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NOTE

Increasing the accuracy of surface area estimation using single wax dipping of coral fragments

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Abstract The measurement of coral surface area is critical to normalising a suit of physiologically significant parameters to greater understand how corals interact with the surrounding environment. The surface area detection from skeletal fragments subsequently needs to be both as accurate as possible, yet practical and robust enough to be performed with minimal laboratory equipment. By using X-Ray CT technology, as a highly accurate surface area standard, 12 coral specimens from 4 different genera were studied using single wax versus double wax dipping methods. Our results reveal that the single wax dipping is far more accurate than the more commonly practised double wax dipping, thereby leading to more accurate estimation of the physiologically active surface.

Keywords Wax dipping · X-ray CT · Surface area

Introduction

The quantification of biotic surface area is a key to understanding corals interaction with their surrounding environment (Edmunds and Gates 2002). Furthermore, the morphological complexity of scleractinian corals and their interaction with the aquatic medium cannot be fully explained without reference to their surface area (Dahl 1973). The normalisation of biological parameters to surface area is required for calculations of, for example: chlorophyll concentration, biomass, respiration rates and dinoflagellate density, thereby underpinning the physiological processes which occur within coral colonies (Helmuth et al. 1997; Hoegh-Guldberg and Williamson 1999; Lesser et al. 2000). The use of coral branches and nubbins often sub-sampled from adult colonies forms the basis of many coral reef studies investigating, for instance, an organism's response to varying environmental stress, either in situ or in controlled aquarium conditions (Jones et al. 2008). The extraction of algal cell material from coral tissue required for a plethora of physiological assessments is often needed to be normalised in relation to surface area or tissue biomass (Edmunds and Gates 2002). Corals are one of a small number of colonial modular taxa which are theoretically isometric, thereby facilitating the formation of proportional relationships of size to biological concentrations (Edmunds and Gates 2002). Excluding a small number of corals with very thick tissues, most scleractinian coral species have a thin layer of tissue which conforms closely to the coral skeleton (Edmunds and Gates 2002). This tight coupling of coral tissue to skeleton facilitates the approximation of tissue surface area from the skeletal surface area, otherwise known as skeletal "primary" surface area (Hoegh-Guldberg 1988).

Methods for primary surface area estimations of corals have been attempted with a multitude of techniques over the past 60 years. Coral skeleton can be foil wrapped (Marsh 1970), wax dipped (Stimson and Kinzie 1991; Vytopil and Willis 2001), latex dipped (Meyers and Schultz 1985), geometrically approximated (Naumann

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et al. 2009) or sealed with varnish and immersed in dye (Hoegh-Guldberg 1988). In each case the displacement, adherent dye concentration or increase in mass of the measuring material can then be used to interpolate the surface area. Alternatively, surface area can be measured with a passive or active three dimensional visualisation platform such as photogrammetric reconstruction (Bythell et al. 2001), laser scanned (Holmes 2008), structured light scanner (Veal et al. 2010) or X-Ray CT Scanned (Laforsch et al. 2008). The use of X-Ray computer tomography (CT) technology to measure surface area of corals with a high degree of accuracy and resolution has enabled the inter comparison of a variety of surface area extraction methods (Naumann et al. 2009; Veal et al. 2010). Despite the accuracy of X-Ray CT surface area measurements, their field applicability and cost can be prohibitive, making cheaper and easier methods such as wax dipping still widely applicable.

Wax dipping was first recorded in the coral literature by Stimson and Kinzie (1991) who used paraffin wax at 59°C to single dip corals for a brief period of time (undefined) and then make a comparison to calcium carbonate calibration blocks of known surface area. Chancerelle (2000) sealed their corals with a varnish and single dipped at 85°C in paraffin wax (35°C above the melting point). Naumann et al. (2009) compared a double wax dipping technique (adapted from Stimson and Kinzie 1991 and Vytopil and Willis 2001) to X-Ray CT to produce a set of correction factors for 6 common coral morphologies. Veal et al. (2010) found double wax dipping to be the most accurate method of surface area detection, compared to X-Ray CT determined surface area. The practice of double wax dipping a coral skeleton fragment to obtain the approximate surface area of coral tissue is the more commonly practice (Vytopil and Willis 2001; Hoegh-Guldberg et al. 2005; Holmes et al. 2008; Naumann et al. 2009; Veal et al. 2010), when compared to the single dipping method (Stimson and Kinzie 1991; Jones et al. 2008).

This paper compares single wax dipping to the more commonly practiced double wax dipping of coral skeletons with two different morphological formations, with varying corallite structure, to determine which method yields more accurate surface area estimates, compared to X-Ray CT surface area.

