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Review Paper

A review of methods for measuring the surface area of stream substrates

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Abstract

Surface area measurement is a common component of benthic research, especially in the quantification of chlorophyll. Multiple techniques are available and 10 are described: artificial substrates, area-specific sampling, geometric approximation, stone shape equations, foil wrapping, grids, stamps, wetted layer, particle layer, and planar area measurement. A literature search of 130 papers indicated the most common methods: using artificial substrates of known area, subsampling a specific area using a template or sampler, measuring stone dimensions and using an equation to derive area, and using the weight of foil wrapped on stones. Methods were compared using spheres of known area, smooth and rough granite stones, and plastic macrophytes. Most methods produced highly correlated measurements and accurately estimated surface area. The wetted layer method was sensitive to stone roughness and plant complexity, but may overestimate the area of complex surfaces. Replication of one method by 10 biologists indicated that individual differences in technique can affect surface area values. Factors to consider in choosing an appropriate method include ease of use, characteristics of the substrates (e.g., porosity and flexibility), fineness of scale in measuring area, and whether methods must be field-based or can include laboratory techniques.

Introduction

Most benthic organisms in freshwaters are small; these organisms include meiofauna, most species of algae and macroinvertebrates, and some fishes. For such taxa, it is often appropriate to scale surface area measurements by using a three-dimensional measurement over the surfaces of substrates, rather than considering benthic habitats as two-dimensional, flat areas. For example, it may be of interest to measure the fine-scale surface area of stones in calculating chlorophyll concentrations, the surface area of mussels as habitat for meiofauna, or the surface area of plants in assessing chironomid densities. Additionally, surface area measurements of organisms may be needed in physiological studies.

The objective of this paper is to describe and compare the various methods used to measure the surface area of objects. We first describe each of 10 potential methods, present results of a literature search on methods actually used by freshwater benthologists, compare several area measurements on a sets of spheres, stones, and macrophytes, and describe some of the advantages/disadvantages and special uses of the various methods.

Description of methods to measure surface area

Artificial substrates

Using artificial substrates provides not only repeatable and abundant substrates, but also simplifies surface area measurement because of their consistent size and generally geometric shape (reviewed by Aloi, 1990). Examples of artificial substrates include glass slides, unglazed clay tiles, bricks and pavers, and filter paper. Clay flower pots are frequently used in nutrient diffusion studies and the surface area of pots can be calculated as a set of geometric shapes.

Area-specific sampling

Sampling a portion of a substrate simplifies area measurement by incorporating area measurement into the sampling protocol, and is commonly used for determining chlorophyll *a* concentration. Area is delineated by tracing around a template and the delineated area variously brushed or scraped. Alternatively, a sampler may enclose a portion of the substrate (see Aloi, 1990 for examples of samplers). Because the sampled area is generally small, the area is more two-dimensional than three-dimensional, and the overall size and shape of the substrate may be irrelevant.

Geometric approximation

Some natural substrates are approximately the shape of simple geometric objects. Examples include cylindrical segments of sticks and logs, and the tapered cases of some caddisflies (Insecta: Trichoptera). Similarly, water-worn stones may approximate the shape of an ellipsoid, but the equations for symmetric ellipsoids are complex, and stone shape equations are generally based on spheroids (see below). Geometric approximations have also been used to estimate the surface area of aquatic plants, especially those with simple architectures (e.g., Rosine, 1955; Harrod & Hall, 1962).

