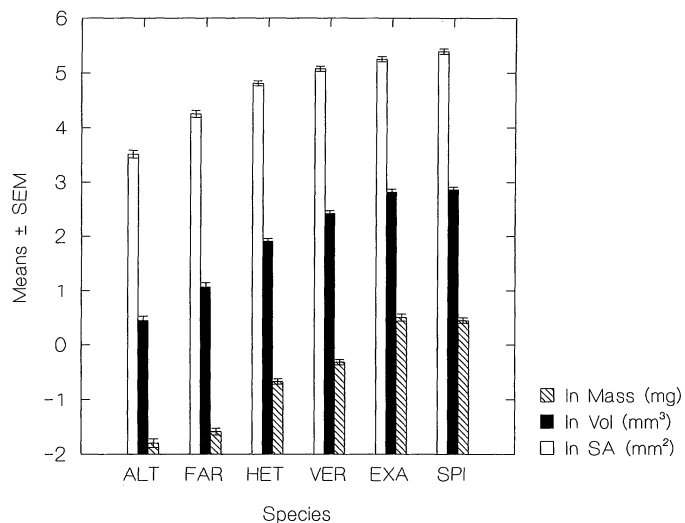


Fig. 1. Logmass, volume (Vol), and surface area (SA) ± standard error for leaves of six *Myriophyllum* species where ALT = *M. alterniflorum*; EXA = *M. exalbescens*; FAR = *M. farwellii*; HET = *M. heterophyllum*; SPI = *M. spicatum*; VER = *M. verticillatum*.

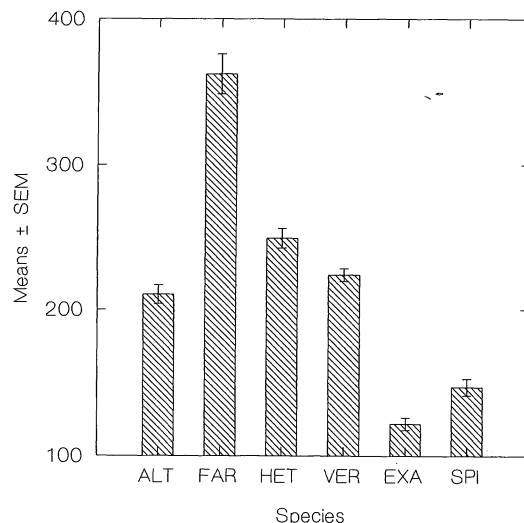


Fig. 2. Means of specific leaf area (mm<sup>2</sup>/mg) ± standard errors for leaves of six *Myriophyllum* species. Species abbreviations are the same as in Fig. 1.

leaf mass, volume, and surface area than the species within the widespread unit (Table 1; Fig. 1). Conversely, the northern species had significantly higher ( $P < 0.05$ ) surface area to volume ratios than the widespread species (Table 1). No significant difference was found between the northern and widespread species for specific leaf area (Table 1). The geographic/habitat units explained the largest proportion of total variance for surface area, volume, dry mass, and surface area/volume ratio (Table 1). A significant species × position interaction was found for each of the above variables; however, the interaction explained <4% of the total variance.

**Allometric analysis**—We compared six regression lines (one per species) from the bivariate plot of surface area with volume (Fig. 4) for homogeneity of slopes using paired comparisons of regression slopes (Table 2). Slopes ( $P > 0.05$ ) were found to be nonsignificantly different between several species (Table 2); these species were then tested for equality of y-intercepts using an analysis of covariance. Species having different regression slopes or having nonsignificantly different regression slopes and significantly different y-intercepts were considered to have different leaf shapes and growth patterns (Hammond, 1941;

Gould, 1966). Isometry, the condition in which changes in leaf size but not in leaf shape occur (Gould, 1966), did not exist within any of the six species. All species differed significantly in either slope or y-intercept (Table 2) and therefore had distinct leaf shapes.

