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Short communication

Potential for estimating macrophyte surface area from biomass

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Abstract

Relationships between macrophyte surface area and biomass were studied in four lakes on Canada's Boreal Plain, representing a gradient of nutrient concentration and light penetration (mean summer total phosphorus 13–60 $\mu\text{g l}^{-1}$; ammonium 4–35 $\mu\text{g l}^{-1}$; vertical light extinction coefficient 0.7–1.6 m^{-1}). We used colorimetry and dry weight to establish relationships between surface area and biomass for six species (*Ceratophyllum demersum*, *Potamogeton richardsonii*, *P. zosteriformis*, *P. pusillus*, *Utricularia vulgaris* and *Myriophyllum exalbescens*). Except for *P. pusillus*, a positive relationship existed between surface area and biomass. The relationship was species-specific; general groupings could not be made based on leaf morphology. For three of the four species found in more than one lake, relationships did not differ across lakes. Only *C. demersum*, which may be sensitive to fluctuating water levels, exhibited a lake-specific relationship between surface area and biomass.

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1. Introduction

Macrophyte surface area estimates are critical to quantitative study of epiphytic organisms, however, current methods are laborious, involving colorimetry (Cattaneo and Carignan, 1983; Watala and Watala, 1994), image analysis (Gerber et al., 1994) and physical measurement of leaf area (Spence et al., 1973; Brown and Manny, 1985). Sher-Kaul et al. (1995) suggest that establishment of standard relationships between surface area and

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biomass would allow researchers to convert readily available macrophyte biomass estimates into estimates of surface area for epiphyton colonization.

The general applicability of relationships between surface area and biomass assumes consistent patterns among lakes. However, previous work has noted variation in macrophyte morphology and specific leaf area ($\text{cm}^2 \text{g}^{-1}$) with changes in nutrient availability, light penetration, temperature and water level. *Nymphaeae odorata* had larger leaf surface areas in eutrophic versus oligotrophic ponds (Sinden-Hempstead and Killingback, 1996). Leaf area can also increase as light decreases (Spence et al., 1973). Leaf dissection in *Ranunculus flabellaris* was inversely related to water temperature (Johnson, 1967), whereas declining water levels were associated with shorter *Potamogeton lucens* leaves (Clason, 1953). Macrophyte surface area versus biomass relationships may differ among lakes, limiting their suitability for application to all lakes.

Relationships between surface area and biomass may also be species-specific (Sher-Kaul et al., 1995; Nielsen and Sand-Jensen, 1991) and depend on leaf morphology and the ratio of leaves to stems. Differences in dissected, fenestrate and entire leaf morphologies are expected to yield different specific leaf areas. Further, the proportion of leaves to stems may affect the surface area to biomass relationship; for most species, leaves provide more surface area than stems (Sher-Kaul et al., 1995). To assess potential species-specific differences, comparison of the relationship between macrophyte surface area and biomass requires a single methodology.

Four lakes (SPH20, SPH200, SPH800 at $55^\circ 23' \text{N}$, $113^\circ 40' \text{W}$ and Long Lake at $54^\circ 35' \text{N}$, $113^\circ 39' \text{W}$) on the western Boreal Plain of Canada were chosen to maximize disparity in variables thought to influence the macrophyte surface area versus biomass relationship, specifically trophic state (meso- to hyper-eutrophic) and light penetration. Lakes varied from relatively shallow to deep (mean depths of 2.2, 3.3, 4.6 and 9.4 m for SPH800, SPH200, SPH20 and Long Lake, respectively). We tested whether macrophytes have more surface area per gram dry weight (1) in lakes with relatively high nutrient concentrations and low light penetration and (2) when leaves are dissected versus entire.

2. Methods

In late August 1999, six submersed species (Table 1) were randomly collected by a scuba diver at 1 m depth, transported to the laboratory in darkened coolers and sorted

Table 1
Morphological characteristics and presence (×) or absence (–) of six macrophyte species in the study lakes

