Estimating the Standing Biomass of Aquatic Macrophytes

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The accuracy, precision, and cost-efficiency of estimation techniques for macrophyte standing biomass are examined, and solutions are offered to problems encountered in the design of efficient sampling programs. Five sizes of quadrats (100 cm² to 1 m²) were compared on seven occasions and all five yielded equivalent biomass estimates in six of these tests. Published and unpublished values of biomass sampling variance measured around the world were predictable from average standing biomass and size of sampler. The sampling cost was predictable from sampler size. Analysis of these relationships allows optimization of sampling design. The use of small quadrat samplers with great replication is recommended. Adherence to this protocol can result in a 30-fold reduction in sampling cost. Recommendations are compared with techniques actually used by aquatic ecologists. A brief analysis of spatial heterogeneity of aquatic macrophyte biomass is presented.

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Recent research underscores the importance of aquatic macrophytes to the functioning of freshwater ecosystems (e.g. Carignan and Kalff 1982; Cattaneo 1983; Howard-Williams and Allanson 1981; Jacoby et al. 1982; Landers 1982). Assessments of biomass, production, nutrient cycling, and community dynamics all rely on estimates of macrophyte standing stock (Landers 1982; Westlake 1965, 1969). In addition, the estimation of macrophyte standing biomass is essential to studies of the epiphytic invertebrate fauna (Downing 1984; Downing and Cyr 1985), and these measurements are in turn necessary for the assessment of fish food resources in lakes (e.g. George and Hadley 1979; Laughlin and Werner 1980). Precise measurement of these factors is limited by the heterogeneity of macrophyte beds and resultant sampling errors. In spite of the importance of macrophyte biomass estimation, no systematic evaluation of estimation techniques has been made.

Standing macrophyte biomass is usually estimated by quantitative harvest of macrophytes contained in randomly placed samplers of known area. Direct harvest of macrophytes from quadrats provides the most accurate estimates of standing biomass (Grace and Tilley 1976; Rich et al. 1971; Sheldon and Boylen 1978; Westlake 1969), although grabs (e.g. Unni 1977) and hooks (e.g. Bayley et al. 1978) have sometimes been used. Macrophytes are usually collected by SCUBA divers because they can see whether plants have been missed, can avoid entangling plants not growing within the quadrat area, and loss rates are very small.

The field researcher must decide what size of quadrat to employ, and how many replicate samples to take for each biomass estimate. The size of quadrat is important because it may affect both the accuracy and precision of macrophyte biomass estimates. Terrestrial ecologists have concluded that small quadrats yield overestimates of plant biomass due to edge effects (Hanson 1930; Hendricks 1956; Sukhatme 1947; Van Dyne et al. 1963; Wiegert 1962), and large quadrats yield higher precision for the same level of replication (Pechanec and Stewart 1940; Smith 1938). While many ecologists have used large samplers (Green 1979, p. 39), other researchers have
programs are needed in order to increase statistical power and
eliminate wasted effort. The purpose of this study is to compare
accurate, precision, and relative cost of harvest techniques
for the estimation of total aboveground standing macrophyte
biomass were made on seven occasions during 1982 and 1983.
Comparisons in 1982 were made during July and August in
Lake Memphremagog, Quebec-Vermont (45°0'N, 72°17'W),
near Cove Island. The substrate was sandy clay to clayey allu-
vium, and dominant macrophyte species were Cabomba carolin-
iana (Gray), Elodea canadensis (Michx.), Heteranthera dubia
((Jacq.) MacM.), Myriophyllum spicatum (L.), Najas sp.,
Potamogeton richardsonii ((Benn.) Rydb.), and Vallisneria
americana (Michx.). Four further comparisons were made in
the same geographical area during 1983 using greater replica-
tion and a wider range of total biomass levels and substrate
types. From lowest to highest macrophyte biomass, these sites
were as follows: (1) Lake Orford, on sandy substrate supporting
Chara sp., E. canadensis, Isoetes sp., Najas sp., Nitella sp.,
Potamogeton crispus (L.), and P. praehlongus (Wulf.); (2) South
Lake Memphremagog, on sandy substrate supporting E. cana-
densis, Eriocaulon septangularis (With.), H. dubia, Isoetes
sp., M. spicatum, Najas sp., Potamogeton gramineus (L.), and
V. americana; (3) North Lake Memphremagog, on mud and
clay supporting C. caroliniana, E. septangularis, H. dubia, M.
spicatum, Najas sp., Nitella sp., P. gramineus, P. praehlongus,
P. robbinsii (Oakes), and V. americana; and (4) Lake Massa-
wicki, on mud supporting a monospecific stand of M. spicatum.
Comparisons in 1983 were all made near maximum seasonal
biomass, at the end of August.
The five quadrat sizes compared covered a geometric series
from 100 cm² to 1 m². This range includes over 90% of the
sample sizes used in the published literature (Fig. 1). Samples
were collected using square quadrats made of (1982) steel or
(1983) PVC pipe (J. A. Downing, unpubl.). At each site, the
quadrats were placed at randomly drawn points along either side
of a numbered 50-m line stretched at uniform depth across the