Stone shape equations

This is a subset of geometric approximation in which easily measured dimensions of stones are used to estimate area. Equations are based on formulae for geometric shapes and/or are derived empirically using regression of dimensions with surface area. Measurements of mutually perpendicular axes are made with a ruler or, more accurately, with calipers. Multiplying length and width (LW; Carlsson et al., 1977) gives a crude estimate of planar area, but is based on a rectangular shape and fails to account for the area effects of stone rounding and stone shape. Uehlinger (1991) used a modification based on the area of a circle:

$$\text{Projected area} = LW(\pi/4)$$

A planar area shape equation was developed by Behmer & Hawkins (1986) to use orthogonal length, width and height (H) to estimate both planar and total surface area:

$$\text{Planar area} = 112.48 + 0.810LW - 0.356H$$

$$\text{Total surface area} = -23.50 + 0.723L^2 + 1.886W^2 + 1.744H^2$$

Coler et al. (1989) found a strong association between surface area and rock weight for crushed limestone ranging from 56 to 178 g. A different and weaker association was found for traprock, indicating that weight may be used to estimate stone area for a homogeneous set of stones, but requires evaluating a set-specific association of weight with surface area, using another method (e.g., foil weight) to determine area.

Calow (1972) used the product of the maximum length (L) and maximum perimeter (P) to estimate the area of variably sized and shaped stones.

$$\text{Surface area} = 2.22(LP).$$

This equation is nearly equivalent to the surface area of a sphere, as length×perimeter is $2r \times 2\pi r$, or $4\pi r^2$, which is the typical equation for the surface area of a sphere. The added constant of 2.22 adjusted spherical area to the more irregular stone area. Calow found large variation in his data, which was attributed to variation among stones; and suggested that the equation would need to be adjusted for other sets of stones.

Dall (1979) suggested that the area of a spheroid was an appropriate model for estimating surface area and presented an equation equivalent to spherical surface area, but which also accommodates differences in diameter within the stone; that is, differences in length, width and height:

$$\text{Surface area} = \pi/3(LW + LH + WH),$$

where L = length, W = width, and H = Height

For a sphere, $LW + LH + WH = 4r^2 + 4r^2 + 4r^2 = 12r^2$, or $r^2 = LW + LH + WH/12$; which can be put into the equation for a sphere: $4\pi r^2 = (4\pi/12)(LW + LH + WH) = \pi/3(LW + LH + WH)$. If $L = W = H$, Dall's equation is equivalent to the surface area of a sphere.

Graham et al. (1988) used this equation for a spheroid and regressed the summed products of lengths ($LW + LH + HL$) and the summed products of perimeters ($ab + bc + ca$) against the surface area of stones measured using the grid method (see below) and obtained the following equations:

Surface area $= 1.15(LW + LH + WH)$;

Surface area $= 0.098(ab + bc + ca)$, where a, b, and c are mutually perpendicular perimeters.

The slope of 1.15 is based on water-smoothed greywacke and may be different for other stone types (Graham et al., 1988). The lengths equation is preferred over the perimeters equation because lengths are more easily measured (Graham et al., 1988). The length-based equation of Graham et al. (1988) is very similar to that of Dall (1979), with forced intercepts of zero and slopes of 1.15 and 1.05 ($\approx\pi/3$), respectively.

Foil wrapping

Stones can be wrapped with aluminum foil, taking care to trim excess foil, so that there is a single layer covering the stone. Use of heavy-weight foil reduces tearing and fine, curved scissors are helpful for trimming the foil on smaller stones. Although the area of foil (= area of the stone) was originally determined by planimetry (Shelly, 1979), area is now commonly determined by weighing the foil and using the density of foil to calculate the area. Foil density needs to be determined for each roll of foil, because density may differ among rolls (Morin, 1987). Plastic wrap can be used instead of foil, and is more flexible and more resistant to tearing but care must be taken not to stretch the wrap (Doeg & Lake, 1981). An earlier, related technique entailed coating a stone with latex, slitting and pressing flat the latex mold, and cutting out and weighing a paper copy of the mold (Minshall & Minshall, 1977).

Grids

A grid system can be marked on a stone and the length of the gridlines measured with a map wheel or string, and a flattened projection of the stone constructed on paper, from which area is measured (Graham et al., 1988). A similar procedure has been used to measure the surface area of uneven oyster shells (Morales-Alamo, 1993). Parallel lines, 1 cm apart, where drawn along the length of shells, the length of the lines were measured using string to follow the topography, and the area calculated using the mathematical Trapezoidal Rule (Morales-Alamo, 1993).

