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A method for determining the surface area of corals

Ove Hoegh-Guldberg

Department of Biology, University of California, Los Angeles, California 90024, USA

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Abstract. Although there are several techniques available that can accurately determine the surface area of simple branching corals, there is an absence of techniques that can be applied to finely branching species like *Pocillopora damicornis*. This paper describes a rapid, spectrophotometer-based technique that can accurately determine the surface area of a range of coral species, including *P. damicornis*. The technique involves dipping corals coated with plastic varnish into a solution that contains a small amount of detergent and the dye Methylene Blue. The amount of dye solution clinging to the surfaces (as a thin-layer) is proportional to the total surface area.

Introduction

Surface area is an important variable in biology. In studies involving corals, surface area is used estimate the sizes of individual corals in order to standardize variables such as chlorophyll a and tissue protein, and physiological rates such as respiration and excretion. There have been several approaches to the problem of measuring the complex surfaces of corals. Kanwisher and Wainwright (1967) used the planar projection of corals (usually measured from photographs taken directly above coral colonies) as a measure of their surface area. However, this approach is unsatisfactory for estimates of total surface area. The aluminium foil wrapping technique of Marsh (1970) has been used by the greatest number of coral physiologists (Burris et al. 1983; Crossland 1987; Davies 1980; Hawkins and Lewis 1982; Muscatine et al. 1984; Porter et al. 1984; Peters and Pilson 1985; Szmant-Froelich and Pilson 1980), and is accurate and easily applied to simple coral surfaces. The amount of foil wrapped over a skeleton is quantified by either weighing the pieces after they are removed and converting the resulting weight into an area by using a measure of foil area per unit weight, or by directly measuring surface area of the pieces after flattening them. Meyers and Schultz (1985) used a similar approach, substituting a thin coat of latex rubber for foil. Although these two techniques have produced rapid and reliable estimates of the area of simple coral surfaces, their applicability to the complex topography of finely branching species like *Pocillopora damicornis* remains to be assessed. A method employing a dye was briefly described by McCloskey and Muscatine (1984). This method uses the amount of dye being absorbed directly onto coral skeletons as a measure of their surface area. Some problems were experienced with this method due to the irreversible absorption of dye onto the coral skeletons. Solutions to this particular problem along with details of the technique are soon to be described (McCloskey personal communication).

In this paper I describe a rapid field technique that accurately determines the surface area of corals. This method also uses a dye. It can be successfully applied to the simplest or the most complex coral skeletons. The method described here is an extension of the thin-film technique originally suggested by Weiser (1951) to measure the surface areas of plants, and later refined by Harrod and Hall (1962). I modify this method by sealing the surfaces concerned with a common plastic varnish and by employing a dye to quantify the volume of liquid adhering to the surfaces in question. These modifications are advantageous for several reasons. Sealing the surfaces eliminates the problem of different surface properties influencing the amount of fluid clinging to a surface (Harrod and Hall 1962) and hence allows a direct comparison between objects having different surface properties. Using a dye to quantify the amount of solution clinging to a surface also eliminates the important problem of obtaining accurate weight measurements (part of the procedure used by Harrod and Hall 1962) on heavy objects like coral skeletons. Dye samples resulting from the application of this technique also have the advantage that they can be prepared with relatively simple equipment in the field and can be transported to the laboratory for analysis at a later time.

Although this technique is used here to measure surface areas of coral skeletons, it is applicable to other types

of surfaces. A preliminary study has shown that this technique will also produce accurate measurements of the area of surfaces as highly diverticulated as the juniper plant *Juniper californicus*, (Swift and Hoegh-Guldberg unpublished).

Methods

Using a Water-Pik (Johannes and Wiebe 1970), coral tissue was removed from skeletons of three species of coral (*Stylophora pistillata, Seriatopora hystrix* and *Pocillopora damicornis*). After air-drying, the skeletons were weighed on a Sartorius top loading balance and a small length of fine copper wire was tied around the base of each coral skeleton. The skeletons were then dipped in "Varathane: Clear Gloss" (The Flecto Co., Oakland, Calif; USA). This was repeated up to five times, letting the skeletons dry between coats. The total thickness of five coats ranged between 0.15 and 0.30 mm. An aqueous solution (400 ml) was prepared, containing a small amount of detergent (0.10% Triton X100, w/v) and approximately 0.4 g of the non-toxic dye Methylene Blue (Merck Index 1968). The concentration of the dye solution is not critical and will depend on the size of the specimens, the volume of water used to elute the dye and the sensitivity of the spectrophotometer (see below). The dye solution was filtered (Whatman filter paper no. 42) to remove any particles.

