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Aquatic Botany 48 (1994) 175-182

Aquatic botany

Technical communication

An image analysis technique to determine the surface area and volume for dissected leaves of aquatic macrophytes

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(Accepted 15 February 1994)

Abstract

An application of image analysis (IA) for determining the surface area and volume of dissected, submerged leaves is described. Surface area and volume were estimated for artificially constructed leaves of varying wire sizes (1) directly using calipers and (2) using an IA technique. Accuracy of the technique was estimated by comparison of direct and IA measurements. We found that the size of the calibration standard used in the IA technique significantly affected the accuracy. The utility of this technique was then demonstrated using fresh *Myriophyllum exalbescens* Fern. leaves. The IA technique increases the rapidity in which surface area and volume calculations can be made for dissected leaves.

1. Introduction

The surface area of submerged aquatic plants is intimately related to their ecophysiological function in the aquatic environment. The leaf surface area in highly dissected, submerged aquatics not only functions as an attachment substrate for epiphytes (e.g. attached animals, algae, and bacteria), but also influences primary metabolic processes of the plant such as gas exchange, light photosynthetic response, and nutrient uptake (Sculthorpe, 1967; Hutchinson, 1975; Denny, 1980; Raven, 1981). Changes in the size of the surface area in the dissected leaves of a submerged plant will therefore effect the acquisition of gases, light, nutrients, and

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SSDI 0304-3770(94)00386-Z

ultimately the metabolism of the entire plant. Determination of surface area then is of broad interest.

Several methods of measuring shoot surface area have been developed (Krecker, 1939; Harrod and Hall, 1962; Cattaneo and Carignan 1983). These methods generally utilize correlations between mathematically calculated surface areas and the surface retention of water or detergents. The methods of Cattaneo and Carignan (1983) calculated shoot surface area mathematically using a colorimetric technique to correlate an absorbance unit with the calculated surface area. Rather than using whole plant measures, leaf area indices may be employed as indicators of functional efficiency in productivity (Spence et al., 1973; Hunt, 1982).

Surface area calculations via physical measurements of simple submerged leaves have been performed using calipers and microscopes (Rosine, 1955), by weighing a paper tracing of a leaf calibrated to the paper weight of a known area, or planimetrically (Spence et al., 1973). The drawbacks of these methods are their difficulty and the inaccuracy inherent in the measurement of leaves that are highly dissected (Evans, 1972) or have a large cross-sectional area. Schwabe (1951) described a photometric method for the estimation of leaf area that measured current generated from total transmitted light after a source light was passed through a photographic image of the leaf. Hughes (1964) improved on this method with the use of a 35 mm camera and high contrast film for recording leaf images.

The technique described in this paper improves on the methods of Schwabe (1951) and Hughes (1964) by employing an image analysis (IA) system and constant light source to measure transmitted light through a photographic image of dissected, submersed aquatic plant leaves. The technique provides a method for rapid and accurate estimates of dissected leaf surface area and volume. We also investigate the effect of different calibration standards on the accuracy of the IA technique

2. Materials and methods

2.1. Artificial leaves

Ten artificial leaves, similar in size and morphology to dissected, submerged *Myriophyllum exalbescens* Fern. leaves were constructed of stiff wire. The artificial leaves were created to test this technique because, unlike fresh leaf material, wire leaves are easily measured with calipers, give sharp clear images when photographed, and are not subject to desiccation. Five of the artificial leaves, similar in size to small *M. exalbescens* leaves, were constructed from wire of varying thicknesses: 0.12, 0.25, 0.40, 0.47, and 1.07 mm in diameter. The other five artificial leaves were constructed of varying wire thickness (as above) and were similar in size to large, mature *M. exalbescens* leaves (see Fig. 1C). Surface area and volume estimates were made using a digital calipers for all ten artificial leaves.

