Appendix A

SALT POND WATCHERS

LAB PROTOCOL MANUAL

for the

URI GRADUATE ASSISTANT

Virginia Lee
Joseph J. Schultz Tokos
Nancy Craig
Julia McMahon
Elena Martin
and
David Avery

Coastal Resources Center
Graduate School of Oceanography
University of Rhode Island
Narragansett, RI
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This manual is the result of years of testing sampling methods by Salt Pond Watchers, some of whom have volunteered their time to sample Rhode Island's salt ponds since 1979. Their careful work and dedication to improve the environmental quality of the salt ponds has provided and unprecedented data set on the salt ponds and valuable information for state and town decision makers. We found the Citizens Monitoring Manual developed by Kathy Ellett of the Alliance for the Chesapeake Bay to be very useful and have adopted their format and some of their text.

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Virginia Lee
Joseph J. Schultz Tokos
Nancy Craig
Julia McMahon
Elena Martin
David Avery
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PREFACE

Welcome to the Salt Pond Watchers graduate assistantship. The manual is designed as guidelines for the graduate assistant. It is an introduction to the Salt Pond Watchers program, as well as a quick reference guide for duties and lab procedures. The manual is divided into three basic lab procedures: 1) bacteria analysis; 2) water chemistry analysis; and chlorophyll analysis. There is an additional section on data management. This manual does not contain field protocols for bacteria and nutrient sampling which the graduate student assistant should also become familiar with. Field sampling protocols are all contained in the Protocol Manual for Salt Pond Watchers, CRC Technical Report #12.

INTRODUCTION

Rhode Island's coastal salt ponds have several characteristics which make them prime candidates for ecological monitoring. These characteristics are related to their physical and biological properties as well as to the fact that they are an attractive location for coastal development.

The salt ponds are shallow lagoons along Rhode Island's ocean shore. They receive water from groundwater and overland runoff and from tidal flushing from Rhode Island Sound. Daily flushing only exchanges a small percentage (about 10%) of each ponds' volume. Restricted flushing combined with nutrients draining from the surrounding watershed are the basis for the high productivity of the salt ponds.

The salt ponds are an attractive site for vacationers and local residents. People come to the ponds for fishing, quahogging, oystering, boating, swimming, birdwatching, and picnicking. Coastal development along the ponds has accelerated over the past several decades[1] and all indications suggest that it will continue to do so. Increased development along the ponds means higher nutrient loadings in the ponds from leaky septic systems, runoff from stormwater drains, fertilizer runoff from lawns, and animal feces. Nuisance algae blooms, eelgrass wasting disease and other signs of eutrophication have been associated with high nutrient inflow into coastal waters and contamination of shellfish beds has been associated with high sewage levels. Monitoring bacteria and nutrients in the ponds give an indication of changes in water quality. Measuring other parameters such as chlorophyll and eelgrass wasting disease gives evidence of how life in the ponds is reacting to changes in water quality. Evidence of serious eutrophication and bacterial contamination are signs that the type and magnitude of development around the ponds must be managed to keep the ponds healthy and safe for the activities for which they are so highly valued.

The Salt Ponds also have a long tradition of important field sites for research. They have been the subject of many graduate research theses, undergraduate class projects and major research projects. Basic ecology has been described in Ecology by Conover in 1961 and

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[1]Olsen, S. and Lee, V. 1984. Special Area Management Plan for the Salt Pond Region. RICRMP. Coastal Resources Management Council.

by Nixon, Harlin, Spaulding, Crawford, Lee and others in Estuaries in 1985. Geological processes are discussed by Boothroyd et al in 1988.

The Salt Pond Watchers program recruited its first volunteer monitors in 1979 as part of the URI Salt Pond Study with the institution of routine sampling in 1985 it became one of the first marine water quality programs to involve citizens in environmental monitoring of coastal marine systems. Each year, in Rhode Island's program, about 40 citizens donate their time to monitoring water quality in the salt ponds to assess whether conditions are getting better or worse. The information that is generated is used by state agencies for management decisions, by local municipalities for planning and zoning decisions, and by researchers at URI and local high schools. The role of the graduate student assistant in the Pond Watcher program ranges from organizing of volunteers to laboratory analysis of samples. More important, however, is the invaluable input the graduate student contributes to the final analysis and presentation of the data collected by the volunteer force.

PART 1: BACTERIA LAB ANALYSIS

BRIEF PROJECT OVERVIEW

Bacterial contamination is a major concern. It is important that shellfish harvested from the salt ponds are safe to eat and that it is safe to swim, wind surf or water ski without danger of pollution by disease causing organisms. On-site sewage disposal from rapid expansion of residential development has intensified the problem over the last few years. As a part of a long term monitoring effort to quantify the impact of the increased demand on pond resources, volunteers collect bacterial samples every two weeks from May to October. In accordance with national guidelines, bacterial contamination is assessed according to the concentration of coliform bacteria in the water. Two labs have offered their services free of charge in support of the Salt Pond Watcher Program in the east: Rhode Island Department of Health (DOH) on Orms St. in Providence and the Federal Food and Drug Administration, (FDA) at Quonset-Davisville in North Kingstown. Bacteria samples are collected in the morning on the assigned day of the sampling week from Salt Pond Watcher coordinators and taken to the appropriate lab for inoculation and analysis. Bacteria samples must be kept cold until processing (ice packs in a cooler is sufficient). Samples must be processed within 6 hours of collection to assure sample integrity. Directions to these labs are in the Appendix.

Every other Wednesday, samples are picked up from each pond coordinator by the graduate student and taken to the FDA lab in Quonset. All sample preparation and processing is done by the graduate student picking up the samples. Media is made up the Friday before (refer to appendix for recipe). People at the FDA: Bob Wetherell, Director, Diane Reitz, and Linda Chandler (lab personnel).

Before 1991 the RI DOH was used. Specific DOH procedures are described in the appendix.