Stamps

The surface area of stones can be measured by counting the number of inked stamp prints that cover the surface. The area of any remaining irregular spaces is estimated (Kovalak, 1978) or a conversion equation can be constructed from the number of stamps covering a known area, such as a ball. The stamp can be constructed from a 1 cm^2 piece of thin sponge glued onto the end of a dowel, and stamp size can be varied relative to stone size.

Wetted layer

Harrod & Hall (1962) described a method to measure the surface area of aquatic plants using the weight of the adhering layer of water. Plants were first dipped in acetone and air dried, dipped in a detergent/water solution, allowed to drip for 20 s, and weighed. The detergent acted as a wetting agent. A regression equation based on the wetted weight gain of objects of known area was used to convert solution weight to area. A variation of this approach (Cattaneo & Carignan, 1983) involved dipping plants into detergent/dye solutions, then into clear water and measuring the dye as absorbance with a spectrophotometer. Acridine orange and methylene blue were both tested and the authors suggest that other dyes would also work. Another variation used a detergent/salt solution and the measure of conductivity of the rinse water to determine area. The wetted layer technique has use beyond plants; for example, Calow (1972) used weight gain of latex molds immersed in a detergent solution to estimate stone surface area.

Particle layer

This method, like the wetted layer method, uses weight gain by the addition of a layer, but uses an adhesive and the weight gain of a monolayer of particles. This technique has long been used to measure surface areas of roots and pine needles by coating the objects with glue or adhesive, weighing the sticky object, adding a monolayer of small plastic or glass balls, and re-weighing (Thompson & Leyton, 1971). An equation relating the weight gain to area for objects of known area is used convert weight to area. Disadvantages of this technique are the trouble with working with adhesives and their solvents and the high costs of small balls (< 0.1 mm diameter).

We developed a modification of this procedure to measure the surface area of highly irregular caddisfly cases constructed from plant material. Instead of using an adhesive, we coated cases with a thin layer of petroleum jelly, which was first melted on a hotplate and then painted on with a small watercolor paintbrush. The coated object was weighed using weighing paper. Instead of small balls, we used table salt, which had a relatively uniform crystal size (mean $= 0.43$ mm). The salt was first sieved (sieve size $= 250$ μm openings) to remove fines, which otherwise also coat surfaces. Petroleum jelly coated cases were rolled in a container of salt, which formed a monolayer, and were reweighed using the same sheet of weighing paper. Coating one surface of lightweight cardboard cut to different measured sizes or a series of spheres was used to regress area with the weight of the salt layer.

Planar area

Planar area, or the two-dimensional area enclosed by an object's perimeter, is obtained by tracing the outline of the object on paper (Gíslason, 1985; McCreadie & Colbo, 1991) or on acetate (e.g., Ledger & Hildrew, 1998), and determining the traced area by planimetry or cutting out and weighing the tracing, as described above. Alternatively, substrates can be scanned with a flatbed scanner (with a sheet of clear plastic beneath stones to protect the scanner) and the images either printed or weighed, or the area determined by image analysis (e.g., using SigmaScan, SYSTAT Software, Inc., Chicago, Illinois, to count the number of contained pixels). Simultaneous scanning of a circle or square of known area allows correction for changes in image size. Gregg and Rose (1982) used a similar method to estimate the surface area of macrophytes. Photocopies of plants were cut out and weighed; but the resulting weight-based two-dimensional areas were converted to three-dimensions by multiplying by 2 for leaf areas and by π for stem areas.

Planar area is also used for cores and other enclosed samplers. Sand and other fines are sampled using corers that vary from simple Petri dishes to freeze corers. Gravels within a confining sampler can be similarly collected. A further step involves collecting stones that fill the bottom of a container and using the area of the container.

