The Role of Regenerated Nitrogen for Rocky Shore Productivity

Description

We evaluated the role of mussels by adding 15N-labeled NH4+ to an assemblage of tidepools where they were either present at natural abundance levels, or absent through manual removal. The role of phototrophs was separately examined by conducting these experiments both during the day and at night. The tidal height of pools varied between 1.2 to 1.5 m above Mean Lower Low Water (MLLW). Tidepools were thus isolated from each other as well as the nearshore environment during the low tide period when experiments were conducted. Each experiment included 4 to 5 mussel removal (MR) tidepools (since 2002) and 4 to 5 mussel control (MC) tidepools with natural mussel densities. In June 2010, we performed daytime NH4+ tracer experiments and in August 2010 nighttime experiments using the same tidepools. The following year (July 2011) these experiments were repeated with the addition of bottle incubations (see below) to evaluate the effects of suspended tidepool components and extended sampling for 6 days after the initial 15N addition to test for long-term retention of NH4+. During the 2011 experiments, unforeseen rain reduced the salinity in some pools by up to 51%, and we have attempted to correct for the expected dilution of NH4+ in our tidepool rate calculations.

Because isotope enrichment levels were relatively low, we used the conventional delta notation instead of atom% to describe variations in 15N enrichment (where delta-15NH4+ = {(15N:14N sample ÷ 15N:14N standard) -1}×1000‰, where the standard is atmospheric N2. Tracer labeled ammonium chloride (15NH4Cl) was added to the pools to approximate a 1000‰ enrichment in 2010 (doubling the 15N-NH4+ concentration) and a 2000‰ enrichment in 2011(tripling the 15N-NH4+ concentration). 15N natural abundance is only 0.365% and these tracer additions thus had a negligible effect on the overall NH4+ concentrations increasing them by only ~0.4% and ~0.8%, respectively. Tidepool volumes were estimated spectrophotometrically using varying concentrations of food dye (Pfister 1995). Together with estimates of NH4+ concentration (from 2009 data), we estimated the tracer addition required to achieve the targeted 15N enrichments. However, the actual initial enrichments varied substantially, 684.4 - 2406.4‰ in 2010 and 781.4 - 3880.2‰ in 2011, likely due to error in tidepool volume estimation and natural variations in initial NH4+ concentrations. Fortunately, we sampled immediately following each tracer addition allowing for the determination of the true initial 15N enrichment.

Prior to tracer addition at ebb tide, 100 mL of tidepool water was syringe-filtered (Whatman GF/F) into separate HDPE bottles for natural abundance 15NH4+ and concentration determination. To each pool, tracer 15NH4+ was then added and distributed by stirring with a stick. Water samples were immediately collected for measuring initial 15N enrichment and subsequently at 2, 4, and 6 hour intervals to determine isotope and concentration time courses. All water samples were frozen until analysis. Tidepool oxygen, pH, and temperature (Hach HQ4D) were also collected at ~ 2 h intervals throughout the experiment.

In 2011 we also assessed the contribution of the suspended microbial community to NH4+ cycling by enclosing tidepool water in a 250 mL transparent polycarbonate incubation bottle. Following tracer addition, the bottle was filled, then left to float in the tidepool for the duration of the experiment. Samples from bottles were filtered as described both immediately after containment and at the end of the experiment (~6 h later).

We assessed macroalgal contribution to NH4+ removal by transplanting two tidepool-dwelling algae species. Prionitis sternbergii were sampled 2 weeks prior to the experiment for baseline natural abundance 15N values and transplanted into the pools with Z-Spar Epoxy (Pfister 2007). On the day of the experiment, the red-alga, Corallina vancouveriensis from a single source patch, was also sampled for 15N natural abundance, inserted into pieces of Styrofoam, and floated in each pool.

At the end of each experiment (~6 h sampling point), we sampled tidepool particulate organic material (POM) by filtering through combusted GF/F filters until they clogged (~ 600 mL), comparing these samples with POM similarly sampled from the immediate nearshore. Floating Corallina spp. samples were collected into clean Eppendorf tubes, and similar sized pieces of Prionitis spp. were collected from each pool into clean foil packets.
We evaluated the extent of longer-term 15N tracer retention in 2011 by sampling tidepool water, POM and transplanted Prionitis 1, 3, and 6 days following tracer addition. We sampled at ebb tide and at again at slack water just prior to high tide on the first day after tracer addition (that is, 24 h later) and at slack water prior to high tide on Day 3 and 6.