Materials and methods

Coral specimens

Four coral taxa: *Porites sp., Goniastrea* sp., *Stylophora pistillata* (Esper 1797) and *Acropora loripes* (Brook 1892) were collected from the waters of the Gulf of Aqaba, Israel (29°30′N, 34°55′E). *Porites* sp., *S. pistillata* and *Goniastrea* sp. samples were collected from several colonies, whilst an individual colony of *A. loripes* was collected and then broken into smaller segments to represent each size class required for the study. Ten fragments of each coral ranging from approximately 1 to 10 cm in axial length where cleaned of live tissue using dilute sodium hypochlorite, then soaked in freshwater before being air dried at 25°C inside an air conditions laboratory for 24 h prior to being glued to a base. Of the 40 coral fragments collected, 12 fragments (2 *Porites* sp., 4 *Goniastrea* sp., 3 *S. pistillata* and 3 *A. loripes*) were chosen to best represent a range of size and complexity classes of each of the genera.

Calibration objects

Thirty-four wooden calibration objects were used to construct a surface area calibration curve ranging from 1 to 100 cm^2. Calibration objects were made from wooden cylinders, prisms and cubes of varying sizes, which were all treated with a clear spray varnish (Motip Dupli Group, Netherlands) to seal the wood. Each calibration object was drilled on the long axial corner, and a 2 mm wide wooden tooth pick was glued in place allowing easier wax dipping. Calibration objects were measured using digital callipers (Mitutoyo, USA), accurate to 0.001 mm to determine geometric surface area. The decision to use a range of both cylindrical and cubical calibration objects was made to insure that wax droplet formation on the corners of calibration objects was kept to a minimum and would make minimal impact on the accuracy of the calibration curve. Hoegh-Guldberg (1988) discussed the use of cylinders for calibration objects as they found dye droplet formation on the corners of cubical objects. The application of the shaking the coral fragments to remove excess dye can be likened to the rapid spinning of calibration objects used in this paper to remove excess wax.

Wax dipping

Wax dipping was conducted using paraffin wax (Superkit, Israel) at 65°C as outlined in Holmes et al. (2008). Wax temperature was maintained by placing the beaker of wax in a water bath with water temperature checked using a Cyberscan pH 510 (Eutech Instruments, Netherlands) temperature probe. Corals and calibration objects were maintained at 25°C. Each coral or calibration object was weighed prior to dipping on an AJ-320CE digital pan scale (Shinko Denshi, Japan) (accuracy = 0.01 g), then dipped for 2 s before being removed and rotated quickly in air (10 revolutions over 2 s) to promote even wax coverage and to remove excess wax. Dipped corals or calibration objects were then allowed to stand for 15 min before being

reweighed. This process was repeated again for the double dipping.

X-Ray computer tomography (CT)

X-Ray CT scanning was performed on a Phillips MX8000, (Royal Phillips Electronics, Netherlands) multi slice, 16 slice helical scanner. Corals of ranging size from each of the four species were scanned using Phillips MX8000 presetting protocol for the temporal bone with a base emission setting of 90 kV at 50 mA with a slice spacing of 150 × 150 × 400 μm. Scans were exported for manipulation as Digital Imaging and Communication in Medicine (DICOM) files for post processing in Amira 5.2 (Visual Imaging Inc, USA). Samples were imported using the AMIRA DICOM reader module 5.2, then using the Isosurface creation tool to build a surface based on the most rapid density change at the air-skeleton interface as outlined in Laforsch et al. (2008). Three dimensional distance measurements using AMIRA's 3D Ruler tool were then collected from 10 points on each coral skeleton reconstruction and compared to measurements made on the real skeleton using digital callipers. Differences of less than 100 μm were observed between the two datasets using a threshold setting of −500 as used in Veal et al. (2010). The reconstruction was then exported as a Virtual Reality Modeling Language (.wrl) file for further post processing in Polywork 10, modeller node (Innovmetric Software Inc, Canada). Polyworks' processing (as outlined in Veal et al. 2010) was used to undertake minor hole filling on the reconstructions prior to cropping each model to spatially match the coral surface where tissue would have been and that got covered by the wax in the wax dipping process. The surface area tool was used to determine the reconstructed coral surface area.