Macrophyte species	Leaf morphology	Leaf shape	Long Lake	SPH20	SPH200	SPH800
<i>Potamogeton zosteriformis</i> Fern.	Entire	Linear	×	–	×	×
<i>Potamogeton richardsonii</i> Rydb.	Entire	Oval to linear	×	×	–	×
<i>Myriophyllum exalbescens</i> Fern.	Dissected	Whorl	×	×	×	–
<i>Ceratophyllum demersum</i> L.	Dissected	Whorl	×	×	×	×
<i>Potamogeton pusillus</i> L.	Entire	Threadlike	–	–	–	×
<i>Utricularia vulgaris</i> L.	Dissected	Bladder	×	–	–	–

to species. To dislodge epiphytes, the plants were shaken in a 4-l mason jar and rinsed until the water ran clear (Nicopoulos, 2001). Samples of each species were cut into 15 individual pieces ranging from 0.7 to 10 g wet weight (range of leaf and stem combinations that existed in the study lakes). Surface area was estimated colorimetrically after Cattaneo and Carignan (1983). Each piece was dipped in a 50:50 solution of detergent (Purex Mountain Breeze) and tap water with blue fabric dye (Tintex Royal Blue #6, 15.8 g l^{-1}). The sample was shaken 30 times to remove excess dye, then submersed in a known volume of tap water (25–700 ml). Water color was measured at 597 nm with a Milton Roy Spectronic 501 spectrophotometer and readings adjusted by multiplying by the volume of rinse water used (dilution factor). The mean of the three adjusted color readings was calculated.

A standard curve was calculated from the leaves of *Potamogeton richardsonii* to establish the relationship between water color and macrophyte surface area. Various leaves were selected and photocopied onto plain paper. Surface area was estimated with a planimeter, then doubled to represent both sides of the leaf. Mean adjusted color readings determined above were plotted against the surface area of each leaf (both variables log transformed). The relationship ($R^2 = 0.97$, $P = 0.002$) was then used to convert mean adjusted spectrophotometric readings into surface area.

Dry weights of samples were determined ($\pm 0.005 \text{ g}$) after drying at 60°C to a constant weight. For Long Lake samples, duplicate macrophyte pieces with the same wet weight were used to determine the influence of the detergent/dye solution on dry weight. One piece was analyzed as above for surface area, while the other was dried and weighed without immersion in the detergent/dye solution.

Water chemistry, chlorophyll *a* (Chl *a*) and temperature data at 2-week intervals were obtained from Prepas et al. (2001) for the SPH lakes for June to August 1999. Water samples were collected from the euphotic zone (depth of penetration of 1% of the surface irradiance at 1 m intervals). The light extinction coefficient (E_{PAR} ; m^{-1}) was calculated between the surface and within 1 m of the lake bottom (Wetzel, 1983). Long Lake water chemistry and Chl *a* data were from Crosby and Prepas (1990), and E_{PAR} data from Chambers and Prepas (1990). Since these variables exhibited little year-to-year variation in nearby Narrow Lake (Prepas unpublished; Crosby and Prepas, 1990), the published 1986 data from Long Lake were assumed to be representative of 1999.

Dry weights of Long Lake samples ($n = 75$) dipped and not dipped in detergent/dye were compared with a Wilcoxon Signed-Rank test. Absorption of residual solution did not appear to increase the dry weight of dipped macrophytes, rather shaking samples to remove excess solution may have dislodged pieces, resulting in a lower weight of dipped samples ($P < 0.0001$). Linear regressions of surface area versus biomass were compared among species and lakes with an analysis of covariance (ANCOVA JMP IN[®] Software—SAS Institute, 1996; Zar, 1996). Although residuals for all linear regressions were normal, some heterogeneous variance was present. Since sample sizes were equal or nearly equal, it was assumed that the analysis was robust enough to operate well with heteroscedasticity (Zar, 1996). Power analysis of the ANCOVA was conducted to determine probability of a type II error (β) and power ($1 - \beta$). The sample size required to detect differences or the number of observations needed to drive down the variance of the estimates enough to achieve a significant result with the given values of alpha, sigma and delta (the significance level, the

standard deviation of the error and the effect size, respectively) was also estimated (SAS Institute, 1996). The Newman–Keuls multiple range test was used for multiple comparisons (Zar, 1996). The significance level was 0.05 for all tests.

3. Results

Leaf morphology and locations of the six submersed species are shown in Table 1. Chemical, biological, and physical characteristics of the study lakes are shown in Table 2. Mean ammonium (NH_4^+) concentration in SPH200 was 2–8 times higher than in Long Lake, SPH20 and SPH800 (Table 2), probably because beaver activity and variable runoff caused fluctuating water levels and enhanced decomposition rates (Prepas et al., 2001). Mean total phosphorus (TP) concentration in SPH800 was about 25, 80 and 360% higher than in SPH200, SPH20 and Long Lake, respectively. Light penetrated deepest in Long Lake and SPH20, where the mean E_{PAR} was about half the value of SPH800 and SPH200. Based on Chl *a* and TP concentrations, Long Lake and SPH20 were mesotrophic, SPH200 was eutrophic and SPH800 was hypereutrophic (Wetzel, 1983). The study lakes provide a range of biogeochemical characteristics to compare the relationship between macrophyte surface area and biomass.