Acquisition description

Laboratory analysis

NH4+ and NO3- concentrations were measured at the University of Washington Marine Chemistry Lab (methods from UNESCO 1994). Concentration values from 2011 were corrected for rain dilution using the change in salinity measured over the incubation period assuming no addition of NH4+ or NO3- to the pools from the rainfall.

NH4+ isotopic composition was measured according to a modified version of Zhang et al. (2007) after isotope dilution to less than 500‰ to prevent isotopic contamination of the natural abundance-level mass spectrometer system. Briefly, NH4+ is oxidized to nitrite using hypobromite then reduced to N2O using acetic acid buffered sodium azide before analysis on an isotope ratio mass spectrometer (IRMS). In modification of the prior method, pre-existing NO2- was removed prior to hypobromite addition by reaction with sulfamic acid. To a 20 mL sample volume, 340 µL 20 mmol L-1 sulfamic acid and 10 µL 10% HCl was added and allowed to react for 12 hours at room temperature. A second improvement was the addition of 6 mol L-1 HCl to reduce the pH of the sample below 7 prior to the addition of an azide-100% acetic acid reagent. Isotope determinations were made at U. Massachusetts Dartmouth using a GV IsoPrime IRMS, a custom purge-trap sample preparation system, and a CTC PAL autosampler. Reproducibility was better than ± 0.5‰.

Filters and algal samples were dried at 60°C for 48 h and elemental and isotopic analyses were made at the University of Chicago and at Yale University. Samples were run using a Costech 4010 Elemental Analyzer combustion system coupled to a Thermo DeltaV Plus IRMS via a Thermo Conflo IV interface (University of Chicago), or using the same Elemental Analyzer coupled to a Thermo DeltaXP Advantage IRMS via a Thermo Conflo III interface (Yale University). Reproducibility was better than ± 0.1‰.

Processing description

Data Processing:

Raw NH4+ isotope data were corrected for a background 'blank' using a simple mass balance equation (Zhang et al. 2007) to remove the contribution made to the signal height from reagent and diluent low nutrient seawater (LNSW) NH4+ contamination. Data were then calibrated using a standard curve derived from co-analysis of international standards National Institute of Standards and Technology United States Geological Survey 25 (-29.4‰) and 26 (+52.9‰), and International Atomic Energy Agency N1 (0.5‰) per Zhang et al. (2007). A final correction was made for the initial isotopic dilution.

Instruments List

1. **Isotope-ratio Mass Spectrometer:**

   Short name: IR Mass Spec

   PI supplied instrument name: IR Mass Spec

   Dataset-specific description:

   GV IsoPrime IRMS: Isotope determinations were made at U. Massachusetts Dartmouth using a GV IsoPrime IRMS, a custom purge-trap sample preparation system, and a CTC PAL autosampler. Reproducibility was better than ± 0.5‰.

   Generic description:

   The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

2. **Nutrient Autoanalyzer:**

   Short name: Nutrient Autoanalyzer

PI supplied instrument name: Nutrient Autoanalyzer

Dataset-specific description:

The nutrient autoanalyzer at UWashington was used to determine the nutrient concentrations in the water. Analyses and calibration follow the protocols of the WOCE Hydrographic Program using a Technicon AAII system.

For more information,
see http://www.ocean.washington.edu/story/Marine+Chemistry+Laboratory

Generic description:

Nutrient Autoanalyzer is a generic term used when specific type, make and model were not specified. In general, a Nutrient Autoanalyzer is an automated flow-thru system for doing nutrient analysis (nitrate, ammonium, orthophosphate, and silicate) on seawater samples.

3. CHN Elemental Analyzer:

Short name: CHN_EA

PI supplied instrument name: CHN_EA

Dataset-specific description:

Samples were run using a Costech 4010 Elemental Analyzer combustion system coupled to a Thermo DeltaV Plus IRMS via a Thermo Conflo IV interface (University of Chicago), or using the same Elemental Analyzer coupled to a Thermo DeltaXP Advantage IRMS via a Thermo Conflo III interface (Yale University). Reproducibility was better than ± 0.1‰.

These instruments were used to look at the mass composition and isotopic signatures of the algal and filter material.

Generic description:

A CHN Elemental Analyzer is used for the determination of carbon, hydrogen, and nitrogen content in organic and other types of materials, including solids, liquids, volatile, and viscous samples.