Dry leaf mass for the six species of *Myriophyllum*, excluding *M. tenellum*, made up from 50% to 70% of the total dry shoot mass (Fig. 5). Dry leaf mass made up less than 6% of the total dry shoot mass in *M. tenellum*. In this species the leaves have been greatly reduced to minute scales (length <1 mm; Voss, 1985); therefore, the shoot functions as the major photosynthetic organ.

A one-way ANOVA and Tukey HSD posthoc test were used to make multiple comparisons among the species for percent dry leaf mass/total dry shoot mass. The means for *Myriophyllum alterniflorum* and *M. tenellum* were significantly different ( $P < 0.05$ ) from all other species. All other pairwise comparisons for percent dry leaf mass/total dry shoot mass were not significant ( $P > 0.05$ ) except between *M. exalbescens* and *M. heterophyllum*.

DISCUSSION

The cost of leaf structure associated with increased leaf photosynthetic capacity is important when considering

TABLE 1. *F*-statistics (*F*) and variance components expressed as percentages of total variance (%) for the following variables from a nested-crossed analysis of variance for six *Myriophyllum* species: Geo. = geographic/habitat unit, pos. = position, spp. = species, SA = surface area, VOL = volume, MASS = dry mass, SA/VOL = surface area to volume ratio, and SLA = specific leaf area.<sup>a</sup>

Variance component	SA		VOL		MASS		SA/VOL		SLA	
	<i>F</i>	%	<i>F</i>	%	<i>F</i>	%	<i>F</i>	%	<i>F</i>	%
Within geo.	18.0***	47.2	21.0***	51.4	14.5***	47.6	10.0**	38.2	1.8ns	7.2
Spp. within geo.	4.3	12.4	4.6	11.7	7.4	17.7	10.4**	22.4	14.0**	46.2
Lake within sp. within geo.	17.3***	17.6	19.5***	15.3	19.4***	13.0	17.8**	11.3	18.8**	16.8
Leaf pos. on stem	3.7*	2.9	9.2***	6.8	13.6***	8.9	25.8**	16.6	14.2**	12.5
Spp. × pos. interaction	2.2*	3.7	2.0*	2.4	2.1*	2.3	1.7ns	1.5	2.1ns	3.2
Error		16.2		12.4		10.6		10.0		14.2

<sup>a</sup> ns, not significant at  $P > 0.05$ ; \*,  $0.01 < P < 0.05$ ; \*\*,  $0.001 < P < 0.001$ ; \*\*\*,  $P < 0.001$ .

TABLE 2. Results of analysis of covariance (ANCOVA) for an allometric analysis of six *Myriophyllum* species.

Species	Y intercept	Slope	R	Homogeneity of slopes <sup>a</sup>	ANCOVA <sup>b</sup>
<i>M. spicatum</i>	-1.34	0.87	0.97	A	A
<i>M. alterniflorum</i>	-0.98	0.87	0.99	A	B
<i>M. heterophyllum</i>	0.30	0.81	0.97	AB	C
<i>M. exalbescens</i>	0.53	0.79	0.96	B	D
<i>M. verticillatum</i>	0.88	0.78	0.95	BC	E
<i>M. farwellii</i>	2.45	0.72	0.98	C	F

<sup>a</sup> Species with the same letter have nonsignificantly different slopes ( $P > 0.05$ ).

<sup>b</sup> Species with the same letter have nonsignificantly different regression lines ( $P > 0.05$ ).

the adaptive morphology of aquatic plants. Changes in both the shape and size of submersed leaves will affect their structural cost and photosynthetic efficiency. Spence and Chrystal (1970) and Spence, Campbell, and Chrystal (1973) found an inverse relationship between specific leaf area and light intensity for entire-leaved *Potamogeton* spp. Similarly in the terrestrial, shade-tolerant *Impatiens parviflora*, Evans and Hughes (1961) found sun-adapted leaves had small specific leaf areas (measure of light capturing ability, Berendse and Elberse, 1990), whereas shade-adapted leaves had large specific leaf areas. Åberg (1943; as cited in Ashby, 1948) also found leaf shape changes in submerged leaves of *Lobelia dortmanna* to be correlated with decreased light intensity associated with increasing water depth. Lee (1986) found that some extreme shade-adapted terrestrial species tended to produce lighter and thinner leaves than those of sun-adapted species.