BACTERIA ANALYSIS AT THE FEDERAL FOOD AND DRUG ADMINISTRATION LAB (294-2561)

1. Prepare Medium

Medium is made before samples are collected. It must be done at least one day and less than six days in advance of using it. Directions for making the A-1 medium are in the appendix, as well as in the pond watcher data notebook (to be left at FDA for the duration of the sampling season). Allow three hours to make the media (including travel time to and from the lab, media preparation and cleanup). Reserve a car with GSO maintenance.

- Be neat.
- Be sure to rinse all the counter-tops in 70% ethanol, (ETOH) an effective disinfectant.
- After you make the media up--check the pH. It should be around 6.9 $\pm$ 0.2. There is a meter and the necessary acids/bases in the lab. It is usually best not to adjust the pH with acids or bases unless absolutely necessary.

2. Set Up Test Tubes with Medium

It will take a couple of hours to make the medium. There are usually 23 samples for the Wednesday sampling so you will need about 264 tubes (21 samples x 12 tubes/sample = 252), plus a few extras for screw ups (one row of 12 is sufficient). The test tubes you will use are 16x150mm and 6x50mm (Durham) culture tubes. Use the racks which hold 6 rows of tubes. This is about 3 2/3 racks of tubes (six row racks, twelve tubes per row). Before you make up the media, check your schedule to verify the number of samples you will be running. Conflicts with holidays will cause some shifting in the schedule. When you set-up the racks of test tubes place a Durham tube (small glass tube used to trap CO_2 emitted by the bacteria) into each test tube, open end down. Use the automatic pipet to fill each test tube with medium. Every tube should contain 10 mls of medium. Once filled, the test tubes get capped and autoclaved. All the tubes with medium must be autoclaved for 10 minutes at 121°C. Someone in the lab will run the autoclave. It is a good idea to learn how to operate it because you will need it all summer, but it is a tricky instrument. They usually like to do it themselves.

3. Process Samples

Processing of water samples for fecal coliforms should be done within 6 hours of collection. Starting at 8:30 am you will collect samples from salt pond watchers in the morning, take the big cooler and freezer packs. You will arrive at FDA around 11:00 am on Wednesday with the samples.

- Get data sheets from black "Pond-watcher's" binder stored at FDA. Sample sheets are numbered sequentially starting with 1 at the start. For each sample fill out a sheet with:

Lab #: Date:
Pond:
Sampler:
Station #:
"A1" media used

- Get media tubes from the storage cabinet--make sure the white tape says "sterilized" (the special tape shows visible black stripes when autoclaved). Each rack holds 6 samples (12 tubes/sample), have 12 extra for slip-ups, catastrophes. Label tubes with FDA sample number.
- Shake sample (violently) 25X
- Pipet 1 ml of sample into each of 12 tubes for a total of 12 tubes per sample. Don't touch tips of pipettes with hands.
- Take samples and put into air incubator at 35°C for 3 hours.
- Note time and ask Linda or Diane to put them in the 44°C water bath after 3 hours. If the lab people are not to busy they can usually switch the samples for you.
- Clean up:
  1) Put pipettes in the "discard" box.
  2) Mark extra tubes with blank white sterilizing tape and put in other room to be autoclaved and discarded.
  3) Pour remaining pond water samples down the sink and throw away sample bottles.
  4) Clean off counters with 70% ethanol.

4. Read the samples Thursday afternoon

- Bring a notebook to record results. The black binder with the data sheets stays at FDA lab. The samples must be incubated for at least 24 hours (time starts when the samples go into the air incubator at 35°C).
- Remove samples from water bath and look for gas bubbles in the Durham tubes inside test tube. If sample is cloudy and bubbles are present, that indicates a positive result. Fill in a "+" in the space on the data sheet for that sample. If there are no bubbles present it indicates a negative response. Fill in a "-" in the appropriate space on the data sheet to indicate a negative response.
- Determine the most probable number of fecal bacteria. The number of positive results recorded from the dilution series for one sample indicates that there is a 95% chance that there are "X" number of bacteria present in that particular sample. To determine

the most probable number (MPN) of fecal coliform bacterial colonies per 100ml sample, count up the "+'s" and use them to find the MPN value in the chart in back of the black binder. The MPN chart is also located in the appendix of this manual.

- Fill in data sheet with the MPN value. The FDA method gives us fecal coliform estimations only. The DOH media and method gives us both total and fecal counts.
- Be careful with the live tubes; they are active cultures of potentially toxic organisms. Mark these racks with a piece of white sterilizing tape. Put on trolley in other room. You may be asked to autoclave and dispose of your used tubes.
- Clean up: Wipe down all counters with 70% ethanol.
- Copy data sheets to bring back to CRC. Originals stay in the FDA lab.

DATA PROTOCOL APPENDIX

You can enter the data into the Salt Pond Watchers computer data file or mail a xerox copy of your lab results to Sue Nardone, Volunteer Data Manager, for her to enter into the computer file. At the end of the season she'll send a work disk of all the bacteria data. File the lab sheet at CRC Salt Pond Watchers file.

Waterfowl. Note on the "bacteria tables" tabulations whether the pond watcher reported presence of waterfowl at his/her station. You will find this info on the "field data sheet" for that station. Note the distance and number of birds.

PART 2: WATER CHEMISTRY LAB ANALYSIS

Brief Project Overview

The Water Chemistry protocols for Pond Watchers involves several steps: 1) organizing equipment and keeping volunteers supplied with sampling equipment and nutrient bottles throughout the active season, 2) periodic pick-ups of frozen samples and chlorophyll filters, 3) analyzing for nutrients, salinity, and chlorophyll, 4) quality control of volunteers, 5) entering and analyzing data, and 6) restocking supplies for next season.