concrete decision rules for macrophyte biomass sampling
programs are needed in order to increase statistical power and
eliminate wasted effort. The purpose of this study is to compare
the accuracy, precision, and relative cost of harvest techniques
for the measurement of aquatic macrophyte biomass. We present

suggested that small samples are the most cost-effective (Green
1979, p. 38-39; John et al. 1980; Pechanec and Stewart 1940;
Smith 1938; Ursic and McClurkin 1959). Aquatic ecologists
have sampled macrophytes using a wide variety of sampler sizes
(Fig. 1; median 2500 cm²). Aquatic methods manuals and
handbooks offer little help in choice of quadrat size (e.g.
Westlake 1969, p. 26); suggested quadrat areas are 333 cm²
(Schwoerbel 1970, p. 80), 1332 cm² (Welch 1948, p. 330),
2500–5000 cm² (Lind 1979, p. 178; Wetzel and Likens 1979,
p. 266), or 400 cm² to 1 m² (Wood 1975, p. 19). The effect of
quadrat size on the accuracy and precision of aquatic macro-
phyte biomass estimates is unknown.
The number of replicate samples (n) to be taken depends upon
the spatial distribution and the desired level of precision of
the variable to be estimated. Green (1979, p. 39) suggests that "The
best sample number is the largest number..." because the stan-
dard error (se) of the mean is \( \frac{s}{\sqrt{n}} \), where \( s^2 \) is the sampling
variance. In practice, sampling cost limits sample number
(Cochran 1977, p. 84), and it is therefore important to know in
advance the level of replication that will yield an acceptable
level of precision. Methods manuals offer no rules-of-thumb,
because \( n \) can be calculated for a desired level of precision only
if sampling variance is known or can be predicted (Cochran
1977, p. 243; Elliott 1977, p. 129). A priori, we might guess
that spatial variation of macrophyte biomass follows a Poisson
distribution or the negative binomial family like those found for
benthic invertebrate populations (Elliott 1977, chap. 5). Al-
though macrophyte biomass is thought to be spatially aggre-
gated (Davies 1970; Westlake 1969; Wong and Clark 1979), no
general model has been advanced to predict the expected
variance for replicate macrophyte biomass estimates. Not
surprisingly, aquatic ecologists have employed a wide variety of
sample replication (Fig. 2).

Concrete decision rules for macrophyte biomass sampling
programs are needed in order to increase statistical power and
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for the measurement of aquatic macrophyte biomass. We present

Fig. 1. Sampler sizes used for aquatic macrophytes in studies pub-
lished between 1970 and 1984 in 30 well-known aquatic journals. If
studies used a range of sampler sizes, both the upper and lower limits of
sample size range are included in the histogram. The bars include
observations made at their upper extreme but not their lower extreme.
Note unequal quadrat size intervals.

Fig. 2. Number of replicate samples (n) taken to estimate aquatic
macrophyte biomass in studies published between 1970 and 1984 in 30
well-known aquatic journals. If studies used a range of n, both the
upper and lower limits of the range are included in the histogram.

Methods

Accuracy

Comparisons of the relative accuracy of various quadrat sizes
for the estimation of total aboveground standing macrophyte
biomass were made on seven occasions during 1982 and 1983.
Comparisons in 1982 were made during July and August in
Lake Memphremagog, Quebec–Vermont (45°0'N, 72°17'W),
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were collected using square quadrats made of (1982) steel or
(1983) PVC pipe (J. A. Downing, unpubl.). At each site, the
quadrats were placed at randomly drawn points along either side
of a numbered 50-m line stretched at uniform depth across the
macrophyte bed. We took between 3 and 26 replicate samples with each quadrat size at each site. The number of quadrats used in 1983 was adjusted to yield a precision of \( \frac{\text{se}}{\bar{x}} = 0.1 \) (\( \text{se} \) = standard error; \( \bar{x} \) = mean biomass in grams dry weight per square metre), determined by a preliminary sampling survey. Macrophytes were harvested from within the quadrats by divers using SCUBA. Plants were cut with grass-clippers or broken directly at the sediment surface and then were placed carefully in 100-\( \mu \)m-mesh nylon bags. Six different divers were assigned to a given biomass level to obtain an acceptable level of precision.

