Protocols for measuring ammonium inside vs outside kelp forests

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# Background

The goal is to find out whether ammonium concentrations vary inside vs outside kelp forests!

Ammonium measurements are tricky because contamination can happen very easily, so please be careful with things touching each other.

# Materials needed in the field:

* 6 labelled 60 mL syringes inside one EXTRA Large ziplock bag
  + Inside 1, Outside 1, Inside 2, Outside 2, Inside 3, Outside 3
* Small red cooler full of crushed ice
  + Get ice from the dry lab next to the chem lab
* 6+ luer lock 0.45 um filter tips
* Notebook + pencil
* Nitrile gloves x2 pairs
* 13 x 100 mL brown Nalgene bottles (6 standard bottles, 6 sample bottles, 1 spare)
  + These bottles should either be acid washed OR still containing the last sample + OPA (the old OPA keeps them clean)
* A can-do attitude

# Field methods: Subtidal collection

* Once Team Kelp has established where their 4 kelp transects will run, we want to take ammonium samples at their first three transects
* These samples will be 5 meters apart, Inside and Outside 1 is closest to the 0 m end of the tape, and Inside and Outside 3 is closest to the 50 m end
* Take the XL ziplock bag of syringes down while the hydrophone is being set up, “burp” the ziplock at the surface to get the extra air out and make the bag less floaty
* Once the hydrophone and kelp transect locations have been established, start at sample 1.
* Note the depth of the RLS transect, then swim 5 meters into the kelp
* Note the depth at this spot, and decide an appropriate sampling depth based on an approximate average between the two
  + Goals: inside vs outside syringes must be at the exact same depth for each sample and samples shouldn’t be taken right on the bottom or in the middle of the water column
* Fill inside 1 syringe, then flush it, then fill it to just past 60 mL maintaining the target depth
* Put the syringe back in the bag, reel in the transect, and return to the RLS transect.
* At the same depth as inside 1, flush and then fill outside 1 syringe
* Repeat for samples 2 and 3, trying to keep all of them at the same depth (within a meter at least)
  + This makes it A) easier to remember the sampling depth
  + And B) makes it more likely that all samples have the same salinity and thus same matrix effects
* Return to the outside sample 2 location and fill and flush the XL ziplock with water at an average of all the sample depths
* Carefully close the ziplock and commit the depths of each syringe and the bag to memory, tell someone on the boat ASAP to write them down

# Field methods: Boat processing

The goal is to get the samples filtered and iced in a reasonable amount of time, filtering the standard curve water which comes from the ziplock bag is less urgent. Wear gloves, be careful not to touch the syringe tip or filter tip to anything, don’t breathe or shed hair into the bottles

* On the boat, carefully swish and empty the old working reagent and sample from a sample bottle
* Carefully open the ziplock and pull out a syringe, we will use the water in the ziplock later!
* Gently tap the syringe to get the bubbles out, screw a filter tip onto the syringe and push out the bubble this introduces
* Push 20 ml through the filter tip into dark Nalgene, shake to rinse and pour out. Push 40 ml into Nalgene, cap, place on ice in sample cooler (you will have to pour out the melted ice and scoop some out so the cooler is half full of ice
  + The 20 mL rinse is a target/approx but the **40 mL sample must be accurate**
  + Ensure the bottles get capped immediately after they have been filled with filtered water to reduce contamination
* Write down the syringe number, depth, and bottle number in the notebook
* Repeat for all 6 syringes, being careful not to spill the water in the ziplock
* For the standard bottles: Keep the last syringe used clean, and use it to pull 20 mL of water from the ziplock to rinse the syringe. Pull the plunger all the way to the end of the syringe to rinse the whole thing, then discard of the 20 ml
* Then pull 60 mL of water, tap out bubbles, screw on the filter tip, and filter 20 mL into a new bottle, rinse the bottle and discard the 20 ml, and filter the remaining 40 mL into the bottle and put on ice
* Repeat for all 6 standard curve bottles!
* If you don’t have time to filter the standard curve bottles just bring the ziplock bag of water back to BMSC and do it there. Make sure the bag is chilled on ice!

# Lab methods: Evening processing

* All of the bottles must be the same temperature! Refresh or add ice to the cooler as needed
* Step 1: calibrate all three pipettes
  + 1 mL of DI = 1 gram
  + Use the scale and DI to make sure the pipettes are accurate
* Pour out the old 200 uM NH4+ bottle in the wet lab
* Rinse the bottle with DI 5+ times, being careful to pour it down the drain and avoid contaminating the sink
* Shake any droplets of water out of the bottle once rinsed
* Add 490 mL DI to the bottle using a clean 100 mL cylinder
* Using the calibrated 10 mL pipette, add 10 mL of the concentrated NH4+ stock solution
* Using the 100 and 200 uL pipettes, add 0, 100, 300, 600, and 800 uL of 200 uM NH4+ stock to the standards-additions bottles
* Write down the bottle numbers and added volume of stock NH4+ on the fluorometry datasheet
* Turn off the lights and turn on Lamp 2
* Add 10 mL of OPA to the 5 standard-additions bottles, then spike the 6 samples as well. Leave the BF bottle unspiked. Write down the time when you spiked the samples.
* Shake bottles gently
* Leave the bottles on the counter and go home!

# Lab methods: Next morning processing

The following procedure is copied from Fiona Francis’s protocol:

Running a standard curve

* Make sure you have everything you need in the dark room, turn out the lights and avoid turning them on if any sample bottles are open as the sample will degrade very quickly with even a few seconds of light exposure
* This first step calibrates the fluorometer and creates your standard curve
* Before reading a sample shake the bottle well and keep the cap on as much as possible to avoid contamination
* Set up the fluorometer by placing the minicell adapter into the slot, closing the cap and turning on the device
* b) hit "cal" and then hit "enter" on the fluorometer
* Pipette ~ 200 ul of your zero sample into a minicell, wipe the minicell clean on the outside using a microfiber cloth (kimwipes scratch the minicells) and place the minicell into the adapter and close the lid (NOTE: to avoid bubbles place the pipette tip vertically into the minicell not on an angle and pipette out all the liquid quickly while lifting the pipette. This is a bit of an art and takes a few practices)
* hit "enter" again
* insert your standard. Use the 800 ul spike for this.
* You are aiming for a ratio of 5-35% between your blank and std
* after calibration hit the down arrow to keep the calibration and hit "diag" to get the %blank and %std
* After you have created your calibration ratio rad the rest of your standard curve. To read a sample you just hit "read" after you have placed it into the adapter
* As a rule of thumb: use a new minicell every time you read a sample that is likely a higher concentration than the previous one as it is very very hard to get all of the material out of a minicell

Running your samples

* Using the same process as for the std curve, read each sample
* All three readings must be within 3 flu units of each other. Keep running new samples in the same mini cell until you get three good readings
* After all of the samples are read, dispose of waste material down the sink with water running
* Throw out used gloves, minicells, and pipette tips into harzardous materials waste
* Keep all of the sample bottle closed and DO NOT throw out remaining contents as this will keep them sterile for the next use