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PHOTOGRAPHIC TECHNIQUE FOR THE STUDY OF CORAL MORPHOMETRICS

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ABSTRACT. – Photographic imagery offers the study of coral morphometrics four principal advantages: speed of sampling, non-destructiveness, an instant permanent record and the opportunity for repeat studies of the same polyps and colony. In this paper, these advantages are weighed against the reduced number of polyp characters that can be accurately measured and the limited number of corals to which the technique is applicable. We were able to measure eight coral traits from images of *Favia speciosa*, a coral with large circular polyps. Taking readings from slides projected onto paper at 10× actual size proved to be more effective than using computer based image analysis. Corals with small or poorly defined polyps, such as most branching, foliaceous, columnar and laminar species, are not suitable for this approach. The speed of sampling equates to less time in the field, and as the technique is non-destructive, sample size is limited only by the number of colonies present at a site. The technique can identify intraspecific morphological variation in corals with well-defined polyps and could be used, for example, for research into phenotypic plasticity.

KEY WORDS. – Photographic technique, small-scale morphology, *Favia speciosa*, Singapore.

INTRODUCTION

Intraspecific variation in the morphology of scleractinian corals has long been recognised (e.g. Quelch, 1886; Stephenson & Stephenson, 1933; Yonge, 1936; Wijsman-Best, 1974). This variety can sometimes be correlated with the organisms' environment, as a consequence of a specialisation or a plastic response (Bradshaw, 1965; Foster, 1979), or it may simply be attributed to stochastic differences between genotypes. Recording this taxonomically important variation involves close examination of coral morphological features from samples taken over a range of habitats.

As many characters can only be measured from tissue-free coral skeletons, the majority of morphometric studies have required colonies, or segments of colonies, to be taken from their natural habitat for treatment and analysis (Dustan, 1975; Foster, 1977, 1979; Dodge, 1982; Beltran-Torres & Carricart-Ganivet, 1993; Amaral, 1994). Treatment usually entails soaking the samples in bleach to clean away the coral tissue before inspection under a microscope. Sometimes the number of colonies necessary is large, with up to 72 removed for one study (Beltran-Torres & Carricart-Ganivet, 1993). Even breaking off a small section of coral can be deleterious to the colony as distribution of metabolites is disrupted and invasion by disease, bacteria or algae is encouraged (Talge, 1992). Sometimes data can only be collected through the sacrifice of specimens, as is the case for most taxonomic work. However, this approach may not always be necessary for studies of phenotypic variation and plasticity. As the growth and recruitment rates of scleractinian corals are slow (Barnes, 1982), it is clear that non-destructive methods should be explored when possible.

In the early 1960s *in situ* coral research became possible through the availability of SCUBA equipment. Since then photography has been widely used in the study of coral communities with photo-quadrats and, more recently video transects, now frequently employed by reef ecologists (Done, 1981; Foster et al., 1991; Carleton & Done, 1995). Photography also provides a relatively cheap and easy way of gathering information at the colony level. Simple size comparisons between colonies can be achieved with photographs or with video stills as demonstrated by West et

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al. (1993) in their study of gorgonian plasticity. Gross morphology of *Montastrea annularis* was examined by Graus & Macintyre (1982) using 20 cm x 25 cm photographic plates. Furthermore, as photography does not damage the coral it can be constructively used for repeat studies of the same colony (Nagelkerken et al., 1998). In Oren et al.'s (1997) study of lesion regeneration in *Favia favus*, time-lapse photography was coupled with image analysis software to make measurements of emerging tissue.

A number of small-scale measurements, important to morphometric studies, may be taken from photographic images, e.g. the number of tentacles in each cycle, size of polyp and the number of polyps per unit area. Lasker (1981) employed close-up photographs of living *M. cavernosa* to help calculate polyp size and density. However, as is the case with *M. cavernosa*, the polyps need to be relatively large and well defined if sufficient detail is to be revealed. Photographic imagery offers small-scale coral studies four principal advantages: speed of sampling, non-destructiveness, an instant permanent record and the opportunity for repeat studies of the same colony. But, to date, there has been no attempt to fully explore its potential and there is no detailed description of a best approach. In this paper we test a photographic technique for studying small-scale coral morphology with particular reference to its application in the field and analysis of collected images. Morphometric readings from images of living coral are also compared to those taken by traditional methods that use bleached skeletons. The technique described here has been successfully utilised to identify intraspecific morphological variation in corals around Singapore (Todd et al., 2001).