We have built such a variance function from our data and those supplied to us by macrophyte ecologists throughout the world (Table 1). The data collected consisted of arithmetic means \( \bar{x} \), 1 weighted variances \( s^2 \), and quadrat sizes \( A \) for aquatic macrophytes from lakes, streams, rivers, estuaries, and oceans. Data on total macrophyte biomass and aboveground macrophyte biomass were included for comparative purposes. Data on \( \bar{x} \) (grams dry weight per square metre) and \( s^2 \) (calculated on a per square metre basis) of both single species and total macrophyte community biomass were also analyzed. The model

\[
\log s^2 = \log a + b \log \bar{x} + c \log A,
\]

where \( \log a \), \( b \), and \( c \) are fitted constants, was fitted to the full dataset using regression analysis (Draper and Smith 1981; Nie et al. 1975). The residuals were plotted against the independent variables to make certain that the regression model fitted the data well, and that no systematic lack of fit remained after analysis. Comparisons of the fit of subsets of the data (e.g. species or categories) were made using parametric and nonparametric methods.

Cost

The optimal combination of sampler size \( A \) and sample number \( n \) was determined by measuring the cost (in time) of sampling under various sampling conditions. The primary cost...
in estimating total standing macrophyte biomass is the time that it takes to collect the macrophytes. In our comparisons of quadrat accuracy, each diver used a waterproof chronograph to record the time (T minutes) elapsed between the start and finish of macrophyte collection from each quadrat. A nonlinear function was fitted to these data using the Marquardt (1963) method (Robinson 1977):

$$T = q(1 - e^{-dA})$$

where e is the base of natural logarithms, A is the quadrat size (square centimetres), and d and q are fitted constants. The residuals were examined for lack of fit. Predicted costs were then combined with predicted numbers of samples to determine the most cost-efficient method of attaining a given precision.

Taxonomic nomenclature for our collections follows Fassett (1983), while species names for other data are those supplied by their authors (Table 1).

### Results and Discussion

#### Accuracy

We took over 350 samples to determine the relative accuracy of different quadrat sizes for the estimation of aboveground standing biomass. These comparisons covered a range of biomass from 5 to over 200 g dry wt. m$^{-2}$ (Table 2). In six of seven cases, no significant (Kruskal–Wallis test; $P < 0.01$) difference could be demonstrated among frequency distributions of estimated macrophyte biomass. This indicates that no quadrat size tends to yield higher biomass estimates than any other. The lone significant difference was found in Lake Massawippi where small quadrats yielded very low estimates of standing biomass. This effect was predicted by divers who noticed that smallest PVC quadrats, dropped at randomly chosen points, were not heavy enough to penetrate dense clumps of $M$. *spicatum* and tended to slide toward bare spaces between them. This effect could be avoided either by using heavier quadrats or by dropping very dense colored objects at randomly chosen points and orienting the quadrats to these weights in a predetermined fashion (e.g. corner, center, midside, etc.).

#### Precision

The major goal in designing a sampling program is to achieve an accurate measurement with high precision for the least effort. The size of the sampler or quadrat and the number of replicate samples taken can be varied so that the minimum number of samples of a given size may be extracted to yield a specified degree of precision. To date, the only means of calculating a priori the number of macrophyte samples needed to yield a biomass estimate with a given level of precision has been to assume that macrophyte biomass estimates follow the Poisson or negative binomial distribution. The actual variance function, or the relationship of the sample variance to the mean biomass of organisms has never been attempted (Taylor 1984).

To this end, we have collected 1200 published and unpublished observations of $s^2$ and $s$ of dry weight biomass estimates. These data cover the range of habitats for which quantitative observations are available. Only data on true replicate samples (same date/same station) were included in the data set. The range of estimated mean biomass is from 0.002 to 3700 g dry wt. m$^{-2}$, the variance from 0.0001 to $1.8 \times 10^{6}$, sampler area from 100 to 10 000 cm$^2$, and $n$ from 3 to 26. Data from marine systems are rare in the dataset (1%), and most data were collected by quadrat harvest (98%). When considered on a per

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**Table 2.** Average biomass (g dry wt. m$^{-2}$) estimated using five different quadrat sizes on seven different occasions. Means were calculated after fourth-root transformation. $x^2$ values are for the Kruskal–Wallis test (Conover 1971, p. 256). Number of samples is indicated in parentheses. $P$ is the significance level for rejection of the hypothesis that all quadrats in the row yield identical distributions of untransformed estimated macrophyte biomass.