Water chemistry samples are taken every other week at several stations each on the major salt ponds and at one station on Maschaug pond. (See Water Chemistry Station Maps in Pond Watchers Field Manual.) In the "water" months, November to April, each pond is sampled once a month at only one station. All pond watchers who sample for water chemistry test for temperature, chlorophyll, salinity, and nutrients as well as take down information on tide, cloud cover, and boating and waterfowl activity (See Field Data Sheet). Volunteers who sample deeper stations also take Secchi depth readings and dissolved oxygen (DO) tests with a LaMotte field kit.

The summer sampling season is usually begun with a spring kick-off meeting where supplies and sampling schedules are distributed to the Pond Watchers and spirits raised for the upcoming season. This meeting takes careful preparation, establishing the year's sampling schedule and counting out the exact number of supplies to be distributed to the Pond Watchers. A wise rule to remember is that people hoard, so keep track of every single gasket and sample bottle that is handed out. Nancy Wetherell, the volunteer pond watcher coordinator, is invaluable in preparing for the kick-off meeting.

In the first few weeks of the season, Nancy Wetherell and the graduate assistant go out on station with each Pond Watcher to verify the station location and to do a Quality Assurance/Quality Control check on the Pond Watcher. Nancy or the graduate assistant filters 3 chlorophyll samples and takes one nutrient sample right beside the Pond Watcher; both sets of samples are analyzed and comparisons done to determine the range of accuracy.

NUTRIENT ANALYSIS LAB PROTOCOL

Introduction

Nutrients are analyzed in Scott Nixon's lab using the Lachat FIA autoanalyzer. This is a complicated procedure which will take at least one training session, depending on your chemistry background, before you attempt a run yourself. For training session and for scheduling to use the autoanalyzer, speak with Betty Buckley, the technician in that lab. Betty is usually in the lab on Mondays and Tuesdays and part-time Wednesdays and can be reached at X6619.

Some helpful bits of advice. Make up artificial seawater and reagents in advance and have clean sample tubes in the oven ready to go. It is a psychological boost to have everything ready to go when you come in to start-up the machine in the morning.

The majority of the samples are run with artificial seawater (29ppt) as the carrier and standards. When running rain gauge samples or nearly fresh pond samples (Maschaug Pond, for example) substitute deionized water (DI) for ASW.

The background method is programmed in the machine as no. 19 for nitrate plus nitrite and phosphate.

Recipes for standards and reagents are included in this manual to guide you. However, these are occasionally revised by Betty, so it is best to follow the protocols on hand in the lab.

Start-Up Procedure for the Autoanalyzer

1. Check levels of artificial seawater (ASW) and reagents. Reagent receipts are on Table 1. (These are best prepared in advance so that you are ready to run when you arrive in the morning.)
2. Pull samples to be run that day out of the freezer to thaw. If you get an early start and everything is running smoothly, it is possible to run 50-60 samples in a day.
3. Switch on computer master switch.
4. Switch on machine master switch and allow to warm up.
5. Turn heater power up to 100% to warm up. Make sure water level is high. When machine is warmed up, turn heater back to 40% to save heating coil.
6. Make up standards. (Table 1) Pond Watchers background method only uses one set of standards. Nitrate, nitrite, and phosphate all go in the same standard. See standard serial dilution in the lab manual.
6. Load background method on computer by going to Load Background Method on the main menu. Hit Return. Key down to method no. 19 and hit Return again.
7. Set pump on minimum and snap modules onto pump rollers. Take pump off minimum.
8. Run D.I. H2O through all lines. Check for leaks.
9. Degas ASW and phosphate reagents with helium. Helium tank is in room 114.
10. Add 3 mls of laurel sulfate per liter to phosphate carrier and reagents.
11. Check that green valve light is on, then place reagent and carrier lines in proper bottles. Cover openings with parafilm.
12. When all air bubbles have passed through the lines, put pump on minimum and hook up cadmium column. (Make sure valve lights are on green.) Take pump off minimum.
13. Set baselines on NO2 + NO3 and PO4 channels using baseline adjustment controls.
14. Set fine gain. Manually put sampler needle into culture tube of high standard. Gently press valve lights to red on channels 1 and 3, wait 30 seconds and switch lights back to green. Adjust fine gain control up or down so that high standard peak fills window without going off the screen.

Calculating standard curve

15. You are ready to calculate your standard curve. Fill culture tubes with standards A through E and place in properly labeled holders in the sample tray. Fill a tube with ASW and place it in the sample holder marked "F". Fill another tube of ASW and place it in sample holder no. 1.
16. On main menu, move cursor to Load Sample Tray Information. Return. Return again. There is no need to label tray--the tray is automatically assigned a sequence number. Enter ASW next to no. 1 on the screen and hit [Esc]ape.
17. Move cursor to Start analysis. Return. Place cursor next to option for SAMPLES and STANDARDS. Check that holders A through F and 1 are full of samples and that the sample needle is set on the automatic arm. Hit Return.
18. Hit [Ctrl] and [Esc]ape together to display peaks. Watch screen for any irregular peaks which you might want to remove later.
19. When curve is complete, you will be given the option to [R]ecalibrate or [G]o on. First hit [Ctrl] and [Esc] keys together and move cursor to Display Calibration Data.
20. Check Nitrate and Phosphate channel curves for outliers. Delete any points which should be removed and print curve information.
21. Return to screen ([Ctrl] and [Esc]) and hit [G] to go ahead.

Running samples

22. Enter sample tray ID.'s and fill sample tray with a batch of samples. The 20th sample is a Check Standard (Stnd). Fill cup with stnd. B and place in holder 20. Make sure tray I.D.'s match up with samples in the tray.
23. Move to Start Analysis and select option for SAMPLES ONLY. Hit Return. You are running!
24. Press [Ctrl] and [Esc] to display peaks.

Recording results

25. The data printout will be in the form of two columns, one for NO2+NO3 and one for POx concentration readings; with three entries for each sample cup, reading 1, reading 2, and an average ("*2*").