MATERIALS AND METHODS

A Nikonos V underwater camera fitted with a 35 mm lens and a single SB 105 strobe was used with Fujichrome Velvia slide film. Extension tubes and framers (Ocean Optics, London) provided 1:1.5, 1:1 and 2:1 macro images. Slide images were taken of all the major scleractinian coral types found in Singapore's waters; including massive, branching, laminar, columnar, encrusting and foliaceous forms. These trails demonstrated that a photography-based method for studying small-scale morphology could not be applied to the majority of the corals sampled, as their polyps were too small or indistinct. This was particularly true for most branching, columnar, foliaceous and laminar corals.

The most suitable taxa were the massive and/or encrusting forms of the family Faviidae. The coral *Favia speciosa* (Veron, pers. comm.) was chosen as the test species due to its large and clearly defined circular polyps; it was also common around Singapore's southern islands. Two pictures of each colony were taken to ensure enough polyps were available for analysis, and that more than just one portion of the colony was sampled. Images were gathered in a variety of conditions over a depth range of 3 - 6 m. Accuracy was lost if measurements were taken from a polyp not perpendicular to the camera, so only the flattest areas of the colony were photographed. For size reference, a millimetre scale bar was incorporated into every shot. The 1:1.5 and 1:1 images gave great detail of each polyp, but at this size only a few individuals were available on each slide for analysis. The 2:1 images captured many more polyps that were still clear enough to measure. If the polyps of the study species had been smaller, the use of 1:1.5 and 1:1 framers would have been preferable.

Image analysis. – Measurements taken from projected slides were compared to those made with computer image software applied to scanned slides. The main objectives were to discern what polyp characters were identifiable from the images and which approach provided the most reliable results.

Projection based measurements were made from 2:1 coral images enlarged to10× actual size. After assigning all suitable individuals (mature polyps perpendicular to camera) a number, five polyps for analysis were randomly chosen. The outlines of the polyps' observable characters were drawn directly onto A2 size plain paper with a 0.3 mm retractable pencil. A fresh sheet of paper was used for each new image, creating a permanent record. Measurements were taken from the inside edge of the pencil line with vernier callipers (± 0.1 mm). The projector lens was found to be distortion free after projecting 1:1 slides of a 1 mm grid and checking for irregularities.

With the computer-based approach (Pentium PC, 300 MHz, 64 Mb RAM), slides were converted to digital images using a Nikon Coolscan II at a range of resolutions producing file sizes from 12 Mb to 0.2 Mb per image. Drawing the polyps (as described above) created an accurate record of their position on the slide image; therefore it was a simple process to identify the same individual for analysis on the computer monitor, enabling direct comparisons to be made. Two software packages were used, SigmaScan/Image (Jandel Scientific) measurement software and the Scion Image image processing and analysis program.

To test the precision of the projection method, ten randomly selected polyps were re-drawn and re-measured at daily intervals for ten days. The combination of re-drawing and then re-measuring captured two possible areas of error, i.e. the inherent problems of drawing around the polyp characters perfectly every time and the slight error expected from measuring between two lines on a piece of paper. A similar approach was applied to the computer-based method, where the same ten polyps were re-digitised ten times. For each polyp, the ranges as a percentage of the mean and coefficients of variations (CVs) were calculated for the characters measured and then averaged over the ten polyps.

To establish how the photographic technique (with-tissue) readings correlated to those using a traditional technique (without-tissue), measurements were taken from 2:1 slide images of thirty living polyps – ten each from three colonies. Similar measurements were then taken from the very same polyps, also from slide images, but after the tissue had been

removed with a 50% solution of household bleach. To discern if it was possible to predict without-tissue values from those with-tissue, linear regression analysis was carried out on the, normally distributed, paired data set.

RESULTS

The projection method proved to be simple and effective, producing large and crisp polyp images suitable for close examination. When applied to slides of *F. speciosa*, six characters were measurable (Fig. 1): polyp maximum diameter, polyp minimum diameter, oral disc maximum diameter, oral disc minimum diameter, number of tentacles and number of polyps per 35.2 cm^2 (the area captured in one 2:1 slide image). Outlining the polyp characters with a 0.3 mm retractable pencil worked very satisfactorily. Taking readings with vernier callipers to a tenth of a millimetre at a 10× scale equated to an accuracy of 10 µm at actual size. More information was lost through image quality than through using a pencil on paper.