The dry plastic-coated corals were dipped in the dye/detergent solution and the excess solution removed by rapidly accelerating the coral specimens at arms length to an abrupt stop (=one "shake") a number of times within 15 s. The solution remaining on the surface of the specimen was washed off by immersing it into a known volume of water. Samples of the solution containing the eluted dye were placed in glass scintillation vials and transported back to the laboratory where their absorbances at 620 nm were measured on a Beckman spectrophotometer (Model 25). The optimal number of shakes was investigated by determining how the amount of dye solution clinging to the surface varied with the number of shakes. In order to determine the relationship between the amount of fluid clinging to a surface and its area, pieces of Varathane-coated graph paper of different sizes were substituted for corals in the above procedure.

The results of the "Varathane" technique were verified for *S. pistillata* and *S. hystrix* in two ways. First, a number of corals were measured using Marsh's (1970) technique before being subjected to the Varathane technique. The results of the two techniques for the same set of corals were compared. A second check was done using small plastic models of defined geometry (composites of clindrical and flat pieces of lucite). The surface area measured using the Varathane technique was compared to that expected from the geometry of the objects and to the results of Marsh's (1970) technique, which was also applied to the objects.

Verifying the accuracy of the Varathane technique for *P. damicornis* proved difficult because it was not possible to measure the complex surface area of *P. damicornis* using another technique. Surface area, however, will scale with weight for objects of similar specific density and geometry. Examining this relationship and comparing it to that determined for *S. pistillata* and *S. hystrix* provided an indirect way of verifying the accuracy of the Varathane technique for *P. damicornis*.

Results

The amount of solution clinging to a surface decreased with increasing number of shakes ($n=2$, Fig. 1). The greatest decrease however, occurred between 0 and 3 shakes and there was little change after 6 or more shakes. The ratio of the absorbance values for the two corals used in this test was 0.609 when the corals were not shaken and was 0.295 when they were shaken between six and 18 times. The ratio of their surface areas as determined by the technique of Marsh (1970) was 0.316. To reduce pos-

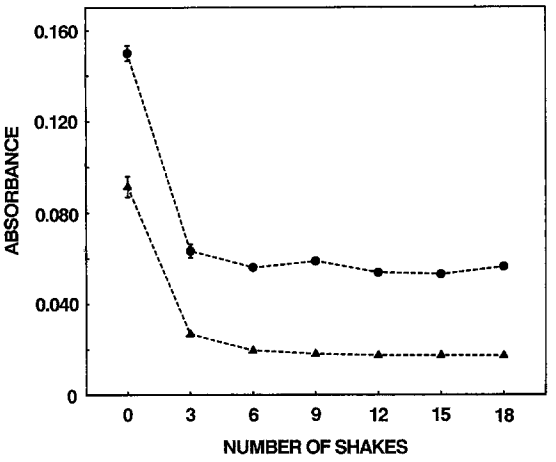

Fig. 1. The effect of shaking on the amount of dye solution adhering to (●) a piece of *Stylophora pistillata* and (▲) a piece of *Seriatopora hystrix*. Each point represents the mean of three replicate washes. Bars indicate standard errors of the mean

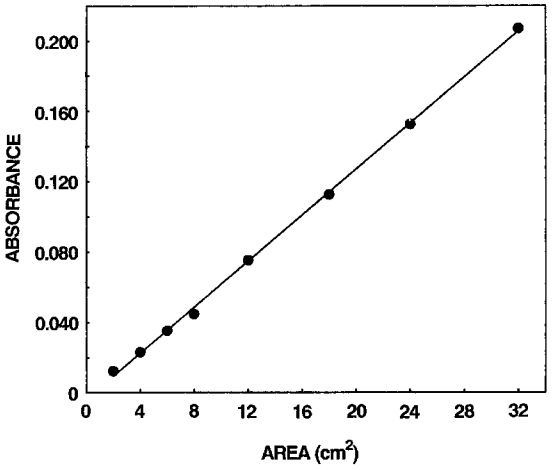

Fig. 2. Absorbance of solutions resulting from application of the technique to pieces of Varathane covered graph paper of different sizes. One of three trials is shown. Each point represents the mean of 5 replicates. Regression line is shown: $y=(6.536(x)-3.716)\,x10^{-3}$, $r^2=0.994$