Literature survey of methods

Methods in use for measuring surface area were surveyed with a literature search of studies quantifying benthic chlorophyll, the most frequent use of surface area measurement in freshwater biology. Papers were selected via the Biological Abstracts database, using the search of 'river* or stream*, and chlorophyll' for the years 1992–2004. Marine, estuarine and lake research were excluded, as were papers that were not readily accessible in the library at the University of Oklahoma or on the internet. The search resulted in a pool of 130 papers from 22 journals (list available by request from EAB), with over half of the papers from three journals: Freshwater Biology (32 papers); the Journal of the North American Benthological Society (23 papers); and Hydrobiologia (22 papers). The papers measured the chlorophyll concentration of periphyton on stones (70 papers), artificial substrates (56 papers), fine sediments (12 papers), nutrient-diffusing substrates (9 papers), or macrophytes (1 paper). Several papers included more than one substrate and more than one method of surface area measurement.

Seven of the 10 surface area methods considered in this review were used among the 130 papers

(Table 1). Grids and stamps were not used, and the two methods described for use in this paper were either not used (particle layer) or were used only for macrophytes (wetted layer). Twelve papers did not include the method used for measuring surface area.

Artificial substrates were the most common ‘method’ and were often unglazed clay or ceramic tiles, but also included glass or plastic microscope slides, styrofoam, bricks and pavers, ceramic eggs, wooden dowels or blocks, and plastic strips or other plant-mimics. Most often, the entire surface was sampled, but substrates were sometimes subsampled by delineating part of the substrate (e.g., ¼ of a tile) or by using a sampler (e.g., coring styrofoam).

Several methods were used for area measurement in stones. Most commonly, a specific area was sampled. Area-specific measurements were built into the sampling protocol by scrubbing or scraping algae within an area delineated with a template (e.g., a bottle cap or a plastic slide frame) or by using a sampler that isolated part of the stone. Foil wrapping was also frequently used and included the variants of wrapping with plastic wrap or paper. Although wrap density was generally used for conversion to area, image analysis, leaf area meters, and planimeters were occasionally employed. Few papers mentioned trimming excess foil, but this detail may have been left out in the manuscripts.

Table 1. Distribution of surface area methods among 130 papers describing benthic chlorophyll concentration. Methods are described in the text

Method	All papers ($n=130$)	Stone sampling ($n=70$)
Artificial substrates	56	
Area-specific sampling	31	25
Geometric approximation	4	0
Stone shape equations	8	8
Foil wrapping	20	20
Grids	0	0
Stamps	0	0
Wetted layer	1	0
Particle layer	0	0
Planar area: cores	17	8
Planar area: tracings	8	8
Not specified	12	8

Planar methods were used for both stones and finer sediments. Stones were traced onto paper or acetate and the weights of the cut-out areas were converted to planar area. Sand was cored in all 12 studies including fine sediments, and large corers were occasionally used for gravel. Corers or trays were also used to delineate an area of stones and the contained stones were scrubbed or brushed.

Stone area was calculated from equations using measurements of two axes (1 paper) or three axes (6 papers); an eighth paper used measurements but lacked further detail. In contrast, geometric approximations were not used for determining stone area. Few natural substrates form common geometric shapes; hence, area was rarely determined directly from shape. The exceptions were subunits of macrophytes, the regular case of a caddisfly, and segments of wood.

Although researchers typically measured the entire surface area of stones (e.g., by foil wrapping and stone equations), the area exposed on the streambed or the area covered by algae were sometimes of interest. Partial areas were obtained by wrapping foil over only the upper surface of stones (1 paper) or over the algal-covered area, which was often a different color from clean surfaces (1 paper). Alternatively, estimates of the entire surface area were mathematically corrected for exposed area (50% of total area: Uehlinger, 1991; Steinman & Lamberti, 1996; 2 papers) or algal-covered area (approximately 64% of total area: Biggs & Close, 1989; 5 papers).

Comparative measurement of area

Methods were first evaluated against the surface area of spheres, which are easily and accurately measured, then compared among each other using the area of smooth stones, rough stones and imitation macrophytes. The six spheres consisted of a glass marble and plastic balls that ranged in diameter from 1.55 and 8.38 cm. The diameter of each sphere was measured with digital calipers to determine actual surface area, using the formula: surface area $= 4\pi r^2$. The following techniques were then applied to each sphere: foil wrapping, grids, stamps, wetted layer (weight gain with a detergent solution), and particle layer (using petroleum jelly and salt). Additionally, sphere diameters were used to calculate areas using equations of Calow (1972), Dall (1979), and Graham et al. (1988). Measured and calculated areas were regressed against actual surface area (Table 2).