26. While running, keep your eye on the peaks and periodically check the data printout. If an air spike is visible on the screen, or the read out for a particular sample is marked with an "A" and the sample should be rerun.
27. If the replicate runs of a particular sample vary more than 0.1 uM, fill another tube with fresh sample and include it on the next tray of samples.
28. Barring the event of an air spike or a large variance in replicates, the average reading, marked by a "*2*" on the data printout, is used as the raw nutrient concentration.
29. In the case of very low POx concentrations, the peak will often read as negative. This is corrected by comparing to Sargasso Seawater (SSW) which tends to have lower phosphate concentrations than ASW made up with DI in the lab. Run a sample of SSW at the beginning or end of a day of running samples. Calculate the correction factor necessary to bring the SSW sample up to a 0.00 reading. Use that correction factor to correct all readings for that channel for that day. The correction factor for the POx channel is usually between +0.12 and +0.20.

    Note: SSW from Kester's lab is the most trustworthy, but it is in short supply. Ask Betty Buckley before using it. The NO2+NO3 channel is usually within 0.02 of the SSW sample and does not need correcting.

30. Enter the corrected readings for nitrates and phosphates for each sample next to its pond, station, and date on a data sheet; include salinity for each sample; and give a copy of the completed sheets to Suzanne Nardone for entering into Excel.
31. Put the printout from the autoanalyzer and your original completed data sheets in the "Nutrients" black ring archive binder at CRC.

Table 1. Nutrient Standards and Reagents

I. Standards
A (5 umol) - add one ml of NO_2, NO_3, and PO_4 standards and fill to 200 mls with ASW. Stopper and invert several times to mix.
B (2 umol) - fill graduated cylinder to 80 mls with Standard A and pour into flask. Fill to 200 mls with ASW and mix well.
C (0.5 umol) - Add 25 mls of B and fill to 100 ml mark with ASW. Mix
D (0.2 umol) - Add 40 mls of C and fill to 100 ml mark with ASW. Mix
E (0.1 umol) - Add 50 mls D and fill to 100 ml mark with ASW. Mix.

II. Reagents
Artificial Seawater (ASW)
In a 4 L volumetric flask, dissolve 109.6g NaCl, 35.0g $MgSO_4+7H_2O$, and 0.75g $NaHCO_3$. Fill to the 4 L mark with DI H_2O.

NO_2 +NO_3 Reagents

Ammonium chloride buffer, pH = 8.5
To a 1 L volumetric flask in a fume hood, add 500 ml DI H_2O, 105 ml concentrated HCl, 95 ml ammonium hydroxide (NH_4OH), and 1.0g disodium EDTA. Dissolve and dilute to the mark.
Note. Do not make up NH_4Cl buffer in Room 112.

Sulfanilamide color reagent
To a 1 L volumetric flask add about 600 ml of DI H_2O. Then add 100 ml of 85% phosphoric acid (H_3PO_4), 40.0g sulfanilamide, and 1.0g N-(1-naphthyl)ethylenediamine dihydrochloride (NED). Shake to wet, and stir to dissolve for 20 minutes. Dilute to the mark, and invert 3 times. Store in a dark bottle. This solution is stable for one month.

PO_4 Reagents:

Stock ammonium molybdate solution
By Volume: In a 1 L volumetric flask, dissolve 40.0 g of ammonium molybdate tetrahydrate [$(NH_4)6Mo7O24-4H_2)$] in approximately 800 ml of DI H_2O. Dilute to the mark. Store in plastic and refrigerate.

Stock antimony potassium tartrate solution
By Volume: In a 1 L volumetric flask, dissolve 3.0 g of anitimony potassium tartrate [potassium antimonyl tartrate hemihydrate K(SbO)C4H4O5-1/2H2O] in approximately 800 ml of DI H2O. Dilute to the mark. Store in a dark bottle and refrigerate.

Molybdate color reagent
By Volume: To a 1 L volumetric flask add about 500 ml DI H2). Add 35.0 ml of concentrated sulfuric acid (CAUTION: The solution will get very hot!). Swirl to mix. When it can be comfortably handled, add 72.0 ml of the Stock antimony Potassium Tartrate Solution (PO4 reagent #3 above) and 213 ml of the Stock Ammonium Molybdate Solution (PO4 reagent #2 above). Dilute to the mark. Degas with helium.

Ascorbic Acid reducing solution
By Volume: In a 1 L volumetric flask, dissolve 18.0 g ascorbic acied in about 700 ml of DI H2O. Dilute to the mark. Mix. Prepare fresh. Degas.

SALINITY LAB PROTOCOL

Salinities are analyzed in the lab using an optical refractometer. Salinities are run at the same time that samples are thawed for nutrient analysis on the autoanalyzer.

There is a refractometer available in Scott Nixon's lab. This instrument is calibrated 1 ppt below the actual level. When using it, add 1 ppt to each reading before recording it.

Place a drop of sample on the refractometer, cover with plastic "lid," aim towards the light and read the salinity value where the refracted line meets the scale. Rinse with deionized water from squeeze bottles, dry and repeat with next sample. Take care not to dilute the reading with rinse water. Record the results on the nutrient data sheet.

Part 3: CHLOROPHYLL LAB ANALYSIS PROTOCOL

Introduction

The amount of chlorophyll on a filter is measured by extracting the pigment with acetone and measuring the fluorescence emitted when the acetone solution is struck by a beam of ultraviolet light using a fluorometer. Not all of the pigment measured initially is chlorophyll *a*, the extracted sample is read in the fluorometer first, then a few drops of acid are added to destroy the chlorophyll; chlorophyll concentration is estimated from the decrease in fluorescence: the remaining fluorescence is due to non-photosynthetically active pigment called phaeophytin. The fluorometer we are currently using is a Turner Designs fluorometer in Ann and Ed Durbin's lab (Aquarium building) and the procedure described below are specific to this machine.