Results and discussion

The calibration curves generated from both the single and double dipping of calibration objects are presented in Fig. 1. The choice to use a Y-intercept function for the equation, unlike that previously documented by Holmes et al. (2008), was to address the issue of wax droplets. The accuracy of wax calibration curves with very small calibration objects can introduce large sources of error relative to the area being measured due to the surface tension of the melted wax. The inclusion of the Y-intercept function was designed to generate a more accurate fit for a range of calibration object size, especially as a large number of coral reef studies use coral nubbins less than 5 cm long; therefore, accuracy at the small scale is critical. The calibration objects selected for this study ranged in size from 5

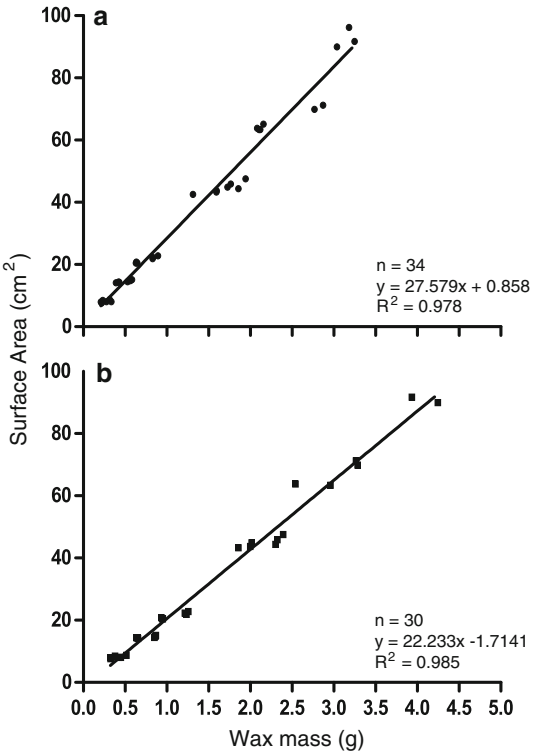

Fig. 1 Graph of relationship between surface area (cm^2) and mass of wax (g) for both single (**a**) and double dipped (**b**) calibration objects, with the number of calibration objects and R^2 of the linear regression fit

to 95 cm^2, with the largest coral in the study having a surface area of 124 cm^2.

The results of wax calculated surface areas to X-Ray CT–derived surface area for the 12 corals used in this study are presented in Table 1. Except for *A. loripes*, the single wax dipping was within ±7% of the X-Ray CT determined surface area. This is in stark contrast to the double dipping estimates, where all predicted surface areas were underestimated by average of 26%, a result supported by Naumann et al. (2009). The underestimation of coral surface area by double dipping occurs as the first dip fills in complex corallite formations, greatly reducing the surface areas measured by a second dip. The results in Table 1 illustrate that for those corals that have small corallite structure (*Porites* sp. and *Stylophora* sp.), the double dipping method only underestimates the surface area by between 2 and 14%, with those corals with complex corallites (*Goniastrea sp*) having their surface area being underestimated by up to 41%. The underestimation of surface area of *Acropora* sp. corals using wax dipping is unusual as both Naumann et al. (2009) and Veal et al. (2010) found very high correlations between X-Ray CT and double wax dipped coral surface area of *Acropora* sp. corals. The justification for this high error in this study can be found in Fig. 2, with *A. loripes* clearly showing very

Table 1 Table of surface areas of 12 different corals determined using both a single and double wax dipping methods, compared to the X-Ray CT surface area, with differences expressed as percentage similarity for both single and double dipped corals

Coral ID	Single wax dipping (cm^2)	Double wax dipping (cm^2)	X-RAY CT (cm^2)	Single difference (%)	Double difference (%)
Gon3	22.37	14.09	22.28	0.42	−36.73
Gon4	41.15	25.63	41.62	−1.12	−38.41
Gon6	40.27	23.03	39.53	1.88	−41.73
Gon8	65.94	46.11	69.60	−5.26	−33.75
Por6	30.28	28.70	30.83	−1.76	−6.90
Por10	123.20	110.05	124.10	−0.73	−11.32
Stylo6	47.22	43.40	44.36	6.44	−2.17
Stylo9	112.44	100.71	113.97	−1.34	−11.63
Stylo10	109.74	91.29	106.48	3.06	−14.27
Acro2	5.63	3.76	6.26	−10.12	−40.04
Acro5	19.72	18.14	23.51	−16.12	−22.85
Acro7	84.70	75.35	93.99	−9.88	−19.83