All techniques accurately measured the surface area of spheres. Foil-wrapping, grids, wetted layer, and particle layer techniques all had regression R^2 values greater than 0.98. These four methods do not provide area directly, but weights were converted to area using the density of the layer of foil, grid projection paper, solution, or particles. Density determination for the wetted and particle layer methods required measurement of a coated surface. Spheres, cubes, and other geometric shapes have been used to calibrate methods (e.g., Dall, 1979; Morin, 1987; Coler et al., 1989). Hence, spheres were not only used to evaluate the accuracy of methods, but to develop equations to convert measured weights to area (Table 2). A specific area of foil can be weighed to determine density; however, because foil wrinkles during wrapping, using the density of flat foil rather than the density of wrapped foil may overestimate area (see below). Direct conversion of stamps to surface area underestimated area because of the unmeasured areas among stamps. Regression of the number of stamps and surface area, however, provided a good conversion to area ($R^2 = 0.97$).

Dall's (1979) equation predicted actual surface area because the equation is equivalent to that for spherical area. Graham et al. (1988) and Calow's (1972) equations are also based on the area of spheres and consequently were highly correlated with actual area, producing an R^2 of 1.00. However, the difference in the constants used among the equations resulted in different areas. For example, measured areas for the largest ball, which had an actual surface area of 220.6 cm^2, were 221, 242, and 489 cm^2, using the respective equations of Dall (1979), Graham et al. (1988), and Calow (1972). The constant of 2.22 in Calow's (1972) equation clearly overestimated the surface area of spheres.

Evaluating the accuracy of the methods in measuring the surface area of stones was problematic because the true area was unknown. Correlation analysis and comparisons among means were used to for comparison. Twelve water-worn granite stones, ranging from 17.8 to 379.8 g and 3.1–10.3 cm maximum length were collected from the bank of the Cimarron River (Cimarron County, Oklahoma). Stones were measured using the same methods used for spheres. Correlations among methods were high, ranging from 1.00 for Dall (1979) and Graham et al. (1988) equations and the two methods to determine foil weight (because both are based on adjustments of the same foil weights) to 0.956 for the correlation between the Dall/Graham et al. equations and wetted layer method.

High correlations among methods equated with a high similarity of area measurements for most methods (Fig. 1). The exception was Calow's equation, which overestimated surface area by approximately the 2.22 constant in the equation.

Table 2. Comparison of measured surface areas and actual surface areas of six spheres, including regression coefficients and regression equations, where SA is area derived using each method and B is the actual surface area of the sphere

Method	R^2	Regression equation	Conversion equation
Calow's	1.00	$SA = 0.138 + 2.223*B$	
Dall's	1.00	$SA = 1.003*B$	
Graham's	1.00	$SA = 1.098*B$	
Foil wrapping	0.999	$SA = 0.015 + 0.007*B$	SA = 2.33 + 134.41*wt or SA = 158.06*wt
Grids	1.00	$SA = 0.377 + 0.962*B$	SA = −0.384 + 1.039*wt
Stamps	0.972	$SA = -6.581 + 0.832*B$	SA = 9.355 + 1.175*count
Wetted layer	0.982	$SA = -0.013 + 0.003*B$	SA = 5.55 + 312.17*wt
Particle layer	0.998	$SA = -0.228 + 0.053*B$	SA = 1.13 + 21.7*wt

Conversion equations are given for methods in which weight change or number of stamps must be converted to area. The two conversion equations for foil wrapping are based on wrapped-foil density (longer equation) and flat-foil density (shorter equation). Equations will vary with different densities of materials and, possibly, with different operators.

