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Measures of Plant Surface-areas for Eurasian Watermilfoil and Water Stargrass

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ABSTRACT

Plant surface areas were measured from samples of two common submersed aquatics with widely diverging morphologies: Eurasian watermilfoil (*Myriophyllum spicatum* L.) and water stargrass (*Heteranthera dubia* (Jacq.) MacM.). Measures for the highly dissected leaves of Eurasian watermilfoil involved development of a regression equation relating leaf length to direct measures of a subsample of leaf parts. Measures for the simple leaves of the stargrass were sums of measured triangles. Stem surfaces for both species were calculated as measured cylinders. Though the means of the stem length and leaf length were larger for stargrass samples, their mean surface area was 95 cm^2 which was less than the 108 cm^2 recorded for Eurasian watermilfoil samples. Relating surface area to dry weight for the stargrass was straightforward, with 1 mg of dry weight yielding an average 0.678 cm^2 of surface area. Biomass measures for the water milfoil were confounded by the additional weight of epiphytic algae persisting on cleaned samples. The results suggest that a less-time consuming method for surface area measures of plants with highly dissected leaves and a caveat for using biomass measures to estimate surface area in such plants.

Key words: Myriophyllum spicatum, *Heteranthera dubia*, plant surface area, leaf morphology, submersed aquatic plants.

INTRODUCTION

Surface area is the most commonly used variable to evaluate the relationship between submerged macrophytes and invertebrates (Korinkova 1971, Soszka 1975, Gerrish and Bristow 1979, Cattaneo and Kalff 1980, Peets et al. 1994). Several methods for surface-area measures have been developed, including planimetric techniques (Cattaneo and Kalff 1980, Peets et al. 1994), image analysis (Gerber et al. 1994), colorimetric methods (Cattaneo and Carignan 1983), weighed-images measurements (Biochino and Biochino 1979, Gregg and Rose 1982), electric surface meter (Brown and Manny 1985) and photometric techniques (Watala and Watala 1994).

In 1998, we initiated a study in experimental ponds to investigate macroinvertebrate communities found on native and exotic macrophyte species. This paper is a part of this study that describes and refines the methods used to estimate the surface areas of Eurasian watermilfoil and water stargrass. Eurasian watermilfoil is a submersed perennial plant with finely dissected leaves whorled on the stem. It is native to Europe, Asia and Northern Africa (Weldon et al. 1977). Introduced into North America over 50 years ago, Eurasian watermilfoil is now widely distributed throughout the United States and portions of Canada. Water stargrass is a native plant that is widespread in the central and eastern United States (Muenscher 1944). The slender branching stems, long ribbon-like leaves, and yellow flowers are distinctive characteristics of this species (Stutzenbaker, 1999).

MATERIALS AND METHODS

Collection of Plant Samples. This study was conducted at the University of North Texas Water Research Field Station located in Denton, Texas, USA. The facility has 47 earthen ponds, each measuring 30 m in length and 16 m in width. The ponds can be filled to a maximum depth of 2 m, but water depth was maintained at approximately 50 cm during our study. Well water was used to fill the ponds and well water was added as needed to compensate for evaporative losses during the collection period.

During May through July 1998, five ponds were planted with Eurasian water milfoil and eight ponds were planted with a mix of native aquatic plants, including water stargrass and eight other submersed species and six emergent species. Plants were taken out from the pots and planted in the sediment. Eurasian watermilfoil and the mixed-native plants were monitored by estimating surface coverage in each pond. Three of the ponds with the greatest similarity in surface coverage were selected for both mixed natives and Eurasian watermilfoil (6 pond total) as replicate sources of plant samples.

A stratified random design was employed for individual plant collection. Each pond was divided into 12 sections. One of these sections was randomly selected for each sampling date for each of the six ponds. Eurasian milfoil occurred in thick beds and placing a net around a single plant was cumbersome and likely to dislodge the invertebrates. Therefore, we used two different methods for collection of individual plants. Eurasian watermilfoil samples were collected from March to December 1999 by cutting a 25 to 30 cm portion of the leafy stems from the plants *in situ.* Following methods described by Beckett et al. (1992), the plant stem was cut and the plant gently raised through the water column and placed in a sampling container.

Water stargrass first appeared in late spring, and collected from June to December 1999 and from March to June 2000. Winter die-off of the Eurasian watermilfoil and water stargrass occurred between late December and March, and no plant samples were collected during that period. Water star-

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grass was collected by placing a plexiglass tube which was 29 cm in diameter with one end covered with a 100-μm mesh nylon net over the plant. The plant stem was broken off at its base and the sampler was brought to the surface with approximately 30 to 50 cm of plant length inside.

In the laboratory, after the removal of macroinvertebrates, plants were washed and cleaned thoroughly using a fine brush under a current of tap water to remove sediments, detritus, and mineral deposits.