2.2. IA technique

Each artificial leaf and a wire calibration standard (diameter 0.50 mm) were photographed with a 35 mm camera using a constant fibreoptic backlighting source (see Schwabe, 1951) and Tmax 100 film. A developed negative of each artificial leaf image and calibration standard image was placed under a camera with an AF Micro Nikkor 55 mm lens. Both images were captured on a video monitor using a PC-Visions AFG Frame Grabber Board and OPTIMAS software (BioScan Inc., 170 West Dayton, Suite 204, Edmonds, WA, USA 98020). To optimize the resolving power of the IA system and to reduce measurement error, each leaf was enlarged to fill the maximum area of the video field. The video image of each artificial leaf and calibration standard was sharpened by varying brightness, contrast, and threshold levels using OPTIMAS.

Next, the perimeter and area for each two-dimensional artificial leaf image and its calibration standard image were made using OPTIMAS. The perimeter and area measurements for each artificial leaf image were then adjusted by multiplying both by a calibration factor (A/B) where A is the image area of the calibration standard determined using a digital calipers, and B is the calculated image area of the calibration standard using the IA system. After adjustment using the calibration standard, perimeter and area measurements from each artificial leaf image were used to calculate the observed surface area (C) and volume (D) of each three-dimensional artificial leaf. $C=(2\pi r_o h_o+2\pi r_o^2)$ and $D=(\pi r_o^2 h_o)$ were calculated by making h_o=perimeter of the adjusted leaf image area/2 and r_o=adjusted leaf image area/$2\times h_o$. A comparison was made for each artificial leaf between the surface area and volume estimates made using the digital calipers and those made using the IA technique. Expected surface area (E) and volume (F) measurements were calculated from dimensions measured using a digital calipers. $E=2\pi r_e h_e+2\pi r_e^2$ *and* $F=(\pi r_e^2 h_e)$ were calculated by making h_e=total length of wire used to produce an artificial leaf and r_e=radius of the wire used. A percent error for surface area was then calculated for each artificial leaf (% error= $[(C-E)/E]\times 100$). A percent error for volume was then calculated for each artificial leaf (% error= $[(D-F)/F]\times 100$).

To determine which calibration standard provided the greatest accuracy in estimating surface area and volume, each of three artificial leaves (produced from wire with diameters 0.25, 0.40 and 1.07 mm) was photographed with four wire calibration standards (diameters 0.25, 0.40, 0.50 and 1.07 mm). A percentage error was calculated for each of the three leaves using the different calibration standards.

2.3. Natural leaves

Fresh leaf material was used to test the precision and robustness of the technique. One fresh leaf of *M. exalbescens* Fern. was spread out evenly on a glass plate and photographed with a calibration standard similar in thickness to the leaf petiole (Fig. 1). To test the repeatability of the technique and error due to

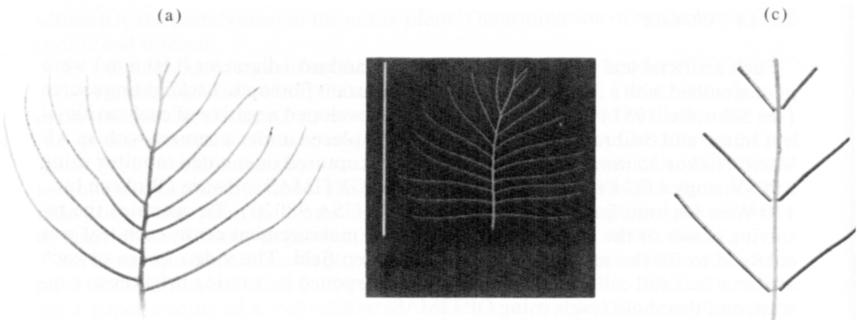

Fig. 1. Natural and artificial leaves: (A) a natural leaf of *M. exalbescens*; (B) the photographic negative of one natural *M. exalbescens* leaf with calibration standard; (C) an artificial model similar in size to a natural *M. exalbescens* leaf.

positioning, the same leaf was repositioned ten times on the glass plate and photographed each time. Adjusted leaf image area and perimeter measurements were made (as above) and C and D measurements were calculated ten times for the same leaf.

To test the variance associated with the IA system, ten different images were acquired from the same negative of a *M. exalbescens* leaf. Adjusted leaf image area and perimeter measures were made for each image then C and D were calculated ten times for this one negative.