When using a computer we found that image files had to be very large if coral features were to be of similar clarity to those provided by the projection method. TIFF (Tagged Image File Format) files of 8 Mb produced sufficient resolution, but these took minutes to load and download. A balance was sought between time to load and download files and the measurements that could be collected. We decided that, given the computational power we had, the computer-based method was not practical for measuring dimensions. However, acceptable area readings could be taken from relatively small files and eventually all slides were scanned at 236 pixels per cm at 100% producing 1.42 Mb TIFF files stored on ZIP Discs. The outline of the polyp and oral disc were digitised and their areas calculated (Fig. 1).

The precision results fall into three distinct groups (Table 1). The dimensional readings taken from the projected slides (polyp max and min diameter and oral disc min and max diameter) all have very similar CVs and ranges. The next group comprises the area measurements which have a higher CV and range – a consequence of poorer quality images and the inherent problems of digitising using a computer mouse. Finally, the number of tentacles and number of polyps per 35.2 cm^2 predictably show the lowest variation as these are straightforward counts. The range values are included to show the more extreme error observed. The figures appear high due to the disproportionate effect outliers have on this statistic; the CV's more accurately reflect overall precision.

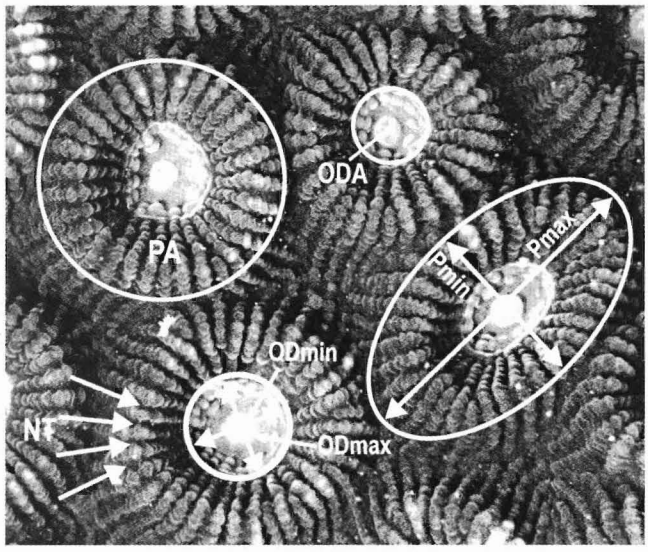

Fig. 1. Photograph of *F. speciosa* polyps indicating measurements taken. PA = polyp area, Pmax = polyp max diameter, Pmin = polyp min diameter, ODA = oral disc area, ODmax = oral disc max diameter, ODmin = oral disc min diameter, NT = number of tentacles (from Todd et al., 2001).

Some polyp measurements correlate well to their equivalent corallite readings (Table 2). The R^2 values derived from linear regression analysis are relatively large for the min and max polyp/corallite diameter. As there is no direct skeletal equivalent to the oral disc, an attempt was made to correlate the oral disc measurements to columella measurements, however the relation is not strong. Both area results are poor, possibly an effect of the less precise measuring technique for these characters. Again the number of polyps per 35.2 cm^2 and the number of tentacles produce the most significant results.

DISCUSSION

The first stage of this technique, taking the underwater pictures, proved to be simple and efficient with large numbers of samples collected during any one dive. The Nikonos V

Table 1. Precision of measurements taken. Range as a % of the mean and the Coefficient of Variation.

Character	Range as % of mean	Coefficient of Variation
Polyp max diameter (mm)	6.58	1.99
Polyp min diameter (mm)	6.63	2.06
Oral disc max diameter (mm)	6.84	2.04
Oral disc min diameter (mm)	7.58	1.94
Polyp area (mm^2)	8.06	2.43
Oral disc area (mm^2)	11.2	4.59
Number of tentacles	1.80	0.73
Number of polyps (per 35.2cm^2)	3.10	1.05

Table 2. Regression analysis results for character measurements taken from polyps before and after removal of tissue. In each of the following comparisons the living polyp character is given first.

