Protocol for Prada Lab Coral Research 2021

**Isotope Measurements 13C & 15N**

Protocol based on methods used in the following studies

* Swart et al., 2005a
* Maier, Weinbauer & Patzold, 2010
* Muscatine, Porter & Kaplan, 1989
* Einbinder et al., 2009

Materials

* Water pik or air brush
* Filtered sea water
* Deionized water
* Centrifuge
* Drying oven
* Freezer
* Hydrochloric acid
* pre-burned glass fiber filters or combusted Whatman GF/F filters (at least two per sample)
* Glassine envelopes?
* Many sample tubes (need to ask EPA folks about sizes)

Methods

1. Micro-fragmented coral samples removed from habitat should be frozen as quickly as possible. Ethanol and other organic preservatives must be avoided because they will later the carbon isotope ratio. Freezing samples stops photosynthesis, which would also alter the ratio.
2. Remove soft tissues from skeleton using a water-pik or air brush with a reservoir of filtered seawater and homogenize samples
3. Centrifuge tissue samples at 2000 x g for 5 minutes
4. Decant supernatant containing host coral tissue and centrifuge again at 4000 x g to pellet remaining zooxanthellae. Discard this pellet and freeze the supernatant until future analysis
5. Rinse zooxanthellae pellet with filtered seawater and resuspend then recentrifuge at 3000 x g for 5 min. This process can be repeated multiple times. Resuspend the pellet and freeze until future analysis.
6. Before analysis, rinse organic tissues with 1 N HCl to remove carbonate, rinse clean with filtered seawater/deionized water
7. All samples filtered on pre-burned glass fiber filters (combusted Whatman GF/F filters)
8. Combust samples in oven at 50˚C for 24hrs (or at a much higher temp for a shorter period of time).
9. Filters with samples can be kept in glassine envelopes or transferred to sample tubes for storage.

Notes

* Both the host supernatant and the zooxanthellae pellet can be visually analyzed to check for remaining contamination
* I need to ask EPA folks about what size tubes they use in the Mass spec and what volume of dry sample they typically need
* Should we do isotope analysis of skeleton? Should we do isotope analysis of particulate organic matter (to analyze carbon ratios of food)?

**Calice Morphology**

Desired morphometrics:

* Calice diameter
* Calice depth
* Calice spacing
* Calice density

Methods

1. Bleach skeletons to remove organic tissues

From Matias: “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.”

**Chlorophyll content**

**Antioxidants**

**Lipids**

**Carbohydrates**

**Symbiodinium Identification**