<table>
<thead>
<tr>
<th>Site</th>
<th>100</th>
<th>316</th>
<th>1000</th>
<th>3162</th>
<th>10000</th>
<th>$x^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake Orford (1983)</td>
<td>5.3</td>
<td>4.4</td>
<td>2.9</td>
<td>5.9</td>
<td>6.2</td>
<td>4.5</td>
<td>0.34</td>
</tr>
<tr>
<td>Cove Island (1982), Exp. 1*</td>
<td>23.2</td>
<td>15.0</td>
<td>16.0</td>
<td>10.2</td>
<td>9.9</td>
<td>2.7</td>
<td>0.60</td>
</tr>
<tr>
<td>Cove Island (1982), Exp. 5*</td>
<td>50.5</td>
<td></td>
<td>55.7</td>
<td></td>
<td>53.3</td>
<td>0.1</td>
<td>0.95</td>
</tr>
<tr>
<td>Cove Island (1982), Exp. 8*</td>
<td>54.5</td>
<td></td>
<td>51.7</td>
<td></td>
<td>43.2</td>
<td>1.6</td>
<td>0.45</td>
</tr>
<tr>
<td>Memphremagog South (1983)</td>
<td>65.1</td>
<td>67.8</td>
<td>71.7</td>
<td>74.7</td>
<td>63.2</td>
<td>2.3</td>
<td>0.68</td>
</tr>
<tr>
<td>Memphremagog North (1983)</td>
<td>171.7</td>
<td>140.0</td>
<td>183.4</td>
<td>129.0</td>
<td>138.4</td>
<td>4.3</td>
<td>0.37</td>
</tr>
<tr>
<td>Lake Massawippi (1983)</td>
<td>0.9</td>
<td>50.8</td>
<td>189.5</td>
<td>166.1</td>
<td>243.4</td>
<td>15.2</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Quadrat sizes were 112, 569, 991, 2540, and 10002 cm$^2$. 

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*The data are available, at a nominal charge, from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Canada K1A 0S2.*
Table 3. Analysis of variance table for regression of $\log_{10} s^2$ as a function of $\log_{10} \bar{x}$ (mean macrophyte biomass, g dry wt. mg$^{-2}$) and $\log_{10} A$ (sampler area, cm$^2$) (equations 1 and 3). $F'$ (partial $F$-value) is calculated as the increase in model SS when each variable is entered into the multiple regression as the last variable. $F'$ is a measure of the variation in $\log_{10} s^2$ accounted for by each independent variable. The probability of obtaining any of these $F$ or $F'$ values by chance is <0.001.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>SS</th>
<th>$R^2$</th>
<th>$F'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model (equation 1)</td>
<td>2</td>
<td>4255</td>
<td>1.00</td>
<td>123</td>
</tr>
<tr>
<td>Error</td>
<td>1197</td>
<td>219</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1199</td>
<td>4475</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Independent variable

- $\log_{10} \bar{x}$
- $\log_{10} A$

Fig. 3. Relationship between observed variance of replicate macrophyte biomass estimates (1200 sets of estimates) and the variance predicted from equation 4. Plotted digits indicate the number of observations at a given location.

square metre basis, macrophyte biomass appears spatially aggregated because $s^2 > \bar{x}$ in 83% of the observations collected.

Using multiple regression analysis, we found that $s^2$ of replicate macrophyte samples is predictable from the mean standing biomass ($\bar{x}$) and that a significant amount of residual variation in $s^2$ is accounted for by the sampler area ($A$) (Table 3). The regression equation ($r^2 = 0.95$) to predict the sampling variance of macrophyte biomass estimates is

\[ \log s^2 = 0.759 + 1.567 (\log \bar{x}) - 0.157 (\log A) \]

or

\[ s^2 = 5.75 \bar{x}^{1.567} A^{-0.157}. \]

Observed and predicted variances are very similar and highly correlated (Fig. 3). Other regression models were attempted, including models containing interaction terms, but this one proved to be the most accurate and precise. Neither the Poisson distribution nor the negative binomial with common $k$ could yield as accurate predictions of spatial variation of macrophyte biomass (Fig. 4) because in the former case $s^2 = \bar{x}$, while in the latter $s^2 = \bar{x}^2/k + \bar{x}$ with a single $k$ common to all samples. Tested on independent data, the Poisson distribution severely underestimated $s$ and the negative binomial ($k = 2$) severely overestimated $s$, while equation 4 predicted standard deviations very close to those actually observed (Fig. 4).