With the exception of *Potamogeton pusillus*, which was excluded from subsequent analyses, macrophyte surface area was positively related to biomass ($P < 0.05$). Whereas macrophyte surface area could be predicted from biomass for the remaining five species, the percent of variance in surface area explained by biomass varied. On average, more variance in *Ceratophyllum demersum* surface area (86%) was explained by biomass as compared to the remaining four species, *Utricularia vulgaris* (79%), *Potamogeton richardsonii* (75%), *P. zosteriformis* (65%) and *Myriophyllum exalbescens* (62%). The proportion of variance in surface area explained by biomass appeared to be independent of leaf morphology, since *C. demersum* (dissected) and *P. richardsonii* (entire) had higher average R^2 values than *M. exalbescens* (dissected) and *P. zosteriformis* (entire).

For three of the four macrophyte species found in more than one study lake (*P. richardsonii*, *P. zosteriformis* and *M. exalbescens*), the relationship between surface area and biomass was not detectably different among lakes, despite the wide range in nutrient concentration and E_{PAR} (Table 3). However, a relatively small sample size contributed to low

Table 2
Mean summer chemical, biological and physical characteristics of the study lakes

Lake	NH_4^+ ($\mu\text{g l}^{-1}$)	TP ($\mu\text{g l}^{-1}$)	Chl <i>a</i> ($\mu\text{g l}^{-1}$)	Temperature ($^{\circ}\text{C}$)	E_{PAR} (m^{-1})
SPH20	15	33	9	17.4	0.8
SPH200	35	48	23	17.3	1.4
SPH800	17	60	13	18.4	1.6
Long Lake	4 ^a	13 ^a	3.9 ^a	Not available	0.7 ^b

Sources: Prepas et al. (2001) for 1999.

^a Crosby and Prepas (1990) from 1986.

^b Chambers and Prepas (1990) from 1986.

Table 3

Probability values from an ANCOVA comparing the slope and intercept of the relationship between macrophyte surface area (dependent) and biomass (independent) among lakes (independent) for each species

Macrophyte species	P_{Slope}	$P_{\text{Intercept}}$	n
<i>Ceratophyllum demersum</i>	0.001	–	54
<i>Potamogeton richardsonii</i>	0.74	0.19	43
<i>Potamogeton zosteriformis</i>	0.10	0.61	43
<i>Myriophyllum exalbescens</i>	0.15	0.19	44

(<0.5) power. Therefore, data from all lakes for each species were pooled to increase sample size to compare surface area to biomass relationships between species (Table 4).

The remaining macrophyte, *C. demersum*, had less surface area per unit biomass in SPH200 compared to SPH20, SPH800 and Long Lake ($P < 0.001$). Detection of this difference may be related to the high power (0.96) associated with this analysis. For comparisons of the relationship between *C. demersum* surface area and biomass across species, data from SPH20, SPH800 and Long Lake were pooled (*C. demersum* P), whereas data from SPH200 were kept separate (*C. demersum* 200).

Surface area to biomass relationships differed among species (*P. richardsonii*, *P. zosteriformis*, *M. exalbescens*, *U. vulgaris*, *C. demersum* 200 and *C. demersum* P) (Table 4). Of the 15 multiple comparisons of the relationship between surface area and biomass, 14 yielded differences among species. Only the relationship between surface area and biomass for *C. demersum* P and *M. exalbescens* was not different. Average specific leaf area was higher for dissected-leaf *U. vulgaris* ($1779 \text{ cm}^2 \text{ g}^{-1}$) than dissected-leaf *C. demersum* P and *M. exalbescens* (427 and $522 \text{ cm}^2 \text{ g}^{-1}$, respectively), which may be attributable to the numerous bladders on *U. vulgaris*. Although both *P. richardsonii* ($766 \text{ cm}^2 \text{ g}^{-1}$) and *P. zosteriformis* ($108 \text{ cm}^2 \text{ g}^{-1}$) have entire leaves, the thin, long, linear morphology of *P. zosteriformis* may have accounted for its lower surface area than *P. richardsonii*, with a wider, oval leaf morphology. When deriving relationships between surface area and biomass, grouping of morphological similar plants such as *C. demersum* and *M. exalbescens* may be possible.