In many laboratories, chlorophyll is extracted by grinding the fibers with a pestle, in acetone. We have recently adopted a procedure used by the Durbin's, in which the filters are soaked in cold acetone for 12-24 hours in order to extract the pigments. Tests done by the Durbin's with bay water samples, and by former Pond Watcher assistant Sharon Larimer with salt pond samples indicate that soaking is as effective as grinding. The new procedure has the added advantages of being much less time consuming than grinding and reduces the worker's exposure to acetone.

Sample preparation (Day One)

Before you start, make sure that no one in the Durbin's lab is planning to use the fluorometer at the same time you are planning to read your samples on the next day.

You will need:

1. 1 or more plastic test tube racks
2. adhesive dots for marking test tube rack
3. disposable glass test tubes VWR
4. test tube caps
5. blunt filter forceps
6. a spatula
7. 1N HCl (in bottle with droppers near fluorometer,)
8. Check to be sure there is 90% acetone in the dispenser bottle in the hood.

NOTE: Frozen filters, individually wrapped in tinfoil, are kept in S. Nixon's freezer in the basement of Horn once they have been collected from the Pond Watchers. It is helpful to put them in some kind of a logical order before analyzing them rather than spend time unscrambling the data afterward.

Take Pond Watcher samples out of the freezer and arrange them in order by station, and by date; Each sampling station and date normally has three replicates. Make a row of three tubes in the test tube rack. On a data sheet assign a number to each tube, and write in pond, station, date, and filter number (1,2) for each corresponding sample. Using adhesive dots, mark every other row of the rack with the number of the first tube of that row, e.g.. row 1 starts with tube 1, row 2 (not marked) with tube 4, row 3 with tube 7, etc. Do this carefully, otherwise you may read a sample and not be able to tell where or when it was taken!

Now turn off the lights, and lower the blinds (chlorophyll is light sensitive.) Open the chlorophyll packets, being careful not to tear the filters, and using blunt forceps place each filter in the bottom of the appropriate tube. Using the dispensing bottle in the hood, add 5 ml of 90% acetone to each tube, and cap the tubes tightly. Cover the rack completely with aluminum foil and leave it overnight in the refrigerator (not the freezer!) in the Durbin's laboratory. Turn on the fluorometer (or else wait 3 hours for it to warm up the next day).

Measurement (Day Two)

The fluorometer should have warmed up for at least 3 hours before reading samples. The first step is to calibrate the machine: fill one test tube with 5 ml of 90% acetone as a blank. Two other standards: an HCl blank and a copo reagent blank are run for calibration. These solutions are generally kept prepared in beakers in a cabinet below the fluorometer (the COPO solution is in a beaker labelled "0.005." Keep this in the dark when not using it.)

The fluorometer has two sensitivity settings, 1x or 100x. Scale can be set at 1, 3.16, 10, or 31.6 using the switch marked "STEP". To read a tube, lift the cap for the sample port, insert the tube, and replace the cap. Wipe tubes with a ChemWipe to remove fingerprints and condensation before inserting.

To calibrate the machine insert the HCl blank, set the controls to 100x at the 31.6 scale and adjust the needle to zero with the "BLANK" knob. Next, read the "COPO" tube at 100x, 1 scale. This should be about 2.85: Record this reading; this is the "U" in the chlorophyll calculations. Finally, read the 90% acetone blank at 100x, 31.6 scale; adjust the needle to zero with the "BLANK" knob. Now you are ready to read samples!

With the lights off, remove the sample tubes from the refrigerator. Insert a tube. Adjust the scale settings so that the needle is between 2 and 8 (most salt pond samples will read at 1, 10, or 31.6 scales). Record the settings (1,10, etc.) and the needle reading (fo). Then add 5 drops HCl and record the new needle reading (fa). Repeat this for the other samples. when you are done, clean up the area and turn off the fluorometer, unless you need it the next day.

Calculations

Copies of an Excel worksheet for chlorophyll calculations are available on the "Chlorophyll determinator" disk in the "Da[illegible]Bases" notebook. This contains all the formulas needed to calculate chlorophyll a. Note that these are specific to the calibration proceedings used in the Durbin's lab and may not be applicable to another fluorometer or another method of calibration. Enter the pond, station and filter number in columns A-C. In columns D-F enter the volume filtered (50 ml for pond watcher samples), sensitivity (1x or 100x), and scale. In G-H enter fo , fa, and U. Chlorophyll *a* will be calculated in column J for each sample, and the average chlorophyll for each set of replicates, in column M. If one value is extremely divergent from the other two replicates, or a mistake was made in sample collection or preparation, delete that value. Phytoplankton abundance can frequently vary by a factor of 2 or more due to patchiness, but variables of 3 fold or more, especially on the low side, may be due to defective filtration. Samples which receive less than 5 ml of 90% acetone may read high, and should not be recorded.

Enter the chlorophyll data for each pond, in order from Point Judith to Winnapaug, by station and date, i.e. for each station leave spaces for all 12 or 13 sampling dates for the year, and arrange stations by number. Insertions and deletions in the worksheet are time-consuming, so its best to have the data organized from the start. If organized properly, the chlorophyll data can be pasted directly in to the master water chemistry file. Put raw data

sheets and chlorophyll printouts in the black ring binder notebook labelled "Chlorophyll *a*" at CRC.

Part 4: DATA MANAGEMENT

Brief Overview

Separate databases are kept for bacteria and water chemistry results. Currently both databases are entered and kept as Microsoft Excel files with a separate file for each Salt Pond. The data is manipulated in this format and tables and charts made either directly from Excel or converted into Cricket Graph or one of the drawing programs, whatever is most appropriate for the job. Copies of these files are kept on the Mac hard drive in the CRC office and on 2 sets of floppies as backups.