Plant Surface Area Measurements. Eurasian watermilfoil leaves typically are arranged in whorls of four to six along flexible underwater stems. Each leaf is featherlike, with finely linear segments occurring in pairs along a central axis (Figure 1a). Leaf surface area of collected Eurasian watermilfoil was determined from a leaf length - leaf surface regression equation. A sub-sample of ten randomly selected plants was used to develop the regression equation. Ten leaves were randomly selected from each of ten plants for a total of 100 leaves. The length and diameter of each leaf segment and the central axis of a leaf were measured for the 100 leaves. To calculate surface area we assumed that the leaflets and central axis were cylinders with conical tips (Sher-Kaul et al. 1995). Total surface area for an individual leaf was obtained by summing the leaf part calculations. This area was then multiplied by the total number of leaves in the whorl to estimate the total leaf surface area per whorl. This procedure was repeated for each whorl on the stem. Plant stem length and diameter was measured and regarded as a cylinder in the calculation of its surface area. Total plant area was estimated by adding all stem and leaf calculations.

The leaves of water stargrass were considered as isosceles triangles with the bases attached to each other (Figure 1b) (Beckett et al. 1992). Surface area was calculated for the upper leaf surface and multiplied by two to account for lower surface of the leaf (Raven, 1984). A total of 278 leaves was measured taken from 20 randomly selected plants. The formula for a cylinder was used to estimate the surface area of the stem of water stargrass. Leaf and stem surface areas were summed to obtain a total surface area for this species. The leaves and stems were then dried at 105 C for 24 hours, and weighed to the nearest ±0.01 mg. Regression analysis was used to determine the relationship of plant surface area to biomass. All stem and leaf measurements were carried out with an Olympus Series Cue-2 image analyzer (Olympus, Tokyo) and Olympus SZH dissecting microscope.

RESULTS AND DISCUSSION

Morphological characteristics and surface area estimations of Eurasian watermilfoil and water stargrass are given in Table 1. Eurasian watermilfoil leaf surface area was calculated using the following regression analysis ($n = 100$, $r^2 = 0.92$): Leaf Surface Area = -25.26 + 8.41 (Leaf Length) (Figure 2).

We measured dry weights of ten Eurasian watermilfoil with and without epiphytic algae (Table 2) to illustrate the percent increase in weight caused by epiphytes. Percent increase in dry weight ranged between 7.38 to 36.10.

The relationship between whole plant biomass and surface area of water stargrass was established using the equation ($n = 20$, $r^2 = 0.82$) of Dry Weight = 19.62 + 0.0128 (Plant Surface Area) (Figure 3). On average, 1 mg dry weight of whole plant of water stargrass developed 0.678 cm^2 surface area. Though the means of the stem length and leaf length were larger for water stargrass samples, the mean surface area (95 ± 3 cm^2) was less than that for Eurasian watermilfoil samples (108 ± 3 cm^2). The variations observed in the surface area measurements are related to inherent variation in individual plant sample for number of leaves per plant, stem length, leaf length, etc. Plant surface area per unit of leaf and stem lengths were calculated as 40 cm^2 and 4 cm^2 for Eurasian watermilfoil whereas they were 7.9 cm^2 and 2.5 cm^2 for water stargrass, respectively. Surface area per leaf also yielded a higher value of 1.08 cm^2 for Eurasian milfoil than the 0.34 cm^2 that was found for water stargrass.

The literature indicates that the abundance of invertebrates found in submersed vegetation has been related to different units such as bottom area (Menzie 1981), plant surface area (Harrod and Hall 1962), dry or wet plant weight (Cox 1990) or sample volume (Korinkova 1971). Although plant surface area is usually considered as the most appropriate unit when it is utilized as a substrate (Gerrish and Bristow 1979, Biochino and Biochino 1979), its measurement is arduous and time-consuming. Sher-Kaul et al. (1995) provided the relationship between biomass and surface area of six submerged aquatic plant species including Eurasian watermilfoil. Such a relationship is useful in quantitative studies

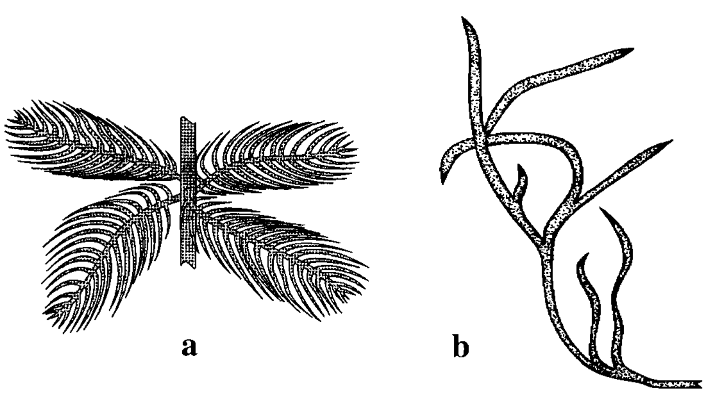

Figure 1. Illustrations of Eurasian watermilfoil (a) and water stargrass (b) leaves.