In the leaves of submersed aquatic plants, there is no trade-off between carbon gain and water loss; foliar stomates are either lacking or nonfunctional (Sculthorpe, 1967; Hutchinson, 1975) with respect to carbon gain and transpiration. Carbon uptake occurs mainly through the leaf surface (Sand-Jensen, 1987). The leaf surface area in submerged aquatics is not only important in light capture

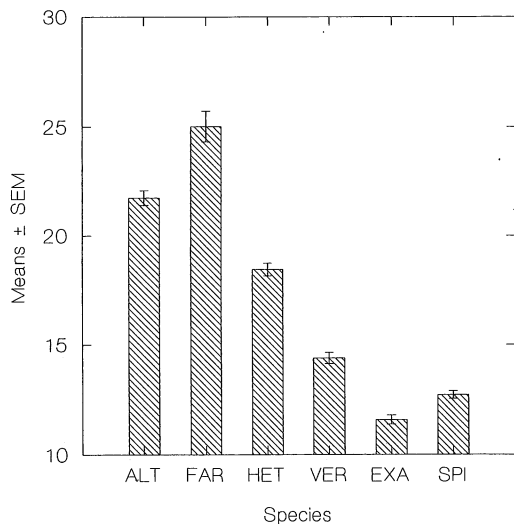


Fig. 3. Means of surface area/volume ratio ( $\text{mm}^{-1}$ )  $\pm$  standard errors for leaves of six *Myriophyllum* species. Species abbreviations are the same as in Fig. 1.

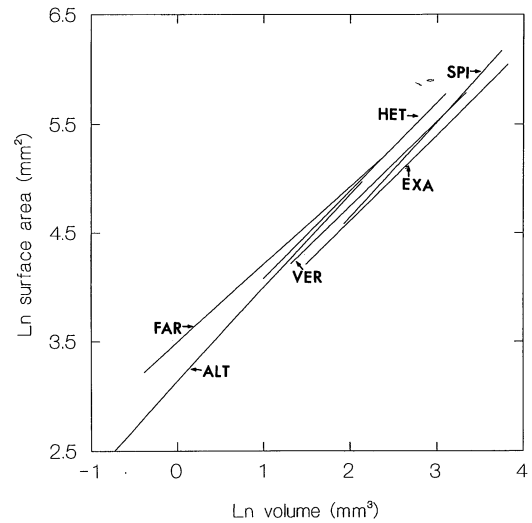


Fig. 4. Regression lines plotting  $\ln$  surface area ( $\text{mm}^2$ ) against  $\ln$  volume ( $\text{mm}^3$ ) for the leaves of six *Myriophyllum* species (abbreviations same as in Fig. 1). Line length indicates the range of values for each species.

and  $\text{CO}_2$  uptake, but also functions in mineral nutrient uptake (Sculthorpe, 1967; Hutchinson, 1975; Denny, 1980; Raven, 1981). Caffrey and Kemp (1992) found an increased reliance on the shoots of *Potamogeton perfoliatus* for nitrogen absorption as the growing season progressed. They suggested that increases in shoot surface area and biomass were responsible for the increased shoot uptake of nitrogen.

Due to low light levels and limiting resources (especially carbon and nitrogen) in aquatic environments (Stumm and Morgan, 1981; Wetzel, 1983, 1988; Barko, Gunnison, and Carpenter, 1991), numerous presumed adaptations for light capture,  $\text{CO}_2$ , and mineral uptake (e.g., high surface/volume ratios, reduction in thickness, thin cuticles, epidermal chloroplasts) are found in submerged leaves