Because different species of macrophytes possess differing modes of reproduction and dispersal, as well as differing ecological requirements, we might expect the biomass of aquatic macrophyte species to be differentially distributed in space. Analysis of the residuals should indicate the relative degree of spatial variability of the biomass of macrophyte species. Table 4 shows the residuals from equation 3 summed over a variety of taxonomic groups. The $t$-values indicate that many taxonomic groups have residuals that differ significantly from the overall
mean residual. These analyses suggest that the biomass of the large macrophyte *P. praetongus* is more highly variable, while the biomass of dense floating plants such as *Eichhornia crassipes* and marine macrophytes such as *Zostera capricorni* are less variable than average. Nonparametric analyses of the residuals also suggest that the variance of marine macrophyte biomass is overestimated by equation 4 (Kruskal-Wallis test; *P* < 0.01). On the other hand, equation 4 works equally well for macrophytes growing in lakes, rivers, and streams (Kruskal-Wallis test; *P* = 0.79). In addition, data collected using quadrats and other collection methods (e.g. rakes, rotary cutters, grabs) appear to follow the same relationship (Mann-Whitney test; *P* = 0.08). Equation 4 works well for both aboveground and total biomass estimates (Mann-Whitney test; *P* = 0.19).

The last column of Table 4 shows the coefficient of determination (*r*^2*) for the linear relation between the residuals from equation 3 (in log,0 form) and the logarithm of mean standing biomass (x). This analysis determines whether the overall regression is significant when compared to that seen within each taxonomic group. There are some significant relationships, but only one accounts for >50% of the variation in the residuals (Ranunculus bandooli). This analysis suggests that a few species of macrophytes increase their spatial variation in biomass more rapidly with increased biomass than other species. Graphical analysis (Draper and Smith 1981) revealed no nonlinear trends in the residuals.

