Ammonium Fluorometric Protocol:
AEP 091912
Modified: MA July 03, 2019

Based on Holmes et al 1999, Can. J. Fish. Aquat. Sci 56: 1801-1808

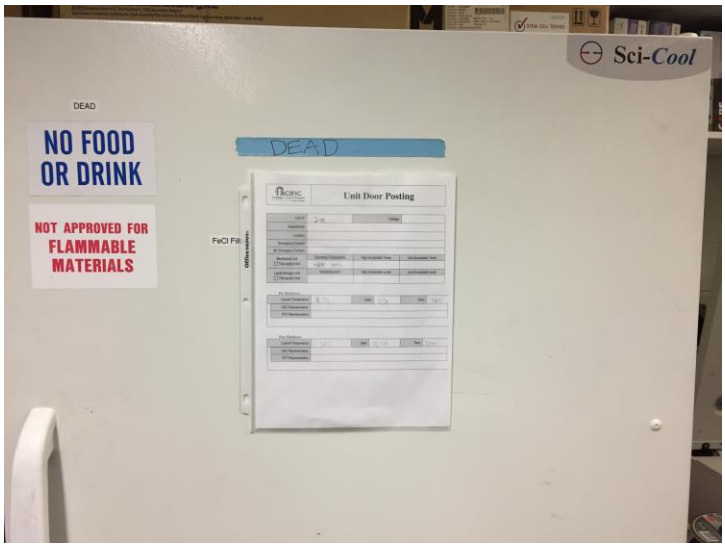
Ammonium is everywhere, so no shortcuts

FOR SPOT:
**Starting January 2015, we will be

- Making up standard curve the day before and keeping on ice in cooler
- Making 4x 0 nM blanks
- Collecting triplicate samples from all depths
- Collecting directly from Niskin bottles
    - No gloves
    - Open spout to allow for slow dripping
    - Wash hands in sample water
    - 3x rinse each triplicate 15 mL tube with sample water, rinsing Niskin nozzle as well
    - Collect triplicate samples to 12 mL
    - Keep in dark place
    - Add reagent at front of boat in shadow, away from RNA Later.
    - Throughout process, keep away from anyone smoking, fishing (especially squid) and ship exhaust.

FOR DAILY TIME SERIES (2019):

- Make the standard curve the morning of and keep it in the 4°C fridge (where the iron chloride filters are stored).



Making Fluorometric Reagent

General notes:

- ***Working reagent must age at least 3 days before use***
- **stable for at least 3 months when stored in the dark at room temperature, but in general discard after 3 months

1. *Borate buffer solution*
   - 2 L Erlenmeyer Flask
   - Stir bar
   - 40 g sodium tetraborate (large chemical bucket)
   - 1 L Barnstead DI water (use graduated cylinder)

Add together and put on stir plate. May take a while (~30 min) to dissolve

2. *Sodium sulfite solution*
   - 125 mL glass bottle
   - 1 g sodium sulfite, anhydrous
   - 125 mL Barnstead DI water (use graduated cylinder)

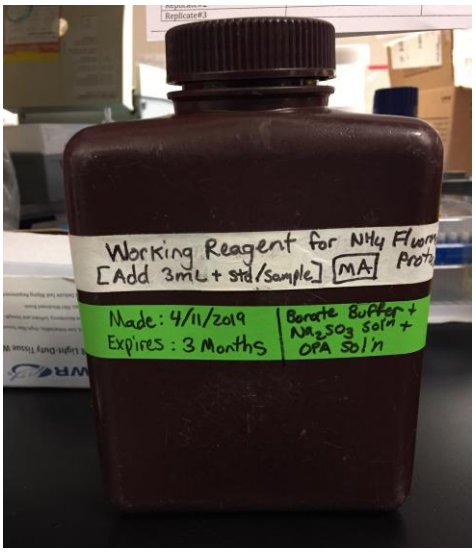
Add together and shake to dissolve. Stable for ~ 1 month when stored at RT in glass bottle.

3. *OPA solution*
   - 50 mL falcon tube wrapped in foil
   - 2 g phthaldialdehyde (aka, OPA)
     - In chemical fridge. Should be in original box or in foil, under desiccation if necessary
   - 50 mL 200 proof Ethanol (100%) (molecular grade not necessary unless nothing else available)

Add together and vortex to dissolve. LIGHT SENSITIVE

4. *Working Reagent* (**what is added to std curve and samples**)
   - 2 L brown polyethylene bottle
     - Stored with 5% HCl inside, so dump and rinse 3 x with DI water
   - 1 L borate buffer solution (all of solution made above)
   - 5 mL sodium sulfite solution (discard remaining solution made above)
   - 50 mL OPA solution (all of solution made above)

Add together and invert to mix.