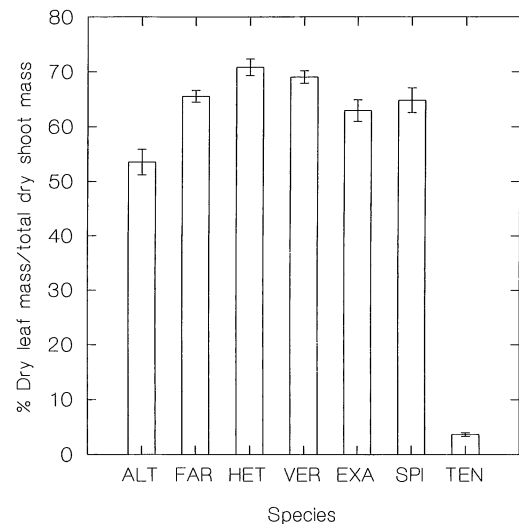


Fig. 5. Dry leaf mass as a percent of total dry shoot mass for seven *Myriophyllum* species. Species abbreviations are the same as in Fig. 1 except TEN = *M. tenellum*.

(Sculthorpe, 1967; Hutchinson, 1975; Bowes, 1987; Sand-Jensen, 1987). These and other putatively adaptive morphological features have led Bowes (1987) to categorize submerged aquatics as "shade plants."

The genus *Myriophyllum* includes 13 North American species (Aiken, 1981a), seven of which are found in Wisconsin (Fassett, 1930b; Trudeau, 1982) and the Upper Peninsula of Michigan (Voss, 1985). Of these seven, *M. spicatum* L. is an introduced species of European origin (Reed, 1977); *M. alterniflorum* DC., *M. exalbescens* Fern., *M. farwellii* Morong, *M. heterophyllum* Michaux, *M. tenellum* Bigelow, and *M. verticillatum* L. are native. Interspecific differences exist for the 1) amount of leaf dissection (number of segments/leaf), 2) leaf segment thickness, and 3) length of leaf segments (Gleason, 1952; Fassett, 1957; Aiken, 1981a; Voss, 1985; Gleason and Cronquist, 1991). These species show considerable phenotypic plasticity in vegetative characters (Aiken and Picard, 1980; Aiken, 1981a); however, taxonomic keys emphasizing vegetative morphology (notably leaf dissection) may be used with success (Voss, 1985), suggesting that each species is distinct vegetatively.

These seven species vary both in their distribution and habitat requirements. *Myriophyllum alterniflorum*, *M. farwellii*, and *M. tenellum* have a northern distribution (sensu Stuckey, 1971) growing in the northern United States and Canada (Aiken, 1981b). In Wisconsin and Michigan, *M. alterniflorum* and *M. farwellii* are found north of the tension zone, which divides these states into two distinct floristic regions and defines the southern limit of many northern species and the northern limit of many southern species (Curtis, 1959). *Myriophyllum tenellum* is not restricted to the northern region. *Myriophyllum alterniflorum* and *M. tenellum* are typically found growing in clear, shallow, soft waters of oligotrophic lakes (Fassett, 1930a; Curtis, 1959; Hutchinson, 1975; Voss, 1985), generally in sandy substrates (Fassett, 1930b; Muenscher, 1944). *Myriophyllum farwellii* grows under low light conditions in bogs with organic substrates and brown-stained water (personal observation). All are found in acidic to neutral waters.

*Myriophyllum exalbescens*, *M. heterophyllum*, *M. spicatum*, and *M. verticillatum* have widespread distributions in the United States and southern Canada (Aiken, 1981a; Engel, 1993). In Wisconsin and Michigan, these species are generally found in the southern portions of each state but are not restricted to areas south of the tension zone. These four species are generally found growing in meso- to eutrophic lakes with harder, more neutral-alkaline waters (Moyle, 1945; Curtis, 1959; Hutchinson, 1970; Beal, 1977; Crow and Hellquist, 1983) and grow on muddy/organic substrates.