### Table 4. Analysis of residuals from equation 3 by taxonomic group. Shown are the arithmetic mean and the variance of residuals in log,0 form. n' is the number of sets of replicate samples of each taxon in the dataset, and t is the *t*-statistic comparing the mean residual of the subgroups (i.e. taxon) with the average of the residuals of all other samples assuming unequal variances (Prepas 1984, p. 298). *r*^2* is the coefficient of determination for the linear relationship between the residuals in log,0 form and log,0 *x* for each taxon. *P* < 0.05; **P* < 0.01. Taxonomy follows the authors cited in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Variance</th>
<th>n'</th>
<th>t</th>
<th>df</th>
<th><em>r</em>^2*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potamogeton praetongus</td>
<td>0.66</td>
<td>0.16</td>
<td>11</td>
<td>5.55**</td>
<td>10</td>
<td>0.34</td>
</tr>
<tr>
<td>Chara sp.</td>
<td>0.42</td>
<td>0.06</td>
<td>8</td>
<td>4.89**</td>
<td>7</td>
<td>0.40</td>
</tr>
<tr>
<td>Phragmites australis</td>
<td>0.35</td>
<td>0.04</td>
<td>3</td>
<td>3.05</td>
<td>2</td>
<td>0.07</td>
</tr>
<tr>
<td>Ranunculus sp.</td>
<td>0.34</td>
<td>0.07</td>
<td>8</td>
<td>3.71**</td>
<td>7</td>
<td>0.43</td>
</tr>
<tr>
<td>Cabomba caroliniana</td>
<td>0.26</td>
<td>0.05</td>
<td>15</td>
<td>4.36**</td>
<td>15</td>
<td>0.07</td>
</tr>
<tr>
<td>Potamogeton robbinsi</td>
<td>0.25</td>
<td>0.21</td>
<td>24</td>
<td>2.75*</td>
<td>24</td>
<td>0.20</td>
</tr>
<tr>
<td>Myriophyllum heterophyllum</td>
<td>0.22</td>
<td>0.15</td>
<td>4</td>
<td>1.15</td>
<td>3</td>
<td>0.32</td>
</tr>
<tr>
<td>Potamogeton richardsonii</td>
<td>0.22</td>
<td>0.06</td>
<td>4</td>
<td>1.81</td>
<td>3</td>
<td>0.46</td>
</tr>
<tr>
<td>Ericaulon septangularis</td>
<td>0.21</td>
<td>0.11</td>
<td>8</td>
<td>1.81</td>
<td>7</td>
<td>0.43</td>
</tr>
<tr>
<td>Potamogeton crispus</td>
<td>0.20</td>
<td>0.11</td>
<td>14</td>
<td>2.26*</td>
<td>13</td>
<td>0.39*</td>
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<tr>
<td>Isoetes sp.</td>
<td>0.20</td>
<td>0.13</td>
<td>11</td>
<td>1.84*</td>
<td>10</td>
<td>0.01</td>
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<tr>
<td>Nitella hyalina</td>
<td>0.19</td>
<td>0.10</td>
<td>18</td>
<td>2.61**</td>
<td>18</td>
<td>0.15</td>
</tr>
<tr>
<td>Ceratophyllum demersum</td>
<td>0.19</td>
<td>0.06</td>
<td>5</td>
<td>1.73</td>
<td>4</td>
<td>0.21</td>
</tr>
<tr>
<td>Myriophyllum spicatum</td>
<td>0.19</td>
<td>0.13</td>
<td>103</td>
<td>5.56**</td>
<td>132</td>
<td>0.22**</td>
</tr>
<tr>
<td>Heteranthera dubia</td>
<td>0.18</td>
<td>0.09</td>
<td>23</td>
<td>2.95**</td>
<td>24</td>
<td>0.01</td>
</tr>
<tr>
<td>Ruppia maritima</td>
<td>0.17</td>
<td>0.14</td>
<td>11</td>
<td>1.51</td>
<td>10</td>
<td>0.05</td>
</tr>
<tr>
<td>Potamogeton cheesemanni</td>
<td>0.12</td>
<td>0.03</td>
<td>7</td>
<td>1.74*</td>
<td>6</td>
<td>0.01</td>
</tr>
<tr>
<td>Chara globularis</td>
<td>0.12</td>
<td>0.24</td>
<td>80</td>
<td>2.24*</td>
<td>88</td>
<td>0.06</td>
</tr>
<tr>
<td>Potamogeton gramineus</td>
<td>0.10</td>
<td>0.15</td>
<td>26</td>
<td>1.33</td>
<td>26</td>
<td>0.30**</td>
</tr>
<tr>
<td>Ranunculus bandooli</td>
<td>0.10</td>
<td>0.17</td>
<td>24</td>
<td>1.15</td>
<td>24</td>
<td>0.71**</td>
</tr>
<tr>
<td>Najas sp.</td>
<td>0.09</td>
<td>0.07</td>
<td>22</td>
<td>1.57</td>
<td>23</td>
<td>0.17</td>
</tr>
<tr>
<td>Myriophyllum trichophyllum</td>
<td>0.09</td>
<td>0.05</td>
<td>8</td>
<td>1.14</td>
<td>7</td>
<td>0.23</td>
</tr>
<tr>
<td>Vallisneria americana</td>
<td>0.07</td>
<td>0.07</td>
<td>32</td>
<td>1.47</td>
<td>35</td>
<td>0.27**</td>
</tr>
<tr>
<td>Elodea canadensis</td>
<td>0.03</td>
<td>0.17</td>
<td>46</td>
<td>0.58</td>
<td>49</td>
<td>0.03</td>
</tr>
<tr>
<td>Nitella sp.</td>
<td>-0.03</td>
<td>0.07</td>
<td>17</td>
<td>-0.49</td>
<td>17</td>
<td>0.05</td>
</tr>
<tr>
<td>M. spicatum/monospecific</td>
<td>-0.05</td>
<td>0.46</td>
<td>17</td>
<td>-0.34</td>
<td>16</td>
<td>0.07</td>
</tr>
<tr>
<td>Chara coralina</td>
<td>-0.06</td>
<td>0.17</td>
<td>21</td>
<td>-0.63</td>
<td>21</td>
<td>0.01</td>
</tr>
<tr>
<td>Zannichellia marina</td>
<td>-0.07</td>
<td>0.01</td>
<td>3</td>
<td>-1.00</td>
<td>2</td>
<td>0.94</td>
</tr>
<tr>
<td>Chara baltica</td>
<td>-0.07</td>
<td>0.09</td>
<td>47</td>
<td>-1.52</td>
<td>54</td>
<td>0.02</td>
</tr>
<tr>
<td>Total biomass/mixed spp.</td>
<td>-0.07</td>
<td>0.20</td>
<td>258</td>
<td>-2.76**</td>
<td>391</td>
<td>0.01</td>
</tr>
<tr>
<td>Potamogeton pectinatus</td>
<td>-0.16</td>
<td>0.16</td>
<td>119</td>
<td>-4.48**</td>
<td>150</td>
<td>0.01</td>
</tr>
<tr>
<td>Chara canescens</td>
<td>-0.17</td>
<td>0.21</td>
<td>35</td>
<td>-2.21*</td>
<td>36</td>
<td>0.08</td>
</tr>
<tr>
<td>Tolyplella sp.</td>
<td>-0.19</td>
<td>0.08</td>
<td>21</td>
<td>-3.21**</td>
<td>22</td>
<td>0.26*</td>
</tr>
<tr>
<td>Zannichellia sp.</td>
<td>-0.20</td>
<td>0.09</td>
<td>14</td>
<td>-2.49*</td>
<td>14</td>
<td>0.06</td>
</tr>
<tr>
<td>Ruppia cirrhosa</td>
<td>-0.27</td>
<td>0.15</td>
<td>91</td>
<td>-7.02**</td>
<td>111</td>
<td>0.03**</td>
</tr>
<tr>
<td>Eichhornia crassipes</td>
<td>-0.42</td>
<td>0.10</td>
<td>12</td>
<td>-4.59**</td>
<td>11</td>
<td>0.06</td>
</tr>
<tr>
<td>Zostera capricorni</td>
<td>-0.45</td>
<td>0.06</td>
<td>8</td>
<td>-5.31**</td>
<td>7</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Requisite Sample Number