Table 4

Regression parameters for the relationship between macrophyte surface area (dependent, cm^2) and biomass (independent, g dry weight) for each species in the study lakes (\pm S.E.)

Macrophyte species	n	Intercept	Slope	R^2	P
<i>Ceratophyllum demersum</i> P ^a	41	63 ± 8	427 ± 29	0.85	<0.0001
<i>Ceratophyllum demersum</i> 200 ^b	13	89 ± 12	230 ± 35	0.79	<0.0001
<i>Myriophyllum exalbescens</i>	43	12 ± 8	522 ± 39	0.81	<0.0001
<i>Potamogeton zosteriformis</i>	43	81 ± 6	108 ± 14	0.58	<0.0001
<i>Potamogeton richardsonii</i>	43	33 ± 8	766 ± 51	0.84	<0.0001
<i>Utricularia vulgaris</i>	15	11 ± 33	1779 ± 256	0.79	<0.0001
<i>Potamogeton pusillus</i>	14	19 ± 3	124 ± 91	0.13	0.20

^a Pooled data from SPH20, PH800 and Long Lake.

^b Data from SPH200.

However, grouping of macrophyte species based on general leaf morphologies (i.e. entire versus dissected) was not supported.

4. Discussion

Except for *P. pusillus*, the strong positive relationship observed between surface area and biomass for all macrophyte species suggests that colorimetry/dry weight can be used to estimate surface area. Since *P. pusillus* was found only in SPH800, it was difficult to determine if the poor relationship was an isolated situation or if the methodology was poorly suited to its small, threadlike leaves. The application of this methodology to macrophytes with threadlike leaf morphology merits further consideration.

Several factors may have contributed to the lower specific leaf area of *C. demersum* in SPH200 than in the other study lakes. The proportion of leaves versus stems on *C. demersum* plants may be relatively small in SPH200. Stems provided a lower specific leaf area than leaves on another dissected-leaf plant, *Myriophyllum spicatum* (Sher-Kaul et al., 1995). Further, dissected leaves may have different degrees of subdivision, length and thickness of segments under different conditions such as fluctuating water level (Sculthorpe, 1985). In SPH200, water level fluctuated by >3 m between 1997 and 1999, with relatively high water levels during the study period. *C. demersum* has a high tolerance for variable water levels (Barrat-Segretain et al., 1999) and may have thread-like rhizoid shoots that penetrate the substrate to aid absorption and anchorage (Sculthorpe, 1985). These were noted on some plants in our study, but were not quantified. Therefore, plants in SPH200 may have consisted of more buds, fragments and rhizoids and fewer whole plants than plants in the other lakes. Thus, a lower leaf to stem ratio, fluctuating water levels and the presence/absence of rhizoids may have altered the relationship between *C. demersum* surface area and biomass in SPH200 versus other study lakes.

Contrary to our hypothesis and despite the wide range in NH_4^+ and TP concentrations and E_{PAR} , no differences were detected in among-lake comparisons of the surface area to biomass relationships for *P. richardsonii*, *P. zosteriformis* and *M. exalbescens*. Differences in plant morphology such as specific leaf area, the proportion of leaves versus stems or other acclimations between lakes did not yield different relationships. Whereas fluctuating water level may have altered *C. demersum* morphology in SPH200, the relationship between surface area and biomass for both *P. zosteriformis* and *M. exalbescens* did not differ.

The surface area versus biomass relationship was highly species-specific, yet differences did not appear to be related to leaf morphology. When dissected-leaf macrophytes were examined based on presence or absence of bladders, bladder-absent *C. demersum* and *M. exalbescens* had lower surface areas than *U. vulgaris* with numerous bladders. Although entire-leaf *P. zosteriformis* had a lower surface area than entire-leaf *P. richardsonii*, the difference can be attributed to long, linear versus wide, oval leaf morphology. These results agree with those of Sher-Kaul et al. (1995), who suggested that the relationship between surface area and biomass was not dependent on leaf morphology. However, grouping of morphologically similar species (i.e. *C. demersum* and *M. spicatum*) could be considered when converting macrophyte biomass to surface area.

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