Making Standard Curve

- Make 12 mL standards of the range in which you wish you measure: I go from 1000nM to ~7nM, plus a blank. I use fresh MQ water from Manahan lab, for the blanks and as the diluent, our Barnstead water has ammonia so don't use, and test any new water source before committing.
- If you really want a seawater version, 0.2 um filter aged seawater (filter about 1liter or 2, aged seawater, I suggest testing it before you use it as a blank because ours always became contaminated with ammonia).
- For the MQ water standards, just make sure all bottles and tubes you used are clean and have been rinsed with MQ 3x, alternatively I have heard that keeping the working reagent in the tubes cleans away ammonium as well. Make sure all this happens in an area that is ammonium free, in our lab we have to stay away from areas where ammonium acetate or RNA Later is used.
- Make sure you label everything well, because it is easy to mix up the standards.

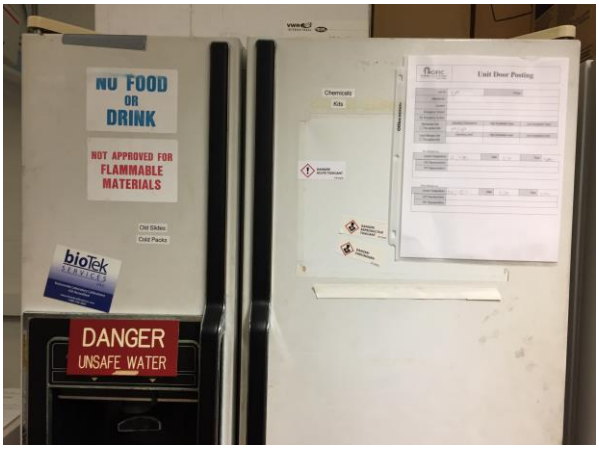
A. Get MQ water from the Manahan lab

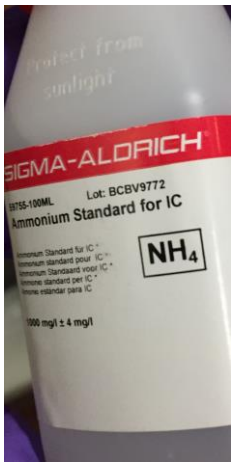
- Bring up the following to the Manahan lab:
  - **9**- 50ml conical tubes (labeled "10X Working Stock", 7, 15, 31, 62, 125, 250, 500, 1000)
  - **20**- 15ml conical tubes:
    - 4 replicates of 0
    - 2 replicates of 7, 15, 31, 62, 125, 250, 500, 1000
  - Graduated cylinder
- Rinse **everything** 3x with MQ water
- Fill 50-mL "10X Working Stock" tube to 50 mL using graduated cylinder.
- Fill 50-mL "1000" tube to 45 mL using graduated cylinder.
- Fill remaining 50-mL to 25 mL.
- Fill all four blanks "0" 15 mL tubes to 12 mL.
- Back in lab, place on bench next to PCR hood (keep away from NH4 standard, instructions below)

B. NH4 standard

10X Working Stock: [10uL Ammonium standard + 50 mL MQ water]

1. Gather materials:
   - **1**- 50 mL Falcon tube labeled "10X Working Stock" rinsed 3x with MQ water from Manahan Lab and filled with 50 mL of MQ water from the previous step.
   - Ammonium Standard for IC, Sigma-Aldrich [1000 mg/L in 100 ml; ≈ 0.055 M] Located in the chemical fridge 4°C





2. Add 10 uL of the Ammonium standard to "10X Working Stock" Falcon tube.

   Note: Do this step far away from the rest of the standard curve to prevent accidental contamination.

   ***** 10X Working Stock should be re-made every SPOT. Discard after use. *****

C. Standard curve

1000 nM Standard [5 ml 10X Working Stock + 45 ml MQ Water]

1. Add 5 ml of the 10X Working Stock to the "1000" 50 mL tube that is already filled with 45 mL of MQ water.

- You are going to make the standards in the 50ml falcon tubes, then add 12ml of each standard into the 15ml falcon tubes. (you could use a serological pipet to be more accurate, but I just pour and use the volume marks on the falcon tubes)

- I do a serial dilution from the 1000nM standard, so in the volumes below the "of Previous" refers to the standard before it, except the 1000nM since you just made it.

Standard (nM)	of Previous (ml)	Water (ml)
1000	50	0
500	25	25
250	25	25
125	25	25
62.5	25	25
31.25	25	25
15.625	25	25
7.8125	25	25

- Once you have the standards, add 12ml of each standard into corresponding 15ml Falcon tubes. Once again make sure all the tubes are rinsed, because it will mess up the standards if you don't.

- Make sure you cap the tubes well.

- Leave the standards in the 4°C fridge (where the iron chloride filters are), until the samples are ready.