Equation 4 can be used to calculate the number of replicate quadrate samples (n) that one must take to yield a given level of precision. The predicted variance can be calculated by rearranging equation 1 (analogous to equation 4):

\[
x^2 = a x^b A^c
\]

where all variables are as in equation 1. Because the standard
error (SE) is calculated

\[ \text{SE} = \sqrt{\frac{s^2}{n}} = (\bar{x}^2)^{1/2} n^{-1/2} \]

the standard error can be approximated:

\[ \text{SE} = a^{1/2} \bar{x}^{b/2} A^{c/2} n^{-1/2}. \]

If we desire a level of precision that is approximately 20% of the mean (\( p = 0.2 \) and \( \text{SE} = 0.2 \bar{x} \)), then the number of replicate quadrat samples needed can be calculated by rearranging equation 7. In the general case \( \text{SE} = \bar{x} \) and substituting into equation 7:

\[ \frac{\text{px}}{\text{px} - \text{SE}} = \frac{a^{1/2} \bar{x}^{b/2} A^{c/2} n^{-1/2}}{a^{1/2} \bar{x}^{b/2} A^{c/2} n^{-1/2} + \text{SE}}. \]

By rearranging equation 8 and combining terms, we find that

\[ \frac{\text{px}}{\text{px} - \text{SE}} = \frac{a^{1/2} \bar{x}^{b/2} A^{c/2} n^{-1/2}}{a^{1/2} \bar{x}^{b/2} A^{c/2} n^{-1/2} + \text{SE}}. \]

where \( \bar{x} \) is the predicted number of samples needed to obtain a level of precision such that the standard error equals \( \bar{x} \).

For aquatic macrophytes in particular, we substitute the coefficients from equation 4 into equation 9 to yield

\[ \bar{f} = a^{1/2} \bar{x}^{b/2} A^{c/2} n^{-1/2}. \]

This equation shows that the requisite number of replicate quadrat samples (Table 5) decreases with increased standing macrophyte biomass, increased quadrat area, and decreased precision (i.e. increased \( p \)). It follows from Fig. 4 and equation 10 that assumption of the Poisson distribution for macrophyte biomass estimates would result in a lack of statistical precision, while assumption of the negative binomial distribution would result in wasted sampling effort.

The predictions of \( \bar{f} \) from equation 10 (Table 5) are made for the average of all types of macrophytes for which data were available. Table 4 shows that the biomasses of some taxa differ significantly from the overall variance relation shown in equation 4. We do not know whether these differences among species will be found consistently in all habitats. The available evidence suggests that it would be prudent to take more samples than predicted by equation 10 if a significantly positive \( t \) is shown in column 4 of Table 4. Correction for such departures can be made by multiplication of \( \bar{f} \) by the antilog (base 10) of the mean residual (column 1 of Table 4). For _Phragmites australis_, \( 10^{0.35} = 2.24 \), and therefore the data in Table 4 should be multiplied by 2.24 to yield more conservative estimates of \( \bar{f} \) for this species. Where \( r^2 \) in column 6 of Table 4 is statistically significant, \( \bar{f} \) might be better calculated as

\[ \bar{f} = a^{1/2} \bar{x}^{b/2} \bar{g}^{2/2} A^{c/2} p^{-2}. \]

### Table 5. Number of replicate samples needed for various sampler sizes and levels of aquatic macrophyte biomass in order that the SE of replicate samples average 20% of the mean standing biomass. Calculations are from equation 10.

<table>
<thead>
<tr>
<th>Sampler size (cm²)</th>
<th>Macrophyte biomass (g dry wt.·m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.2</td>
</tr>
<tr>
<td>100</td>
<td>42</td>
</tr>
<tr>
<td>316</td>
<td>35</td>
</tr>
<tr>
<td>1000</td>
<td>29</td>
</tr>
<tr>
<td>3162</td>
<td>25</td>
</tr>
<tr>
<td>10000</td>
<td>21</td>
</tr>
</tbody>
</table>

where values of \( f \) and \( g \) can be found in Table 6 and all other variables are as in equation 9. Although these corrections can be substantial in some cases (e.g. P. _praelongus_), equation 10 can probably be used without correction for most design purposes.