Among the other area determinations, the foil wrapping method that used simple foil density for conversion produced consistently larger surface areas than using the density of foil wrapped around spheres as a conversion (paired *t*-test: $t_{11} = 4.38$, $p = 0.0011$). This difference is procedural. Wrapping slightly wrinkles the foil, such that a density conversion based on flat foil overestimates area.

Possible effects of substrate size and texture on area measurement were tested by (1) comparing area measurements in the smallest and largest measured smooth granite stones and (2) comparing the area measurements of similarly sized smooth and rough granite stones. Areas of the smallest, pebble-sized and largest, cobble-sized stones varied similarly across the measurement techniques (Fig. 1); that is, area was overestimated by foil wrapping based on using foil density for conversion to area and was underestimated by uncorrected stamping on both sizes of stones. Variation in measurement was greater among large than small stones (standard deviation of 4.92 and 1.17), but this difference scaled with stone size (respective coefficient of variation of 9.20 and 16.85).

Rough, weathered stones were obtained from a granite outcrop in Comanche County, Oklahoma. Two stones of similar size were measured and areas obtained from each method were averaged. One of the smooth granite stones was similarly sized and data from this stone were used for comparison. In comparison to the smooth stone, the grid and stamp methods underestimated the area of rough stones, whereas wetted layer overestimated area (Fig. 2). The string used to measure gridlines in the grid methods tended to go into crevices, thereby reducing the estimated area. In stamping, numerous peaks affected the clarity of stamped marks and resulted in increased error. Wetted layer overestimated area because of capillary retention of liquid in crevices; in contrast, most other methods underestimated area because area was based on maximum dimensions rather than actual contours.

The complex architecture of macrophytes restricts the number of appropriate surface area methods because, for example, shape equations based on ellipsoids are not applicable. Three methods were tested on macrophytes, using plastic plant imitations of *Vallisneria* (eelgrass), *Ludwigia* (false loosestrife) and *Myriophyllum* (water milfoil), which have increasingly complex morphologies. Wetted and particle layer methods were done on entire plants, whereas conversion of scanned images to area (Gregg & Rose, 1982) required dismantling the plants. Particle layer and scanned image techniques produced similar areas

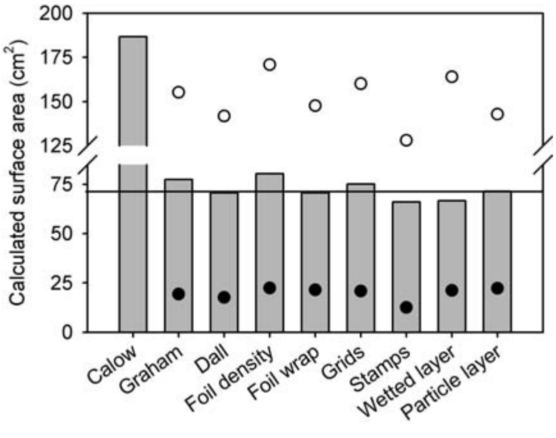

Figure 1. Mean calculated surface areas of 12 water-worn granite stones, as measured using stone measurement and stone-area equations (Calow, Graham, and Dall), foil wrapping, using simple foil density and also foil wrapping on spheres for converting weight to area, grids, stamps, and wetted layer and particle layer techniques. The average of all estimates, exclusive of that derived from Calow's method, is shown with a horizontal line. Solid circles show area measurements of the smallest stone; hollow circles show area of the largest stone.