Bacteria Data

Bacteria results are either read directly by the student assistant (at FDA) or lab sheets containing results are sent to us by the lab (from DOH). In each case it is the student assistant who interprets the raw data and assigns MPN values to each. (See Bacteria data analysis section.) These calculations can be hand written as long as all the necessary data is included such as Pond, Station, Date, Fecal coliform, and Total coliform (where appropriate). These completed sheets are sent to the volunteer Data Manager to be entered into Excel and incorporated into the larger database. Be sure to include < "less than", and > "greater than" symbols where they are called for in the data. Data Manager data entry must be checked for quality control before final entry into the Salt Ponds data base.

Cross reference between final MPN values and the Pond Watcher field data sheets is necessary to pick out such information as waterfowl and boating activity observations and any notes of exceptionally turbid water or other signs of a prominent algae bloom.

Water Chemistry Data

The water "chemistry" database includes a wide range of physical, biological, and chemical parameters that are measured biweekly on the salt ponds. Some of these parameters (temp and DO) are entered directly off the Pond Watcher field data sheets. Salinity and nutrients and chlorophyll are analyzed separately. The water chemistry data base thus comes from several sources and requires some orchestrating to make all of the different pieces match up into one complete file. To pull this off, the student assistant and volunteer Data Manager work closely.

POND, STATION, DATE, TEMPERATURE, and DO (if measured at that station) are all entered directly from the field data sheets. The Pond Coordinator makes copies of these sheets at the time of a periodic sample collection. One set of copies is put in a large ring binder at CRC, and one set is sent to the volunteer Data Manager. The Data Manager creates the "skeleton" of the file by entering the information off the field sheets and setting up the file format.

SALINITY, NITRATES, and PHOSPHATES are read by the graduate assistant. The student transposes this information into a legible and complete table for each batch of samples run (can be hand written or entered onto floppy) and sends the tables to the Data Manager. The standard format for this data is for no decimal places for salinity, one place for nutrients (μM) and one place for chlorophyll a (0.1μgl).

CHLOROPHYLL values are calculated by a computer worksheet program (See Chlorophyll calculations section) and are thus already in floppy format. A printout of the appropriate worksheet columns containing POND, STATION, DATE, and TOTAL CHLA ug/liter as well as a floppy of these same columns are sent to the Data Manager.

A quality assurance check is done on all data entry. When these files are returned by the volunteer Data Manager, the graduate assistant must cross reference the data (both bacteria and water chemistry) with the original field and lab sheets.

Rainfall

Monthly rainfall records for Kingston, Rhode Island are mailed to us by the National Weather Service Station manager at URI in Kingston. These are put in the graduate assistant mail box at CRC for entry into the computer data file. Rainfall gauges are also kept by some of the Salt Pond Watchers. Samples are collected at each rainfall and frozen in a liter bottle, cumulatively. Rainwater samples are collected at the end of the summer and analyzed for nutrient concentrations (using a deionized water standard). Results are entered into the data file. Rainfall patterns are important for interpreting high bacteria or nutrient concentrations. Nitrogen in rain water may be an important input to the Salt Ponds.

Dissolved Oxygen

Dissolved oxygen is measured by the pond watchers at the deeper stations using LaMotte modified Winkler titration field kits. The results are logged on the field sheets and need to be entered into the data base.

Eelgrass Wasting Disease

Once a summer, pond watchers take a sample of eelgrass to record evidence of wasting disease (see field protocol manual). The data sheets are left with the nutrient samples for pick up or are mailed to us. The grad assistant collects the data sheets, puts copies in the CRC pond watcher file, and mails the originals to Dr. Fred Short, University of New Hampshire, Jackson Marine Laboratory to contribute to the national data base he is compiling.

DATA MANAGER

This role has been expanding with the growing proficiency of its operator, Suzanne Nardone. The data Manager has full use of a Macintosh SE computer, purchased by the Pond Watcher Program and kept at the home of the Data Manager. The general duties are to enter and manipulate data for both bacteria and water chemistry sampling and to produce summary graphics in coordination with the Program Head and graduate assistants.

Part 5: FIELD SAMPLING

A. Field Sampling Protocols are described in handbook Tech Report #13.

B. Pond Watcher Coordinator

This role has been filled since the inception of the program by Nancy Wetherell. The following description only represents the nuts and bolts of the function she has played. The innumerable tasks that she performs "in the line of duty" are myriad.

1) Coordinate the activities, sampling, supplies of the Salt Pond Watchers and assist the professional and graduate student staff of CRC in the day to day running of the Pond Watchers Program.

2) Help with planning for each year's activities and ordering of supplies.

3) Help to assemble sampling kits at the beginning of each season.

4) Be the liaison between volunteer Pond Watchers and Pond Watcher staff: Make sure that they always have adequate supplies for sampling. Coordinate for substitutes to cover sampling when a regular Pond Watcher is laid up or out of town.

5) Quality Assurance/Quality Control - visit each station at least once in every season. Verify station positions on the pond maps. Run duplicate tests at water chemistry stations. Grad. student assistants help in this task.

6) Pick up frozen samples and data sheets from pond coordinators and deliver to the Bay Campus at specified intervals in the sampling season. Check data sheets and samples for accuracy in labeling. Deliver copies of data sheets to the Data Manager.

7) Involvement in the community - Speak as a volunteer Pond Watcher to small interested groups. Help new groups who are setting up similar programs and would like input from a nonprofessional.

8) Attend meetings and conferences where possible. Network with other coordinators and monitors. Pass on new ideas to Head of Pond Watcher Program.

C. Bacteria Sampling Supplies

EACH POND WATCHER RECEIVES:

ENOUGH STERILE BACTERIA JARS TO LAST FOR 3 SAMPLES SCHEDULE OF SAMPLING DATES (drop off additional jars as needed. This takes some planning).
NEW LIST OF POND WATCHERS & SAMPLE STATIONS
MAPS OF SAMPLE LOCATIONS - ALL PONDS
CURRENT PROTOCOLS FOR BACTERIA SAMPLING
NEW DATA SHEETS
DOH FORMS (If needed).