Observed versus expected surface area and volume calculations were performed to determine if the error associated with choosing a calibration standard for fresh leaf material was similar in pattern to that for the artificial leaves. One *M. exalbescens* leaf was photographed with four calibration standards (diameters 0.25, 0.40, 0.50, 1.07 mm). Three observed surface area (G) and volume (H) calculations were made using each of three calibration standards with 0.25-, 0.40-, and 1.07-mm diameters. The expected surface area (I) and volume (J) for the same leaf were calculated using the calibration standard (0.50 mm) most similar in thickness to the leaf petiole (0.49 mm). G and I were calculated in the same manner as C above. H and J were calculated in the same manner as D above. A percent error for surface area was then calculated for the fresh leaf (% error $= [(G-I)/I \times 100)$. A percent error for volume was then calculated for each artificial leaf (% error $= [(H-J)/J] \times 100$).

3. Results

3.1. Artificial leaves

The best estimates of surface area and volume using the IA system occurred when the diameter of the calibration standard was similar to the diameter of the wire used to construct the leaves (Table 1). For the artificial leaves with diameters of 0.40 and 0.47 mm, the error produced in estimating surface area was 7% or less when the calibration standard was slightly larger in diameter (0.50 mm). The error produced in estimating volume was 8% or less using the same standard. As the diameter of the wire used to construct the artificial leaves increased or decreased from the diameter of the calibration standard, the % error also increased independently of leaf size.

Four calibration standards of varying thickness photographed with each of three artificial leaves were used to estimate surface area and volume. The lowest error was associated with those measurements calculated using a calibration standard equal to or slightly larger in diameter than the diameter of the wire use to construct each artificial leaf (Figs. 2 and 3).

3.2. Natural leaves

The variances associated with image area and perimeter measurements obtained from ten different negatives taken of one *M. exalbescens* leaf were: image area (49.07 $mm^2 \pm 5.95$ (SD), C.V. = 12%), mean surface area (155.32 $mm^2 \pm 20.39$ (SD), C.V. = 13%) and mean volume (11.75 $mm^3 \pm 2.63$ (SD), C.V. = 22%).

The variances associated with image analysis of ten surface area and volume measurements representing ten different images from a single negative were: mean adjusted surface area (149.45 $mm^2 \pm 0.92$ (SD), C.V. < 1%) and mean volume (12.32 $mm^3 \pm 0.15$ (SD), C.V. = 1%).

The error associated with choosing a calibration standard for fresh leaf material was similar in pattern to that for the artificial leaves (Table 2). The four surface area and four volume measurements of the same leaf ranged from 140.95 to 192.45 mm^2 and from 11.04 to 20.62 mm^3, respectively. The error increased

Table 1
The calculated % error in *C* vs. *E* and *D* vs. *F* (see text for explanation of symbols) for ten artifical leaves made from wire thinner and thicker than the calibration standard (0.50 mm)

Wire leaf	Leaf length (mm)	Leaf wire diameter (mm)	% error *C* vs. *E*	% error *D* vs. *F*
1	29.00	0.12	+36	+82
2	12.52	0.12	+35	+81
3	28.70	0.25	+15	+33
4	12.40	0.25	+13	+17
5	12.95	0.40	+4	+4
6	28.86	0.40	+3	+3
7	24.76	0.47	−4	−6
8	12.30	0.47	−7	−8
9	14.64	1.07	−12	−23
10	28.26	1.07	−26	−45

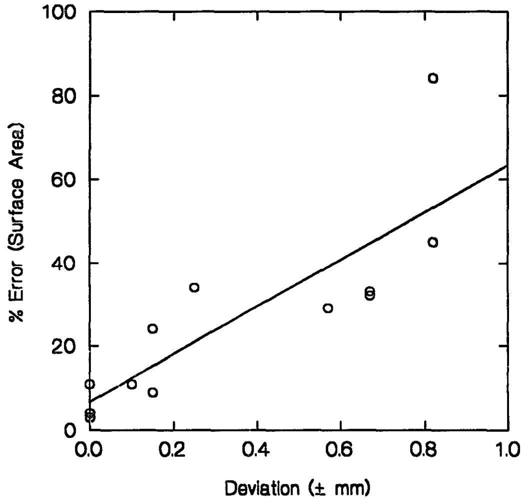

Fig. 2. Percent error in surface area associated with the deviation (± mm) of sample diameter from calibration standard diameter for three artificial leaves.