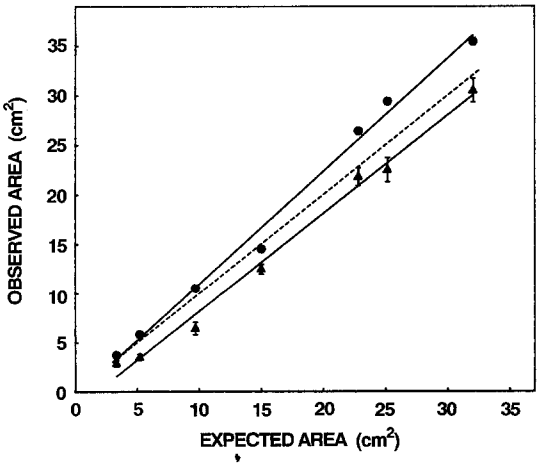

Fig. 3. Comparison of the foil technique ($n=2$, ●, Marsh 1970) and the Varathane technique ($n=5$, ▲) for determining the surface areas of 7 lucite models. The dashed line indicates surface area expected from the geometry of the models. Regression equations are: (●), $y=1.139(x)-0.313$, $r^2=0.991$; (▲), $y=0.982(x)-1.603$, $r^2=0.985$

Table 1. Coefficients of determination (r^2) and ratios of estimates from the Varathane technique (SA_{vara}) and Marsh's (1970) technique (SA_{foil}) as a function of different numbers of coats of Varathane applied to the skeletons of two coral species

Species	Numbers of coats	r^2	Ratio (SA_{vara}/SA_{foil})
S. pistillata	1	0.388	1.653
	3	0.631	1.372
	5	0.934	0.966
S. hystrix	1	0.590	1.643
	3	0.637	0.992
	5	0.865	0.870

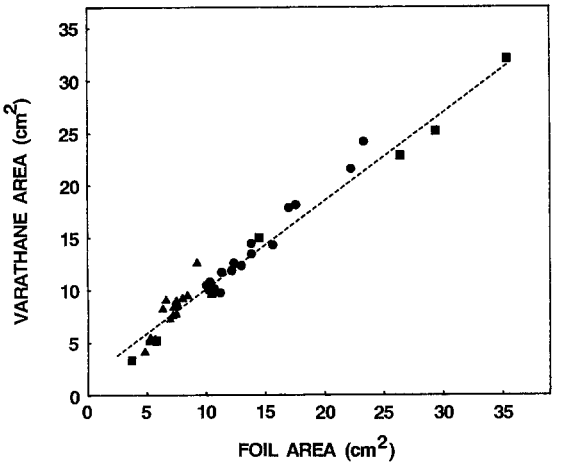

Fig. 4. Comparison between surface areas measured using the Varathane technique (Varathane area) and the foil technique (Foil area) for pieces of *Stylophora pistillata* (●), and *Seriatopora hystrix* (▲) and lucite models (■). All points represent the mean of five replicates. Regression line for all three data sets is shown: $y=0.840(x)+1.689$, $r^2=0.964$

sible errors related to shaking, objects were subjected to 12 shakes within 15 s for all other surface area determinations.

The amount of dye eluted from Varathane-coated graph paper was directly related to the surface area in contact with the dye solution (three trials; $r^2=0.994$, 0.983 and 0.999; Fig. 2). Similarly, the amount of dye eluted from the surface of lucite models was directly related to their surface area ($r^2=0.985$, Fig. 3). The data obtained from the Varathane-coated graph paper were used to convert the absorbance values to estimates of surface area. Surface areas estimated using this technique were slightly though consistently lower than those calculated from the dimensions of the objects. Surface areas measured using the technique of Marsh (1970) were, on the other hand, slightly higher than those predicted from their geometry (Fig. 3).