Submerged leaf morphology varies noticeably, with some exceptions, among the seven *Myriophyllum* species. *Myriophyllum exalbescens* and *M. spicatum* are similar in surface area, volume, and mass. Both species are also similar in overall morphology and in the past have been confused taxonomically (Voss, 1985). However, the leaf segments of *M. exalbescens* are thicker and fewer in number than in *M. spicatum*. These differences may explain the slightly larger specific leaf areas and surface to volume ratios found in *M. spicatum*. These same derived variables were significantly larger in *M. farwellii* than in *M. alter-*

*niflorum*, both of which had very thin segments; however, those of the former were longer. Thus, increases in surface area to volume ratios and specific leaf area can occur by altering the submerged leaves of these species in two ways: 1) segment elongation and thickness reduction; and/or 2) increased dissection (increased number of segments/leaf). Differences in these characteristics are responsible for the interspecific differences in leaf shape (Ashby, 1948) found in this study.

Under conditions of limited resources, maximum leaf surface area to minimum mass and volume should allow for the greatest light and nutrient acquisition. A trade-off exists, however, between increasing surface area/decreasing mass or volume and the ability to withstand the physical stresses of water movement (e.g., currents, waves). Submerged leaves must increase mechanical tissue, and thus increase in mass and thickness (i.e., change shape), to resist these stresses. All plants in this study, with the exception of one *Myriophyllum alterniflorum* site, were collected in relatively "still" waters and therefore grew under relatively similar physical stresses. However, differences in nutrient and light conditions did exist between the lakes where northern and widespread species were collected. The significant larger surface area/volume ratios of northern *Myriophyllum* species represent adaptations to nutrient-poor conditions because large surface area/volume ratios should facilitate nutrient absorption (especially carbon dioxide).

No significant differences in specific leaf area were found between the northern and widespread species. On a relative scale, *M. farwellii* grew in the most light-limited environments (personal observation) and consequently had the highest specific leaf area. The four widespread species grow under relatively higher light conditions than *M. farwellii* but also have lower mean specific leaf areas. However, *M. alterniflorum*, which grew under the highest relative light levels, also had a relatively high specific leaf area. This may be due to the low percentage of leaf to shoot material, relative to the other species, found in *M. alterniflorum*.

Unlike the vittate shoots of its congeners, *Myriophyllum tenellum* apparently has alternatively adapted to oligotrophic conditions by drastically reducing leaf tissue, reducing main stem mass, and growing in dense mats on the substrate. Values of variables obtained for *M. tenellum* shoots (see Results) are very similar to those for leaves of *M. exalbescens* and *M. spicatum* (see Figs. 1–3). With relatively low specific leaf area and surface area/volume ratios, *M. tenellum* had the opposite characteristics of those species in the northern geographic/habitat unit. The dense growth habit of *M. tenellum* provides this species with a large surface area that facilitates growth under oligotrophic conditions.

The differences in leaf morphology, is plasticity, and how it affects resource gain must clearly play a part in interspecific competition, environmental adaptation, etc. However, understanding plant morphology and habitat relationships requires a whole-plant perspective (Grace, 1993).

We conclude that significant differences in submerged leaf morphology exist between the northern and widespread groups of *Myriophyllum* species presumably due to adaptation to different habitats. However, to fully un-

derstand the adaptive constraints placed on the leaf morphology of these seven species of *Myriophyllum*, other aspects of their habitats, physiology, and development must be addressed. We are presently undertaking studies involving comparisons of ammonium nitrogen uptake between northern and widespread groups of *Myriophyllum*. Future comparisons of: 1) chlorophyll content per area of plant surface; 2) leaf area per habitat area; 3) chlorophyll content per habitat area; and/or 4) thylakoid membrane area per habitat area (Raven, 1984) may provide additional evidence for differences between these northern and widespread groups. Raven (1984) stated that evidence exists correlating low pigment content/m<sup>2</sup> of plant surface with high leaf area. Additionally, interspecific differences in total chloroplast volume per cell volume have been observed under microscopic examination of *Myriophyllum* leaf tissue (personal observation).

Computer-enhanced image analysis now makes it feasible to study the architecture of the delicate and complex leaves of submerged hydrophytes. The further application of this approach has great potential for exploring other factors of structure and function in water plants.

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