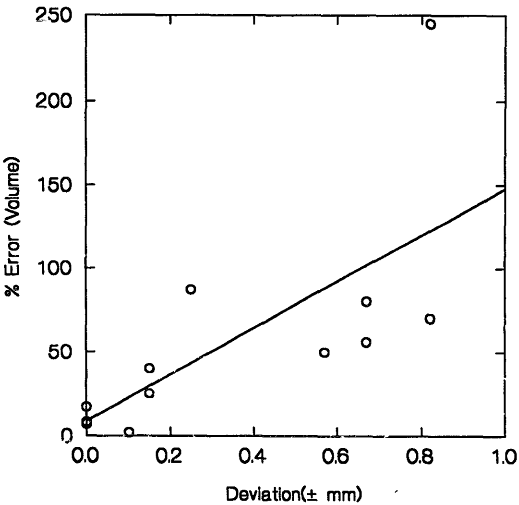

Fig. 3. Percent error in volume associated with the deviation (± mm) of sample diameter from calibration standard diameter for three artificial leaves.

Table 2
The calculated % error in G vs. I and H vs. J (see text for explanation of symbols) for one *Myriophyllum exalbescens* leaf using four calibration standards. The 0.50 mm calibration standard was closest in diameter to the actual leaf petiole diameter

Standard diameter (mm)	% error G vs. I	% error H vs. J
0.25	−12	−22
0.40	−4	−7
0.50	0	0
1.07	+20	+46

in estimating the surface area and volume of the same real (Table 2) or artificial leaf (Figs. 2 and 3) as the calibration standard increased or decreased in diameter from the diameter of the leaf.

4. Discussion

Species of several submerged aquatic genera (e.g. *Myriophyllum*; *Ranunculus* subg. *Batrachium*) have highly dissected leaves with cylindrical or nearly cylindrical segments that are circular in cross-section (Sculthorpe, 1967) in contrast to leaves with thin, undissected, flat blades (e.g. *Potamogeton* spp.). For species in the latter category, surface area measurements may be determined by calculating the surface area for the upper leaf surface (Drew, 1978) and multiplying by two to account for 'both sides' of the leaf (Raven, 1984). Because the thickness of these leaves is very small relative to their width, thickness contributes an insignificant amount to the total surface area. However, surface area measurements for those species with small, highly dissected, cylindrically shaped leaves must take thickness into account because of the much greater influence of the curved leaf surfaces on the total surface area.

We obtained acceptable accuracy of our image analysis technique by reducing two main sources of error: (1) the error associated with measuring the two-dimensional leaf image area and (2) the error produced when calculating the area of a curved surface. Two procedures minimized the error associated with measuring the two-dimensional image area of each artificial wire leaf. First, each leaf image was enlarged to fill the maximum amount of the video screen. Using the largest video image increases the accuracy of image area and perimeter measurements. Second, a cylindrical calibration bar of known area (similar in thickness and shape to the leaves being measured) was used rather than a linear measure to calibrate the leaf image area. By using an areal unit as a calibration standard rather than a linear unit, the increase in measurement error associated with squaring a linear unit to derive a two-dimensional area standard is avoided. The most important factor in this procedure is to use a calibration standard that is similar to the thickness of the petiole in the leaves to be measured. This approach minimizes error due to estimating the expected image area from the adjusted image area. The technique developed in this paper combines the advantages of (1) rapid analysis such as characteristic of the photometric method for measuring highly dissected leaf area and (2) reduced error by use of a calibration standard similar in thickness and shape (i.e. cylindrical) to the leaves.

Few calculations of leaf surface area and/or volume in dissected leaved plants are found (e.g. Uspenskij, 1913 as cited in Arber, 1920; Rosine, 1955; Harrod and Hall, 1962) due perhaps to the difficultly of obtaining these measurements. The technique described above decreases the difficulty and increases the rapidity in which surface area and volume calculations can be made for aquatic plants having highly dissected leaves.

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