Character	R^2
Polyp max diameter : corallite max diameter	0.780
Polyp min diameter : corallite min diameter	0.729
Oral disc max diameter : columella max diameter	0.646
Oral disc min diameter : columella min diameter	0.581
Polyp area : corallite area	0.552
Oral disc area : columella area	0.443
Number of tentacles : number of septa	0.985
Number of polyps : number of corallites	0.988

underwater camera, combined with Fujichrome Velvia film, provided consistently high quality images.

On each slide, finding polyps perpendicular to the camera was difficult if the images were taken haphazardly from any part of the coral. Thus, it was necessary to limit the areas of the colony captured to the flattest sections. Taking more than one slide of each colony ensured a greater area sampled and gave a better chance of collecting sufficient polyps.

Although the polyps of *F. speciosa* were relatively large for corals, the average diameter was still only 12.5 mm. Therefore, it was difficult to produce digitised images of adequate resolution for accurately taking dimensional readings, even when using a good quality dedicated slide scanner. Only files of over 8 Mb per image provided enough detail, but these files took a considerable time to load and download from ZIP Disc. Image files of 1 Mb – 2 Mb were quicker to download, and though not so crisp, were still useful for taking area measurements. As computer speeds increase, it will become more practicable to use large files, permitting dimensional readings to be taken. But it is unlikely that a computer-based method would be able to pick up *more* detail what can be discerned from a good quality projected slide.

Projecting the slides to 10× actual size meant that each polyp was approximately 125 mm in diameter, a large image to work with. However, due to the nature of the coral tissue, margins between tentacles and other body parts were often ill defined and it was impossible to take accurate measurements of traits such as 'thickness of tentacle'. Other characters, including minimum and maximum oral disc diameter, still required a subjective decision based on the information supplied by colouration and shading. This ambiguity was a weak point reflected in the precision results. The absence of hard edges meant repeat measurements of the same character were likely to differ. The extent to which this occurred was not at a level to render the entire method valueless. The diameter measurements made from the projected slides had a coefficient of variation of around 2% and range of approximately 6.5% of the mean. Therefore, if the polyp diameter was 125 mm on screen, we could expect a standard deviation of about 2.5 mm and a range of about 8.1 mm. Although the area readings were not as precise they were still of use, as this measurement was difficult to acquire any other way.

Comparisons of measurements taken from polyps with tissue to those same polyps with the tissue removed were carried out to determine whether standard without-tissue measurements might be derived from living coral, thus reducing the need to remove colonies or sections of colonies from their habitat. Although some of the dimensional R^2 values were high, only the counts of number of polyps per 35.2 cm^2 and number of tentacles could be used to predict their skeletal equivalent with a high degree of accuracy. Thus, only coral morphometric studies utilising similar methods should be compared with each other.

Traditional methods can generate over 15 skeletal traits per corallite for measurement (Amaral, 1994). Dimensions such as height of theca are only possible when the three-dimensional, tissue-less structure is at hand. Two-dimensional images of living tissue considerably limit the corals that can be studied and which characters are available for analysis.

As coral taxonomy is based upon many skeletal traits the photographic technique cannot be used for species identification work. However, useful information may still be obtained from a reduced number of characters if the differences between polyps are significant. Research into intraspecific variation in corals could make use of this technique as disparity in only a few characters can provide statistically viable information (Todd et al., 2001). At the very least it can help determine sites for more intensive study. Expeditions or projects covering a large geographical range, but where collecting hundreds of coral skeletons may be undesirable, could also benefit from the ability to rapidly record polyp detail. Finally, the capacity to take repeat images may facilitate a novel way of studying polyp growth and development, particularly under conditions of environmental stress such as heavy sedimentation or eutrophication.

CONCLUSION

In this study it was clear that the analysis of computer images was not as effective as the projection method for taking dimensional measurements of coral polyps. This situation will no doubt reverse as scanning technology evolves, computer speeds increase and advances in underwater digital photography provide fast, high resolution images. However, even with improved technology, the corals to which a close-up photographic technique might be applied, and which characters may be available for analysis, are unlikely to change. The method proved to be straightforward, inexpensive and provided a simple and permanent way of storing samples. The speed of sampling equated to less time in the field, and as the technique was non-destructive, sample size was only limited by the number of colonies present at a site. The technique can identify intraspecific morphological

variation in corals with large well-defined polyps and in these cases it could be used for research such as studies of phenotypic plasticity. It cannot be employed for taxonomic work nor applied to most branching, columnar, foliaceous and laminar corals where the polyps are too small or ill-defined.

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