Protocol for Prada Lab Coral Research 2021

**Calice Morphology**

**Isotope Measurements**

(DeNiro & Epstein, 1978)

* Measured a variety of non-coral species fed a variety of diets
* 13C and 12C
* Samples were freeze- dried (lyophilized)
* Methods for isolating lipids (BLIGH and DYER, 1959). glycogen (JACORSON et trl., 1972) and protein (MARROQUIN and FAKHER, 1965).
* Prep for isotope analysis:
  + Lyophilized
  + Ground into a powder
  + Shell samples were treated with 50% Clorox to remove organic matter

(Heikoop, et al., 2000)

* Anthropogenic inputs of 15N/14N on reefs
* Prep for isotope analysis
  + 50cm2 of tissue removed from colony
  + Samples treated in 10% HCL to decalcify samples (this has no impact on 15N or 13C compared to the waterpik samples (heikoop et al. 1998)
  + Rinsed in distilled water
  + Freeze – dried
  + 15mg of tissue for each 15N analysis, 5mg of tissue for each 13C analysis
  + Used combined host & zooxanthellae samples
  + Samples combusted for 2hrs at 550˚C the day before analysis
  + Gases cryogenically purified prior to measurement on VG SIRA mass spectrometer

(Lesser, et al., 2010)

* Chlorophyl content analyzed
  + 1.8 – 1.95 cm2 plug from coral replicates
  + placed in 50M tubes overnight in 90% acetone at 4˚C
  + Extracted pigments centrifuged at x1000 g to remove particulates
  + Absorbance measured against acetone blanks at 630 nm and 663 nm
* Measured gross primary productivity
* Isotopes 13C and 18O
  + Tissue removed with water pik
  + Separated by centrifugation 5 min at 5000g
  + Host fraction isolated onto pre-burned glass fiber filters
  + Zooxanthellae acidified to remove skeletal fragments & isolated onto filters
  + Both were combusted in a chamber attached to a mass spectrometer
  + Skeletal material prepared as described by Rodrigues and Grottoli (2006)
    - 75-95 ug sample
* Zooxanthellae analyzed based on Lesser and Farrell(2004)

(Maier, Weinbauer, & Patzold, 2010)

* Isotopes 15N and 13C
  + Tissues removed with water pik
  + Sieved through 20 um net, equivalent to acidifying the sample
  + Half the sample filtered on combusted Whatman GF/F glass fiber filters
  + Half homogenized for 5 -10 mins then centrifuged (2000 x g) to separate tissue & zooxanthellae
  + Host tissue filtered on GF/F filters
  + Zooxanthellae pellet was suspended and filtered on GF/F filters
  + All filters dried in 60˚C in oven for 24hrs
  + Seawater Particulate organic matter also filtered on GF/F filters
  + Finnigan MAT Delta Plus Mass spectrometer

(Muscatine, Porter, & Kaplan, 1989)

* Isotopes 13C
  + Samples frozen
  + Freezing had no adverse affect on speration of zooxanthellae and animal tissue (Streamer et al. 1986)
  + Tissue removed with water pik
  + Homogenate filtered through layers of surgical gauze to remove fragments of skeleton then centrifuged in 10ml centrifuge tubes at 2000 x g for 3 to 4 min to pellet most of the zooxanthellae
  + Supernatant (host tissue) was decanted and centrifuged at 4000 g for 4min to pellet residual zooxanthellae
  + Final supernatant homogenized & put on a glass fiber filter using suction
  + Pellet of zooxanthellae suspended and washed 3-5 times by adding filtered seawater and centrifuging at a low speed.
  + Both host & zoox filters were examined under a microscope and re-centrifuged if they still contained the opposite tissue
  + Filters dried at 50˚C overnight
  + Filters stored in glassine envelopes

(Weber, Deines, Weber, & Baker, 1976)

* Isotopes 13C/12C
  + Skeleton only
  + Soaked in hypochlorite for 24-36 hrs to remove soft tissues
  + 1-3 mm pieces
  + soaked in 5% sodium hypochlorite for 12 hrs & rinsed thoroughly
  + dried in air for 2 days then ground to 0.18mm in size
  + Samples headed to 400˚C for 20 min in purified helium
  + CO2 obtained by reaction & mass spec

(Swart, Saied, & Lamb, 2005) (Swart et al. 2005)

* Isotopes 15N and 13C
  + Samples placed on ice until tissues were removed by air brush
  + Tissue slurries were homogenized for 30s using electronic homogenizer
  + Centrifuged 3-5 min at 1500 rpm
  + Supernatant decanted and frozen for future analysis
  + Pellet re-suspended three times in filtered sea water and skeletal fragments were allowed to settle out and removed before re-centrifuging
  + Final pellet recovered with small volumes of deionized water and frozen
  + Before analysis, organic tissues were rinsed with dilute HCl to remove carbonate, rinsed with water
  + Dried in oven
  + Mass spec
  + 3 replicates of each sample

(Grottoli & Wellington , 1999)

* Isotopes: 13C and 18O
  + Skeleton only
  + Dissolved in 100% H3PO4

(Einbinder, et al., 2009)

* Isotopes
  + Tissue removed with airbrush with filtered seawater (0.20 um filter)
  + Centrifuged for 4min at 1323 x g
  + the supernatant centrifuged for 10 min at 2068 x g
  + supernatant acidified with 1N HCl to remove inorganic carbon
  + pellet was washed with filtered seawater & resuspended and recentrifuged fr 4 min at 2687 x g
  + both samples were lyophilized (freeze-dried) for 24hrs and sent for isotopic analysis

(Klaus , Budd, Heikoop, & Fouke, 2007)

* Methods on IDing zoox
* Isotopes 15N and 13C
  + 0.5mg – 1.0mg tissue samples
  + Decalcified using 1N HCl and rinsed & dried
  + Combined tissue & zoox samples

(Land, Lang, & Barnes, 1975)

* Isotopes
  + Skeletons only
  + Bleached, dried, ground up, bleached, dried
  + Converted to CO2 using H3PO4 technique

Morphology

(Einbinder, et al., 2009)

* specimens bleached with 70% commercial cleaning bleach, rinsed in distilled water & dried
* Traits measured using Nikon dissecting scope equipped with scale built into the eyepiece
* 3 randomly selected corallites were measured
  + Polyp density
  + Corallite spacing (minimum distance to nearest neighbor)
  + Corallite diameter

(Klaus , Budd, Heikoop, & Fouke, 2007)

* Digitized 25 landmarks on calice surfaces using a reflex microscope
  + Polyp density
  + Corallite spacing (minimum distance to nearest neighbor)
  + Corallite diameter

(Klaus , Budd, Heikoop, & Fouke, 2007)

* Digitized 25 landmarks on calice surfaces using a reflex microscope