Nitrification Rate Incubation Protocol for Pelagic Systems
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Notes, caveats, and lessons learned from 7 years of at sea rate measurements:

This method has several important and potentially invalid assumptions. Most importantly it assumes no dilution of $^{15}N/^{14}N$ in the NH_4^+ pool over the course of the experiment. This is a particular concern for incubations done with waters from euphotic zone depths incubated in the dark, or for highly productive coastal systems. See Lipschultz 2008 and references therein for a discussion of these issues.

The timing of timecourse measurements needs to be empirically determined for each study system. It is unwise to rely only on endpoint measurements if you are working in a system for the first time.

Nitrification rates show a strong effect of light, thus experiments in the euphotic zone should be carried out with a consistent reference to the photoperiod under simulated in situ light conditions.

Rate calculations are very sensitive to the starting atom percent ^{15}N enrichment of the source pool, thus precise and accurate measurements of the starting ambient [NH_4^+] and careful preparation of the ^{15}N spike is essential.

The detection limit for most labs using the denitrifier method of NO_3^- isotope analysis require 10 nmol NOx- to make a measurement, which in practical terms is 10 mL of a 1 μM solution. Use this as a guide when determining what volume timepoint samples should be.

Useful reading:

Lipschultz, F. (2008) Isotope tracer methods for studies of the marine nitrogen cycle. In: *Nitrogen in the Marine Environment*, 2nd ed. Capone, Bronk, Mulholland, and Carpenter, eds. Academic Press.

Santoro, A.E., Casciotti, K.L., and C.A. Francis. 2010. Activity, abundance, and diversity of nitrifying archaea and bacteria in the central California Current. *Environmental Microbiology* 12: 1989-2006.

Ward, BB. (2008) Nitrification in marine systems. In: *Nitrogen in the Marine Environment*, 2nd ed. Capone, Bronk, Mulholland, and Carpenter, eds. Academic Press.

Protocol

For each depth to be sampled prepare:

- Three 250 mL or 500 mL polycarbonate bottles, acid-washed and MQ water rinsed; pre-labeled with depth and treatment ID
    - A: $^{15}NH_4^+$ addition
    - B: $^{15}NH_4^+$ addition
    - C: $^{14}NH_4^+$ addition or no addition control
- Time zero isotope sample bottles (20 mL scintillation vial or 60 mL screw top HDPE bottle), pre-labeled with bottle ID #.
- 0.2 µm-filtered 100 µM $^{15}NH_4Cl$ stock, P-200 or P-1000 pipette and filter tips
- 60 mL syringes with 0.2 µm syringe filters
- Ziploc bags for storage of syringe between time points.

When cast comes on deck, from each depth to be incubated:

1) Bring two 250 mL polycarbonate incubation bottles and one 250 mL control bottle per depth.
2) Rinse pre-labeled incubation bottle three times with about 20 mL of water from the appropriate depth; fill with 200 mL of sample water, repeat with other incubation bottle and control. The graduations on the bottle are of sufficient precision, but it is helpful to mark the fill line with a permanent marker prior to filling.
3) Place bottles out of the sunlight and/or on blue ice while remaining samples are collected.

When all water has been collected:

1) Add ^{15}N-NH_4^+ spike to A and B incubation bottles (~10% ambient NH_4^+ concentration or 100 nM if no NH_4^+, make two working stocks of differing concentrations), invert 5 times to mix. Note time that you start adding spikes for future reference.
2) Collect time zero isotope samples (20 or 50 mL volume) from each incubation bottle by pouring into 60 mL syringe with attached 0.2 µm PES syringe filter and pass into scint vial; freeze; note time and sample bottle # on datasheet. Save syringe in ziploc bag labeled with depth and treatment ID to reuse at next time point.
3) Place incubation bottles in environmental room or on deck incubator as appropriate.

Prior to each time point (T4, T12, T18 h for coastal waters; T6, T12, T24h for oligotrophic systems):

1) Place sample bottles in rack; prepare syringes and syringe filters for sample collection.

At each time point:

1) Remove incubation bottles from incubator.
2) Collect samples from each incubation bottle, filter, and freeze as above. Note time and bottle # on data sheet.
3) Return incubation bottles to incubator.

Between incubations:

1) Rinse incubation bottles 5 x with DI water
2) Rinse incubation bottles with 10% HCl. Cap and sit > 1 hr, up to overnight if possible.
3) Rinse incubation bottles 5 x with DI water.
4) Store with caps fastened.
5) If possible, try to re-use the bottles for same treatments/depths, etc in both experiments.

Please be conscious of the ^{15}N. This means:

1) Try to keep the frozen samples with ^{15}N separated from the natural abundance (NA) samples during storage, shipping, etc.
2) Plastic ziploc bags are helpful for organizing groups of samples by cast, time point, etc as needed. They help to keep ^{15}N in labeled samples and out of NA samples.
3) DO NOT bring the ^{15}N spike anywhere near the rosette.