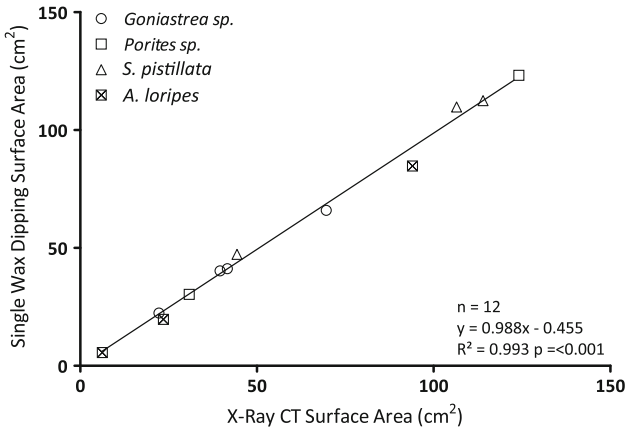

Fig. 2 Graph of single wax dipped predicted surface area to X-Ray CT surface area both expressed in cm^2 for *Goniastrea* sp., *Porites* sp. *S. pistillata* and *A. loripes*

deep corallites in the X-Ray CT image (indicated in grey). These surfaces have been included in the surface area extraction from the X-Ray CT method. The X-Ray CT estimated surface area for corals with the deep corallites would not actually be a valid "primary" surface in relation to the area occupied by living tissue (Hoegh-Guldberg 1988). Kruszyński et al. (2007) used a hole fitting algorithm to reduce the effects of these artefacts on the estimation of coral skeletal surface area thereby enabling the use of X-Ray CT surface areas on a greater range of coral skeletons with varying densities.

The wax dipping protocol used in this paper followed the outline given in Holmes et al. (2008), during which they found that the weight gain between first and second dipping could be expressed as (Eq. 1)

$$\text{Surface Area}\left(\text{cm}^2\right) = 34.32\left(\text{cm}^2/\text{g}\right) \times \text{Mass}(\text{g}) \qquad (1)$$

Despite following the same methods, the use of a different paraffin wax has resulted in a very different estimate of surface area. It would therefore be suggested by the authors that any researcher wanting to use wax dipping to determine surface area of coral skeletons should generate his own calibration curve using objects of known surface area and use a single dipping method as this yields higher accuracy than traditional double wax dipping techniques, with the exception of highly porous coral skeletons such as *Acropora* sp., where there needs to be a sealing of the deep corallites prior to a measurement of the approximate surface area. This sealing is best achieved by using the double dipping wax protocol. Variability in approximated tissue surface area can be further compounded by the high intra-colony and inter-species variability in many coral species if predefined surface area formulas for genera-specific corals (Naumann et al. 2009) were used instead of wax-specific calibration curves (Fig. 3).

Among all accurate techniques, single wax dipping is the fastest and cheapest method to acquire the surface area of coral skeletons and nubbins. The significant time and monetary costs of X-Ray CT scanning restricts its usage to small laboratory studies such as this one, so that it can be used to establish accuracy relationships with other commonly utilised techniques (Naumann et al. 2009; Veal et al. 2010). The use of single dipping, instead of double dipping, greatly increases the accuracy of the surface area detection of coral skeletons, thereby improving the quality of environmental interpolations made from surface normalisation of biological parameters. The greatest limitation still facing coral scientist using these methods is that measurements of coral tissue surface area are based on skeletal surface area and not on actual coral tissue surface area. The future direction of surface area extraction of coral tissues needs to focus on methods of non-destructive measurement of soft tissue surface area to enable actual measurement of absolute coral tissue surface area.

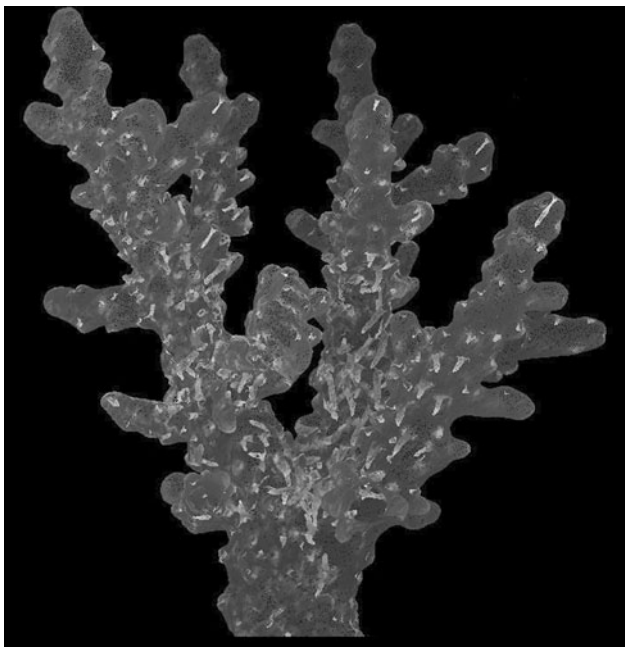

Fig. 3 X-Ray CT image of *A. loripes* displaying the large number of corallites (*light grey*) that penetrate several centimetres into the inside of the coral skeleton, leading to an overestimated X-Ray CT–derived surface area

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