### Cost Efficiency

Table 5 shows that there exist many combinations of quadrat size and sample number that can yield the same level of precision for a given biomass. Decisions as to which combination to use are almost entirely based on cost (Cochran 1977, p. 84). In the estimation of total macrophyte biomass, most of the effort and cost are expended in sampling the macrophytes. We found that the time required for a diver to collect a macrophyte sample was an increasing, decelerating function of quadrat size (Fig. 5A) but was relatively insensitive to the mass macrophyte collected (Fig. 5B). Collection time was poorly fitted as an exponential or power function of quadrat size because this relationship is asymptotic. Divers spend less time per unit bottom area as quadrat area increases. The trend in the data is approximated well (\( r^2 > 0.54 \)) by the asymptotic function

\[ T = 39.95(1 - e^{-0.00539A}) \]

where \( T \) is collection time (minutes), \( A \) is the quadrat size (square centimetres), and \( e \) is the base of natural logarithms. The large amount of scatter in Fig. 5A is due primarily to differences among divers. For example, one inexperienced diver took an average of 16 min longer to collect the macrophytes from a 1-m² quadrat than a highly experienced diver, although no differences in biomass collected were found. The shape of equation 12 is accurate for a diver of average experience, and it provides predictions of average sampling cost for our aquatic macrophyte biomass estimates.

The average cost of different combinations of quadrat size and sample number can be approximated by multiplication of equations 10 and 12 (Table 7). The amount of effort needed to collect macrophyte samples yielding a given precision decreases with standing biomass and increases rapidly with size of quadrat employed. For a given precision, large quadrats (e.g. 1 m²) require over 30 times the sampling effort of small quadrats (e.g. 100 cm²). This finding would be accentuated if samples were to be sorted to species, because 100-cm² quadrat samples could often be sorted in <1 min, while 1-m² samples took several hours (M. R. Anderson and J. A. Downing, unpubl.). Other advantages of small quadrats are that they yield samples that are easier to store and transport, destroy less habitat, and are easier to ash (Wetzel 1965). It has long been suggested that small
samples yield efficient estimates of biomass and populations of other organisms (Downing 1979; Green 1979; Hanson 1930, 1934; Hasel 1938; Hendricks 1956; Justesen 1932; Pechanec and Stewart 1940; Smith 1938; Ursic and McClurkin 1959). Pringle (1984) found this to be true for sampling Irish moss off western Prince Edward Island. We found small samples to be the most efficient for macrophyte biomass research, in general.

Recommendations

The use of quadrat sampling by SCUBA avoids the possible losses using remote samplers. As long as randomization is complete, all quadrat sizes yield the same estimates of standing biomass (Table 2). Caution should be exercised in the placement of small quadrats, however, especially in dense macrophyte beds.

Analysis of the spatial variance of aquatic macrophyte biomass shows that greater replication must be used when sampling macrophyte beds of low standing biomass or when using small quadrats. The use of small quadrats can double the number of replicate samples required for a given level of precision (equation 10; Table 5). Analysis of the cost of sampling macrophyte biomass shows, however, that the use of large numbers of small quadrats can result in 30-fold savings in sampling time (Table 7). Comparison with the sampling habits of aquatic ecologists (Fig. 1 and 2) and the recommendations of handbooks and methods manuals (e.g. Lind 1979; Schwoerbel 1970; Welch 1948; Wetzel and Likens 1979; Wood 1975) suggests that many ecologists could save sampling effort or improve levels of precision by using smaller quadrats. The number of requisite samples should be estimated in advance, based on the sampling experiences of other ecologists (equation 10). Equation 10 can be applied by guessing the macrophyte biomass to be encountered in the field, or by inspection of published tables of macrophyte biomass data (e.g. Sculthorpe 1967; Wetzel 1983). In addition, equations 3 and 10 simplify preliminary sampling because they provide guidance on choice of quadrat size, and require that the preliminary sample only provide an estimate of \( x \). The use of large numbers of small-sized samples can result in much less costly macrophyte biomass estimates with high levels of precision.

The use of small quadrats results not only in reduced sampling cost but can also result in skewed biomass frequency distributions (Cain and Evans 1952; Evans 1952; Pechanec and Stewart 1940). Skewed frequency distributions can lead to bias in analyses based on the normal distribution (Schefé 1959). Data must be properly transformed to stabilize the variance, as this will often alleviate skewness and increase the probability of additivity (Bliss and Owen 1958; Snedecor and Cochran 1967; Southwood 1966; Tukey 1968). The fourth-root transformation should perform well for most macrophyte data because the general \( b \) in equation 1 is 1.57 (equation 4) (Downing 1979; Taylor 1961). In some cases, transformation to stabilize the variance will not be adequate. The performance of transformation should always be verified, as improper transformation can lead to inferential bias. When there is doubt, parallel analyses should be performed using nonparametric methods.

Acknowledgments

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References