Coral and seawater 15N2 incubations

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

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Pre-incubation preparation

1 Fragment coral colonies in duplicate for control and experimental treatments. Attach to underwater rig to acclimate for 1 week prior to incubation.

2 Collect seawater to filter-sterilize for incubation (can be done on day of fragmentation). Collect seawater in acid-washed 20L or 30L carboys.

Note: Volume to collect will vary with number of incubations. For 22x 1L incubation jars, we recommend collecting at least 60L of water.

3 Filter-sterilize 60L of seawater using 0.22um pore size filters.

Recommended filtration set up:
A peristaltic pump, tubing, 3x Y-connectors and either (1) 4x filter heads with GF/F filters and 4x sterivex (0.22um) or (2) 4x filter heads with GF/F and 4x filter heads with 0.22um filters.

Using the y-connectors, create 4x parallel outflows, each passing sequentially through a GF/F filter and a 0.22um filter.

Collect filtrate in clean, acid-washed carboys.

After filtration, store carboys in cool, dark place until incubation experiment.

4 Prepare $^{15}N_2$ label using filter-sterilized water (see separate protocol on dissolved label preparation).

5 Clean and acid wash (10% HCl) everything for the incubation (chambers, buckets, funnels, graduated cylinders, tubing etc.), rinse thoroughly with Elga water (18.2MΩ).

Note: incubation chambers lids were modified to include 2x rubber septa and a PVC connector piece, which was used to attach coral fragments.

6 Prepare 12mL exetainers by flushing with He for 5 minutes. To avoid bringing ZnCl2 into the field, it can be added to the exetainer beforehand. Add 0.01mL of 50% wt/vol ZnCl2 per mL of sample.

7 Prepare glass bottles to collect alkalinity samples by acid washing with 10% HCl and rising with Elga water (18.2MΩ).

8 Pre-label Whirl-Pak bags for coral sample collection.

9 Acid wash (10% HCl) 15mL falcon tubes to collect seawater for nutrient analysis. Rinse with Elga water (18.2MΩ).

Incubation Day 1

10 *Treatment Summary:*

Experimental

4x coral species#1 incubation chambers with filtered, $^{15}N_2$ enriched water

4x coral species#2 incubation chambers with filtered, $^{15}N_2$ enriched water

3x unfiltered seawater chambers with unfiltered, $^{15}N_2$ enriched water

Controls

4x coral species#1 incubation chambers with filtered, no enrichment

4x coral species#2 incubation chambers with filtered, no enrichment

3x filtered seawater chambers with $^{15}N_2$ enriched water (to control for labeling loss)

11 Retrieve coral fragments from reef and place in large water bath on boat. Cover water bath with mesh to keep corals shaded.

12 Niskin cast for unfiltered seawater incubations. Empty water in Niskin casts into acid-washed bucket (this bucket is only for the unfiltered seawater samples).

12.1 From the same Niskin casts, collect samples for seawater nutrient analysis. Filter 12mL of seawater into acid-washed falcon tubes using a sterivex filter and syringe. Place falcon tubes in dry shipper. Store at -20C until analysis.

12.2 From the same Niskin casts, filter 1L of seawater through a sterivex filter. Place filter in dry shipper and store at -80C until analysis.

12.3 From the same Niskin casts, collect 3-4L of water to filter onto triplicate GF/F filters. Filter this water and record volume filtered. As a lot of material is typically needed to get a nitrogen isotope signature,

we recommend filtering at least 1L of water per GF/F, depending on the particulate concentration of the water. (This will serve as the t0 value for seawater incubations.)

12.4 From the same Niskin casts, collect water for any remaining biogeochemical measurements.

12.5 Wearing long gloves, fill 3x unfiltered seawater incubation chambers by completely submerging the chamber in the bucket with water from the Niskin casts. Close chambers headspace free, with no air bubbles.

13 Pour filter-sterilized water prepared in Step 3 into clean, acid-washed bucket. Wearing long gloves, fill 3x filtered seawater chambers with filter-sterilized water by completely submerging the chamber in the bucket. Close headspace free, with no air bubbles.

14 Fill coral chambers with filtered seawater, using separate clean, acid-washed buckets for each coral species. Close the coral incubation jars with the coral sample** by completely submerging the chamber in the bucket. Close headspace free, with no air bubbles.

**Before putting coral into the incubation, replace any cable ties and/or PVC with clean cable ties and PVC, rinse corals briefly with filtered seawater, and then attach corals connector on lids.

15 Using a needle and syringe, add 100mL of $^{15}N_2$ from serum bottles into each 1L incubation chamber. Outflow and inflow needles should be placed in opposite septa to prevent loss of the ingoing label. Note the time of injection.

Acid-washed tubing can be using to carefully dispense label from the serum bottle into the syringe. Take care to purge needles prior to use.

16 Take oxygen readings of each jar using Unisense, Oxygen Sensor Spots (OXY1-SMA).

17 Wrap Parafilm around the rim of all lids to avoid sediment accumulation in the gap between the lid and the glass jar.

18 Cable tie chambers to plastic crate with the lid facing downward and coral facing upwards.

19 Deploy incubation on the reef. Lift bags are recommended for transport from the boat to the reef. Note the time.

Incubation Day 2

20 Retrieve incubation crates from reef. Note the time. Place jars in shade or under mesh until processing.

21 Niskin cast and collect water for additional biogeochemical analysis if desired (e.g. dissolved nutrients, particulate nutrients, t0 d15N, microbial community)

22 Take oxygen readings of each jar using Unisense, Oxygen Sensor Spots (OXY1-SMA).

23 Before opening jars, rinse lids with DI water to remove any particles trapped in the small gap between the lid and the glass jar.

24 Open jars over large, clean funnels and clean graduated cylinders to collect any spillage. Note the time each jar is opened.

When each jar is opened, collect the following samples in the following order:

24.1 From all $^{15}N_2$ jars, collect 8mL of water using a syringe, purge 1mL of water and then transfer the remaining 7mL into pre-prepared He-flushed exetainers pre-loaded with $ZnCl_2$ (0.07mL of 50% wt/vol ZnCl2). Place exetainer in 50mL falcon tube and cover with filtered seawater for storage, to prevent any gas loss.

24.2 Collect 180mL sample for alkalinity measurement.

24.3 Pour remaining incubation water into graduated cylinder. Record volume. Filter water using pre-combusted, pre-weighted GF/F filters*. Record volume of water filtered, fold and wrap filters in Aluminium foil, place in dry shipper, store at -20C until isotope analysis.

*Note: While GF/F filters are commonly used in 15N2-based studies of nitrogen fixation, work by Bombar 2018 suggests that Advantec filters or silver membranes with 0.2 µm pore size may perform better.

24.4 Using a rotating table, take 40-50 photos of each coral fragment for surface area using photogrammetry (Autodesk Recap Photo). Sterilize workspace between samples.

24.5 Using a sterilized chisel, split coral fragment into two pieces for (1) isotope analysis and (2) molecular analysis. Place samples in corresponding Whirl-Pak bags. Transport the isotope sample on ice and store at -20C until analysis. Place molecular sample in dry shipper and store at -80C until analysis.