DOH supplies the sterile bacteria sampling jars for all of the bacteria sampling, whether the samples are analyzed at DOH or FDA. The person dropping samples off at DOH must remember to bring back a new box of jars each trip he/she makes to the State lab. These jars are then allocated between the Pond Watchers so that they are always stocked for their next sampling date.

D. Water Chemistry Supplies

EACH POND WATCHER RECEIVES:

1 SYRINGE
3 NUCLEPORE FILTER ASSEMBLIES
APPROXIMATELY 45 GLASS FIBER FILTERS (in a plastic baggie)
1 SHARPIE WATERPROOF PEN
2 PAIR OF PLASTIC FORCEPS
1 PIECE (10 INCHES LONG) TYGON TUBING
15 NUTRIENT BOTTLES

1 ROLL OF LABEL TAPE (if needed)
A NEW SCHEDULE OF SAMPLE DATES
A NEW LIST OF POND WATCHERS & SAMPLE STATIONS
MAPS OF SAMPLE LOCATIONS-ALL PONDS
NEW PROTOCOLS
NEW DATA SHEETS
LAMOTTE DO TITRATION KIT with new refill (for those volunteers who measure DO)

A full complement of syringes, filter heads, nutrient bottles, forceps, and tubing belong to the program. The syringes, filter heads, and tubing are collected once a year at the end of the season and acid washed and rinsed to prepare them for the next season. Nutrient bottles are recycled after nutrient and salinity analysis by rinsing 3 times and filling with deionized water (DI). The O-rings and gaskets from the filter heads are removed before acid washing and are rinsed only in DI to prevent hardening and breakdown of the rubber.

Below are listed for those items which commonly need to be replaced or refilled each year to some or all of the Pond Watchers.

1) glass fiber filters
2) filter head gaskets
3) LaMotte kit refills
4) Sharpie pens
5) labelling tape

APPENDIX

DIRECTIONS FOR BACTERIA SAMPLE PICK-UP AND DELIVERY

NOTE: Bacteria samples are collected every other week

On the Friday before--make media at FDA, Quonset (see previous section).

(Winnapaug and Point Judith)

Don't forget the ice packs and cooler, the bacteria samples have to be kept cold.

1. Take Rte 1 S to Rte 1A in Westerly. Left exit sort of, the turn is just before the sign "Mary's Restaurant" on right. Continue on this road 4.8 miles. After a sharp curve make a right into Misquamicut Hills (stone pillars at entrance). Cy Morgan's House is 129 Misquamicut--grey house. If no one is home, just go in--the samples are in the refrigerator.

2. Get back onto Rt 1 N. Take the "East Matunuck State Beach" exit. Turn left onto Island View, a development, about 1.5 miles from Rt. 1. It's the corner house, first on the left (#4)--Lars Larson, Succotash Road. The samples are usually in a cooler outside by the garage.

3. Get back onto Rt 1N. Take the Pond St. exit. Turn right. Make the first left onto Quagnut St. Phil Carpenter's house is on the left #83 Quagnut (it has solar panels).

4. Get back onto Rt 1 N. Head to Providence.

(Ninigret and Green Hill)

1. Rt. 1 S to the "Cross Mills, Charlestown Beach, (Breachway)" exit.
   Go straight to intersection. Go straight thru the intersection onto Town Dock Rd.
   Ocean House Marina is at the end of the road.
   Take last left into parking lot. The store is on the left. Pick up samples from Van Ackerman. If the samples are not outside, check inside the Marine Store.

If you should take the "Charlestown Beach, Breachway" exit (the first Charlestown exit, but not Cross Mills):

Take a right at the stop sign on Scenic 1A--follow a few miles until you reach "Realty Connection" a business on the intersection of 1A and Town Dock Rd. Turn left onto Town Dock Road. Ocean House Marina is at the end of the road.

2. From Town Dock Road, go right (North) on Rt. 1A and continue north (1-2 miles) until you see Green Hill Beach Rd. on the right. Turn left onto Green Hill Beach Rd. There is a distinct median at this intersection. Turn left at the end of the road onto Schoolhouse Rd, then immediately right onto the continuation of G. H. Beach Road, south. Go about 1 mile, past Carpenter Dr. (on L) and Maple Dr. (on R). Turn right onto Twin Peninsula. Third road on the left is Wild Goose. Turn left on Wild Goose, then immediately bear right onto Kingfisher. About 20 yards later, take a left onto Cormorant (bear left towards the fireplug). Al Hale is #20, on the left, two houses down Cormorant. Once you have picked up the samples head to Providence.

(Potter and Quonochontaug)

1. Head south on Rte 1--look for the "Matunuck Beach Road" exit. Exit onto Matunuck Beach Road. Take left onto Sycamore Lane--it's right after the school sign, about 1 mile from Rt. 1. Ross Toney's is on the left--550 Sycamore, about 0.8 miles in. Look for the mailbox with his name on it. Cooler is usually on the front porch.

2. Take Rt. 1S until you see the sign for "Mary's Restaurant" on right. Turn around so you are heading north on Rt. 1. Turn right into Shelter Harbor Inn (A big white sign and some stonework marks the entrance).

   Alternatively, you may turn into Shelter Harbor directly from Rt. 1 S. There is a turn-around thru the median on Rt. 1 S right in front of the stonework and sign marking Shelter Harbor.

   Go down the drive 0.35 miles off Rt. 1 S. It is the light grey house on the left (stones in driveway). Jack Tobin's house is #36 (its the house right after mailbox that has Andrew's on it). The cooler is on the front porch.