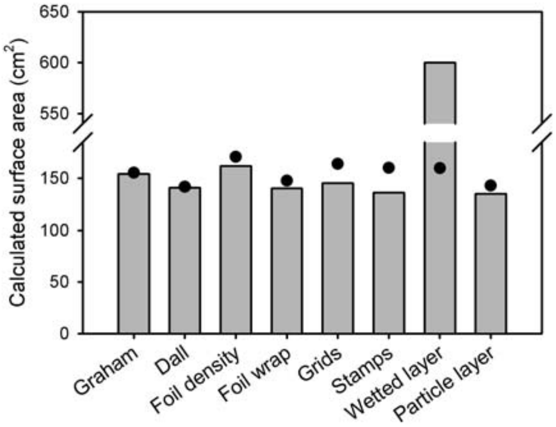

Figure 2. Mean surface area of two rough, weathered granite stones, calculated using the same methods as in Figure 1, with the deletion of Calow's equation. Mean area across methods is shown by a horizontal line. Solid circles are comparative area measurements of a similarly sized smooth granite stone.

for all three morphologies (Fig. 3). In contrast, area derived from the wetted layer method was comparatively small for plastic *Vallisneria* and increased with greater morphological complexity.

Methods for determining the planar area of individual stones were compared by having 10 field biologists trace each of three stones (two of sandstone; one of travertine) onto paper placed beneath the stones and onto plastic transparency sheets placed on top of stones. Images were cut out, weighed, and the areas determined using the density of the respective paper and plastic. There was no significant difference between using paper and plastic tracings to determine area (ANOVA: $F_{1,54} = 0.003$, $p = 0.957$). The scanning technique was not replicated because this technique does not involve hand-tracing and is more objective. Results with scanning were consistent with the two tracing methods; for example, the irregular travertine stone averaged areas of 16.9, 17.2, and 17.4 cm^2 for paper tracing, plastic tracing, and scanning, respectively.

Individual differences in measuring surface area were indicated by the range in values for a planar tracing technique among the 10 researchers (Fig. 4). Standard deviation increased with the means and averaged 12.7% of the mean for paper tracings and 14.8% of the mean for plastic tracings. Use of the more objective scanning method or having all measurements taken by the same person would reduce this undesirable variation.

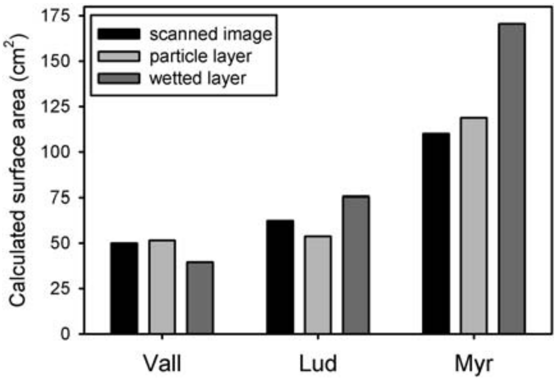

Figure 3. Mean surface area of two replicates of various plastic macrophytes. *Vallisneria* ('Vall') had long, linear blades, *Ludwigia* ('Lud') had ovate, entire, petioled leaves, and *Myriophyllum* ('Myr') had whorls of pinnately divided leaves.

Method selection

All of the methods are useful for stones and their artificial substrate mimics and, indeed, many methods were developed specifically for measuring stones. But not all methods are equally usable (Table 3). In studies that include field sampling of periphyton or stone-dwelling invertebrates, field-based surface area methods may be desired, and include specific area sampling, or sampling the entire stone and either taking measurements and applying stone shape equations or using the planar method of tracing the stone outlines. If stones can be brought to the lab, additional methods that require weighing or other techniques can be used: wetted layer, particle layer, foil wrapping, and planar area by scanning. Measuring the area actually covered by algae or other selected areas can be done with foil wrapping or particle layer techniques.

Measuring the area of non-stone substrates provides greater challenges because of surfaces that may be highly irregular, flexible, and/or porous. The wetted layer method works well for flexible and highly irregular substrates such as macrophytes, but would work poorly for porous wood, for which foil wrapping and particle layer techniques are more appropriate.

