**Calice Morphology Protocol**

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Shape, polygon

Description automatically generatedDesired morphometrics:

ID Intercorallite distance

(We’ll use density as A

proxy for this)

C Calice circumference

H Calice height

(widest distance)

W Calice width

(perpendicular to CH)

A Calice area

CC Corallite circumference

CH Corallite height

(widest distance)

CW Corallite width

(perpendicular to CH)

CA Corallite area

D Calice depth

Materials

* Stereo microscope
* Microscope light
* Microscope camera
* Ruler or measuring device
* Lab Computer with OMEX ToupView Software
* Computer with ImageJ

Microscope set up

1. Turn on stereo microscope and microscope light
2. Set up scope with black side of plate showing and ruler in view
3. Place sample in view and focus to eyes
4. Switch microscope view to the camera by pulling on the silver knob. This will cause one of the eyepieces to go black.

Computer Set up & Photographing

1. Open OMAX ToupView
2. Plug in Omax camera to laptop
3. Make sure that ruler and sample are
   1. In view
   2. In focus
   3. Not over or under saturated (adjust light as needed)
4. Take a photo by clicking the “snap” button
5. Take at least three photos of the sample with varied views. This is especially important when the sample is not level. Make sure all parts of the skeleton are focused in at least one photo
6. Save photos to the desktop, label the photo with the sample name
7. Upload photos to the drive

Measuring Calices

1. Open imageJ
2. Randomly select 5 corallites to measure. Do not measure corallites that are cut off around the edges.
   1. If there are enough full calices, do not use any along the growing edge of the coral fragment. Usually these are much smaller and deformed.
3. Set the scale for each image
4. Use the straight line measuring tool to measure:
   1. Calice height – the widest point of the circle
   2. Calice width – perpendicular to the line used for calice height
   3. Corallite height - the widest point of the circle
   4. Corallite width – perpendicular to the line used for calice height
5. Use the multi-line measuring tool to measure
   1. Calice circumference
   2. Corallite circumference
6. Use the area measuring tool to measure:
   1. Calice area
   2. Corallite area
7. Overlay a 1 cm^2 box onto the coral skeleton. Record the number of calices within the box (if the center of the calice is in the box then it counts.

References

Todd et al. 2004c

Ow & Todd 2010

From Matias’ proposal: “To quantify variation in morphological traits across light gradients, I will collect (after one year) one of the fragments from each environment (two per colony one each from shallow and one from deep). I will bleach skeletal fragments, and characterize calyx morphology from 10 haphazardly - selected corallites per fragment, by a configuration of 48 landmarks to reconstruct space shape from 92 dimensions (48 landmarks x 2 – 4) (Savriama, Y. et al. BMC Evol. Biol. 11, 280 (2011)). The decomposition of shape variation into symmetric and asymmetric components will be done via a full Procrustes fit and Principal Component Analysis to compare shape changes. I will take pictures of each corallite at 2× and 4× objectives using a high-resolution digital camera attached to a stereoscopic microscope. Landmark acquisition and statistical analyses will be done from corallite photographs using the ImageJ, TpsDig2, MorphoJ, and R software.”