Adding Reagent to Samples and Standard Curve

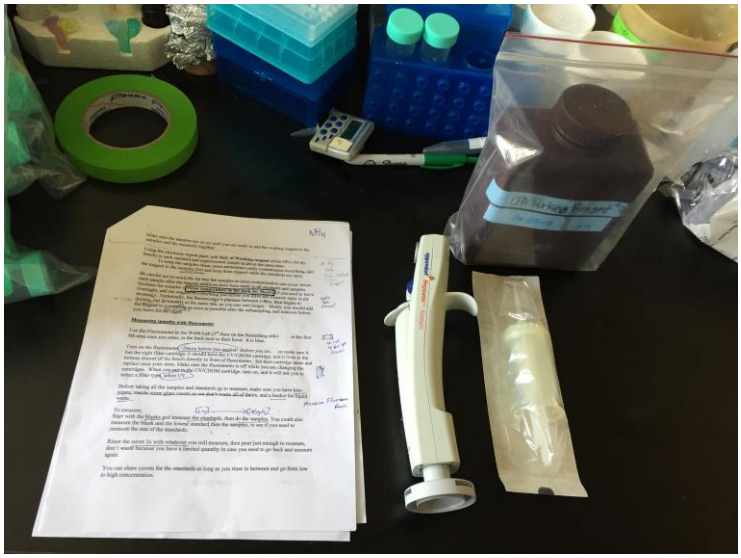
Make sure the samples are on ice until you are ready to add the working reagent to the samples and the standards together.

Using an electronic (fully charged) or mechanical repeat pipettor and a 50-mL combitip, add **3 mL of Working reagent** of the OPA to each standard and experimental sample at about the same time.

Note: Make sure to check the repeating pipettor before taking on a cruise and bring a backup P1000 pipette.

Record the time.

TIP: Pour out the OPA Working Reagent in a disposable plastic reservoir, then use the repeating pipettor to draw up 50 mL of solution. The pipet should be set on "Disp." for dispense. Normally it is set to dispense 1 mL at a time, but can be changed to dispense 3 mL if you wish.



To keep the samples clean, since ammonium easily contaminates everything, add the reagent to the **samples first** and keep them capped while the standards are open.

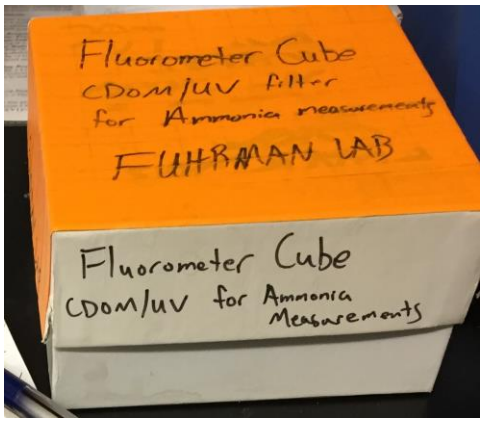
Be careful not to touch the tip into the samples as cross contamination can occur. Invert each sample after the reagent additions have been made to all standards and samples.

Incubate the samples at **room temperature in the dark for 3 hours** (if you need to leave overnight, add the reagent to everything just before you leave and measure early in the morning). Technically, the fluorescence's plateaus between 3-8hrs, then begins to decline, but fortunately at the same rate, so you can wait longer. Ideally you should add the reagent to everything as soon as possible after the subsampling, and measure before you leave for the night.

Measuring samples with fluorometer

Use the Fluorometer in the Webb Lab (3rd floor on the Heidelberg side). It is in the first lab area once you enter, in the back next to their hood. It is blue.

Turn on the fluorometer **~20min before** you need it. Before you turn it on make sure it has the right filter cartridge, it should have the UV/CDOM cartridge, and it lives in the basket next to the sink closest to the weigh scales in our lab.



When you put in the UV/CDOM cartridge, turn on, and it will ask you to select a filter type, select UV.

Checklist:

- ☐ Kim wipes
- ☐ Waste container
- ☐ DI Water squirt bottle
- ☐ Cooler with Ammonium samples + standards (keep in dark!)
- ☐ Sheet to record data.
- ☐ Webb lab key

To measure,
Start with the blanks and measure the standards, then do the samples.

Rinse the cuvette 3x with whatever you will measure, then pour just enough to measure, don't waste because you have a limited quantity in case you need to go back and measure again.

You can share cuvettes for the standards as long as you rinse in between and go from low to high concentration.

Wipe the bottom of the cuvette, and place in the fluorometer.

Press "measure raw fluorescence"

Record the number, and this is what you will use to plot up the standards vs. the concentration in excel.

Do the same for the samples, and figure out the unknown concentrations, using the trendline and intercept equation. I don't trust anything below 10nM, so record these, but if the curve is not linear at the lower end, I would just record this as 0nM or Below Detection Limit in the excel sheet.

Add the concentrations to the excel sheet in the SPOT dropbox
File: WTSS_NH4_measurements.xlsx

PLEASE charge the electronic repeating pipet!