TABLE 1. CHARACTERISTICS OF LEAVES AND STEMS OF EURASIAN WATERMILFOIL AND WATER STARGRASS. VALUES ARE GIVEN AS MEANS ± SD.

	Eurasian watermilfoil	Water stargrass
Number of plants	10	20
Stem length (cm)	27 (±4.8)	38.1 (±6.0)
Number of leaves per plant	28.7 (±7.8)	15.6 (±3.9)
Leaf length (cm)	2.7 (±0.9)	12.2 (±4.2)
Plant surface area[a] (cm^2)	107.9 (±31.2)	95 (±31)
DW of whole plant (mg)	NA	140.2 (±45.4)
Surface area/leaf length	40	7.9
Surface area/stem length	4	2.5
Surface-area/leaf	1.08	0.34

[a]Surface area of one plant (leaves and stem surface areas are combined).

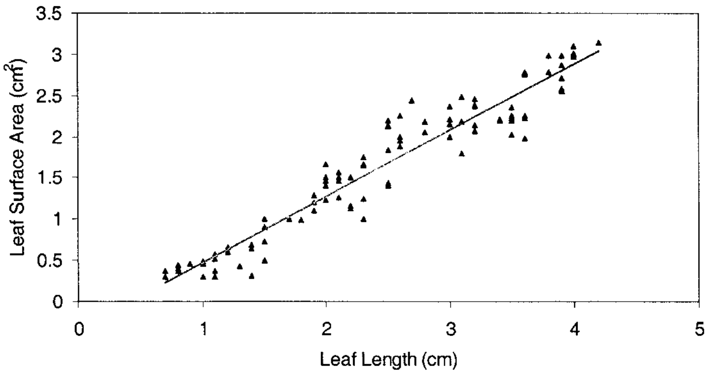

Figure 2. Relation between leaf surface area and leaf length of Eurasian watermilfoil (n = 100, $r^2 = 0.92$).

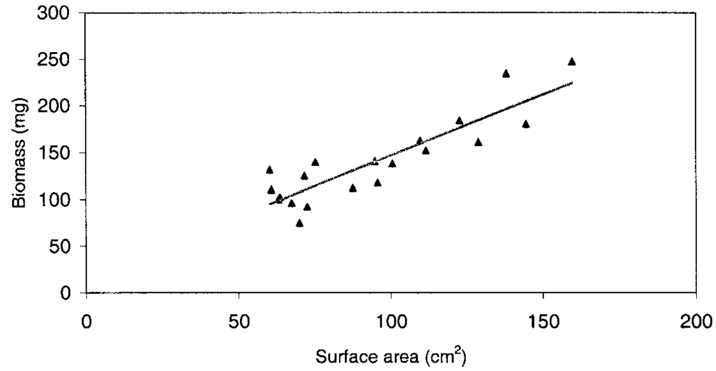

Figure 3. Relation between biomass and surface area of water stargrass (n = 20, $r^2 = 0.82$).

because plant biomass is more easily measured than their surface area. However, we observed that finely dissected leaves of Eurasian watermilfoil tend to retain epiphytic algae even after cleaning under running water with a fine brush. Additional weight caused by the epiphytes could affect the results of the surface area measurements when related to plant biomass. Our results indicated that estimation of surface area of the Eurasian watermilfoil from biomass data could result in inaccurate estimates.

Brown and Manny (1985) compared the surface areas of Eurasian watermilfoil and water stargrass measured with electronic surface area meter to the values obtained by planimetry and weighed-images methods. Surface area measurements were 20.3, 23 and 38.2 cm^2 for Eurasian watermilfoil, and 16.2, 16.9, 20.4 cm^2 for water stargrass with area meter, planimeter and weighed-images method, respectively. These measurements are smaller than those observed for Eurasian watermilfoil and water stargrass in this study. However, these figures are not strictly comparable to our results because the authors provided no morphological information (stem length, number of leaves, etc.) for the plants measured. In additional to that, surface area measurements for species with small, highly dissected, cylindrical shaped leaves such as Eurasian watermilfoil, should take thickness into account because of the much greater influence of the curved leaf surfaces on the total surface area (Gerber et al. 1994). Methods used by Brown and Manny (1985) did not take thickness into account that could result in inaccurate surface area measurements for Eurasian watermilfoil.

Table 2. Dry weight measurements (mg) of Eurasian watermilfoil with and without epiphytic algae.

Weight without epiphytes	Weight with epiphytes	(%) difference in weight
196.7	223.6	13.67
188.2	217.3	15.46
235.7	253.1	7.38
206.1	248.9	20.77
134.6	183.2	36.10
181.3	218.2	20.35
166.3	215.0	29.28
189.5	220.9	16.57
179.1	202.2	17.09
120.2	139.8	16.30

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