Other considerations are the ease of measurement, observer variation, and the scale of measurement. Relative ease and the time needed for measurement vary among methods (Table 3), and

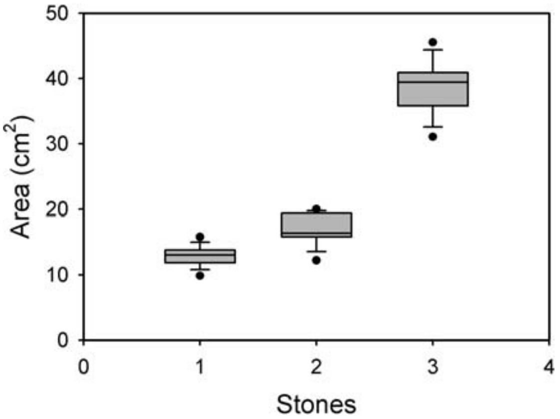

Figure 4. Box plot of planar area for three stones showing the variation among estimates resulting from stone tracings by 10 individuals.

Table 3. Usability of surface area measurement techniques

Method	Artificial substrates	Specific area	Geometric equations	Stone shape equations	Foil wrapping	Grids and Stamps	Wetted layer	Particle layer	Planar area
Object types	mimic all	S, M, O	S, M	S	S	S	S, M, O	S, O	S, M, O
Ease and time	✔✔	✔✔	✔	✔✔	X	X	✔✔	X	✔
Appropriate for field	✔✔	✔✔	✔	✔✔	X[a]	X[a]	X[a]	X	✔[a]
Requires lab work					✔✔	✔✔	✔✔	✔✔	✔✔
Fine-scale measurement			X	X	✔	X	✔✔	✔✔	X
Very irregular objects		X	X	X	X	X	✔✔	✔✔	✔
Partial area		✔✔	X	X	✔✔	✔ (Stamps)	X	✔✔	
Porous objects		✔		✔	✔✔	✔✔	X	✔✔	✔✔

S = stones; M = macrophytes; O = other; ✔✔ = very appropriate; ✔ = usable; X = not recommended; blank = not applicable.

[a] These methods require lab work but can be partially completed in the field. Foil wrapping in the field may require that the wrapping be rinsed and dried prior to weighing (e.g., Doeg & Lake, 1981), the colorimetric methods of wetted layer may be partly field adapted, and field tracings for planar area can later be weighed in the lab.

are also a function of the number of samples. Foil wrapping is commonly used, but is time intensive in requiring careful wrapping and trimming (McCreadie & Colbo, 1991), tracking of specific pieces of foil, weighing, and calculation of roll-specific conversion formulae (preferably based on wrappings of geometric objects rather than simple density). Although time-consuming and tedious for a large number of samples, once the conversion is determined, foil wrapping may be an efficient method for small sample sizes. Grid and stamping methods are also time-consuming. The particle layer technique is faster than foil wrapping, but produces petroleum jelly and salt covered objects, which may need to be cleaned (e.g., mussel shells that will be incorporated into a research collection). At the other end of the spectrum, measurements for area equations and the wetted layer technique are both very fast and can be used to process large numbers of samples quickly, though the wetted layer method requires development of a conversion formula for each batch of soap solution. Although not specifically tested in this review, many of the methods are liable to variation among measurers (e.g., the planar tracing technique, above), hence it is suggested that all area measurements in a study be made by a single person.

The scale of measurement determines whether surface texture contributes to surface area measurement. Surface texture contributes habitat space and refuges for small organisms (Bergey, 2005). Surface area methods range from coarse-scale stone equations, in which stones are considered as ellipsoids, to fine-scale particle and wetted layer methods in which the area measured closely follows the contours of objects. The wetted layer method, especially, produced higher surface areas as roughness or morphological complexity increased; but this method may also overestimate area on non-smooth substrates because of fluid retention in cracks or where surfaces, such as macrophyte leaves, join together.

Combining surface area methods in a single analysis may result in bias (e.g., comparing clay tiles and stones, using geometric equations and foil wrapping, respectively, may result in a spurious, consistent difference among the two sets of samples). Similarly, if samples consist of stones with different surface textures, a fine-scale method may be more appropriate than a coarse-scale method because a coarse-scale method may not adequately measure the fine surface irregularities of the rougher stones.

In summary, there are a variety of good methods available for surface area determination and the choice of method depends on the characteristics of the sampled substrates, the design of the project, and the preference of the researchers.

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