The quantity of dye eluted from the surfaces of *S. pistillata* and *S. hystrix* was influenced by the number of coats of Varathane applied. The correlation between dye elution and surface area was small for pieces of coral coated only once ($r^2=0.388$ and 0.590 for *S. pistillata* and *S. hystrix* respectively, Table 1), but increased as the number of coats increased. The best results were obtained with five coats ($r^2=0.934$, *S. pistillata*; $r^2=0.865$, *S. hystrix*; Table 1). Inspection of the surfaces between coats revealed that whereas five coats were required to create a smooth surface on the exterior of *S. pistillata* skeletons, and that three coats were sufficient to fill in the calices of *S. hystrix*. Combining the data collected for the models and the two coral species after five coats revealed a strong correlation between the results of the new method and that of Marsh (1970, Fig. 4; $y=0.840(x)+1.689$, $r^2=0.964$, $P<0.001$). The average coefficient of variation was $9.05\pm3.772\%$ ($\pm$SE, $n=30$) for surface areas determined using the Varathane technique, as compared with $4.566\pm3.329\%$ ($\pm$SE, $n=30$) for those determined using the aluminium foil technique.

It took 5 h to measure the surface area of 37 waterpiked and air-dried coral skeletons, indicating an average of 8.1 min per specimen. The absorbance of samples measured immediately did not differ from the absorbance of the same samples kept in glass scintillation vials in the dark for 4 weeks.

Surface area and skeleton weight were strongly correlated in all three species ($r^2=0.973$, 0.879 and 0.805 for *S. pistillata, S. hystrix* and *P. damicornis* respectively). The surface area to weight ratio was highest in *S. hystrix* ($5.847\pm0.296\ cm^2\cdot{}^{-1}$; $\pm$SE, $n=15$), followed by *P. damicornis* (3.492 ± 0.271, $\pm$SE, $n=8$) and then *S. pistillata* (2.871 ± 0.074, $\pm$SE, $n=24$).

Discussion

The technique described in this paper accurately determines the surface area of a series of objects that had a wide range of shapes. It is rapid and inexpensive. Due to the stability of aqueous solutions of Methylene Blue, samples can be prepared under field conditions and absorbances measured at a later time in the laboratory.

The quantity of dye solution clinging to the surfaces investigated was influenced by the number of times the object was shaken to remove surplus dye. When objects are not shaken, absorbance values do not yield accurate surface areas. However, when objects are shaken six or more times, absorbance values correlated well with the amount of surface area. The objects in this study were shaken 12 times, which was perhaps overly cautious given the results of Fig. 1. It is instructive to point out however, that the greater the number of shakes the less likely that differences in the vigour of shakes will influence the results.

The surface "micro-topography" of the objects had an influence on the surface areas calculated. Between three and five coats of Varathane were needed to reduce the micro-topography due to the empty calices of *S. pistillata* and *S. hystrix*. After filling the calices of these corals, the amount of unexplained variance decreased significantly, possibly due to a reduction in errors due to the wicking of dye solution into the fine cracks and

crevices of an untreated surface. Five coats smoothed the surface of the corals and estimates of surface area from the Varathane technique compared well with those done using the technique of Marsh (1970). The Varathane technique, therefore, does not estimate the absolute surface area of the skeleton (i.e. that immediately under the bottom or calcifying epidermis) but rather is an estimate of the "primary" surface area occupied by the coral polyps. This latter measurement, however, is a useful index of coral colony size and has been used extensively by coral physiologists and ecologists (Burris et al. 1983; Crossland 1987; Davies 1980; Hawkins and Lewis 1982; Muscatine et al. 1984; Porter et al. 1984; Peters and Pilson 1985; Szmant-Froelich and Pilson 1980).

The thickness of five coats of Varathane was small relative to the thickness of the coral branches, and consequently, increases in total surface area due to the addition of Varathane to the surfaces was minimal. On average, foil-based measurements were 19% higher than those resulting from the Varathane technique. Some of this error is due to the foil technique, where folding foil over rounded surfaces will lead inadvertently to overlapping folds, and consequently, to elevated estimates of surface area. However, as was apparent from using the two techniques to determine the surface area of lucite models, measurements done using the Varathane technique were also consistently lower than that expected from the geometry of the models. It is suspected that some of this error is due to the use of flat graph paper standards which may hold slightly less dye solution per surface area than objects having a more complex topography. Consequently, it is probably better to use a series of small cylinders to standardize this technique to surface area.

It was not possible to independently measure the surface area of *P. damicornis* and thereby verify the accuracy of the surface areas calculated for this species. However, the surface areas calculated for *P. damicornis* correlated with skeletal weight, and the ratio of surface area to weight was similar to values of the same ratio measured for *S. pistillata* and *S. hystrix*. This suggests that the Varathane method can accurately measure the surface areas of a morphologically complex species like *P. damicornis*, as well as the surface areas of species with simpler morphologies like *S. pistillata* and *S. hystrix*.

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