FDA LAB - Quonset

Take Rt. 1N to the Wickford-Quonset exit that bears right (where Rt. 4 begins). Continue north on what is now Rt. 1, thru North Kingstown to the Quonset Air Base exit. Bear right after exiting Rt.1 and go to the signal light. Turn left at the signal in the Quonset Point/Davisville Industrial Park, then turn right over the tracks onto Callahan Rd. Bear right, follow signs to the O-Club. About 1 mile on this road (on the left) is a set of buildings of N. Kingstown Community Health, etc. Turn left on School Street, just before "Little Bee" baseball field. Turn left. FDA is the low white building on the left, about 25 yards in.

Recipe for Medium (A-1 Modified):

This is an edition of the formula given in "Laboratory Procedures for Seawater and Shellfish". The exact reference for this recipe is available from the FDA (Linda or Diane):

The need for uniformity dictates the use of dehydrated medium. Maintain written quality control records on preparation of medium and reagents including results of productivity and inhibition tests, pH. sterilization time and temperature, and other pertinent data.

Recipe for A-1 broth:	**1 L**	**3 L**
Lactose	5.0 g	15.0 g
Tryptone	20.0 g	60.0 g
Sodium chloride, NaCl	5.0 g	15.0 g
Salicin	0.5 g	1.5 g
Triton-100 (Polyethylene glycol *p* isooctylphenyl ether)	1.0 ml	3.0 ml
Deionized water	1.0 L*	3.0 L

***Note** that this is for 1.0 liter of medium. You must make 3 liters so triple all ingredients. With the present number of stations, 3 liters leaves little to spare; if more stations are added you will need 3.5 to 4 liters of broth.

Place a magnetic teflon-coated stirring-bar into the deionized water in a stainless steel bucket and start stirring vigorously. Add all dry ingredients and mix until all ingredients are dissolved. Add Triton ether and allow to mix well. Take the bucket into the main lab and record the pH. The pH should be 6.9 $\pm$ 0.2. The meter is in the lab at the other end of the building. Adjust with acid or base only if absolutely necessary. Dispense into test tubes using pipetting machine. Check to be sure that it is set for 10ml and run it for 20 cycles before filling tubes to clear machine. After use, run hot, deionized water through the machine to clean. Sterilize the medium by autoclaving at 121 C for 10 minutes. Store labelled medium in a cabinet provided until inoculation with sample the following day.

DOH APPENDIX

BACTERIA ANALYSIS AT THE RHODE ISLAND DEPARTMENT OF HEALTH - pre 1991

Preliminaries

Notify DOH lab (274-1011) ahead of time as to how many samples you will bring in the up coming week and on what day. (Note that holidays may affect the schedule.) Reserve a car from GSO Maintenance for the required number of days. If it is easier for you, you may have a car for a continuous period (all the days you collect samples). Take the big cooler with you with the frozen ice packs from the freezer in the Marine Resources Building. It is important to keep samples cool in transit to the labs.

Processing Samples at DOH

Enter door at the loading dock on the right side of the building. Bring with you 1) samples in cooler, and 2) lab submission forms filled out by pond watchers. You should check that there is one sheet for each sample when you pick them up and fill them out if pond watchers forget. The lab submission forms (filled out in triplicate) should be given to the person in receiving (at counter on right as you enter the building.) The forms should be arranged in order of station. The receiving person numbers each submission form. Separate the triplicates and leave one copy with the receiving person, give one to the lab, and keep the third for our files at CRC.

Arrange the samples, in order of pond and station, on the counter and write on their caps the assigned DOH number (last two digits) which was punched onto the lab submission forms. Repack samples on ice and take to the Microbiology Lab, 4th floor. You will be expected to help in sample processing. Ask for Ted Pliakas or Pat McNulty. Once you have finished inoculating and processing Salt Pond Watcher Samples, please offer to help with any other general assistance to the lab the rest of the afternoon, including media preparation or clean up. The deal is that the lab offers free analysis in return for your assistance if needed.

Things to watch out for: (1) The person operating the numbering meter will often get confused and misnumber the lab submission forms, most commonly by duplicating a number. Watch closely for this. (2) The Pressure Differential Phenomenon: watch out when someone opens the door to the outside. This results in a big gust of wind and your tidy pile of data sheets ending up in the other room!

BACTERIA DATA ENTRY AND ANALYSIS

Data from DOH

Data Tabulation. Coliform data sheets are mailed from DOH about one to two weeks following sampling dates and will usually be placed in your mailbox at CRC. These data must be entered into "Bacteria tables" notebook. When the data set is updated, a photo copy of the tabulations should be sent to Suzanne Nardone, Volunteer Data Manager, for data entry onto floppy disks.

Reading MPN Index. The total coliform and fecal coliform data from DOH are reported in a rather cryptic fashion. The tabulated results are in the center column of the DOH data sheets and consist of a series of 1 to 4 numbers followed by a letter. The meaning of these numbers and letters are as follows:

(a) Numbers: Usually three numbers are reported, such as, 3-1-1. If a zero follows this sequence, it is ignored for our purposes. If only one or two numbers are reported, we assume the remaining one or two are zeroes. For example, the reported number 3-2 should be assumed to be 3-2-0. These three numbers are converted to number of bacteria (total coliform or fecal coliform; see (b) below) per 100 ml simply by reading from the MPN index table which will be found in the sleeve of the "Bacteria tables" notebook. For example the combination of positives, 3-1-2 reported by DOH, corresponds to 120 coliform bacteria per 100 ml of water. Ignore, for purposes of reporting your data, the column for 95% confidence intervals.

(b) Letters: (P,C and F). These letters will follow the sequence of numbers and will identify the data as total or fecal data. Often more than one letter will follow a series of numbers, such as 3-2-1-F,C. This means that 3-2-1 is the number for both fecal and total coliforms.

P=preemptive - ignore this

C=total coliform
F=fecal coliform

Saving data sheets. Save the DOH data sheets and the field data sheets in the large red three ring notebooks. Order them chronologically as well as by pond and station.

Save the third copy of the lab submission form in a folder at CRC. These are to be given to Virginia at the end of the sampling season. They will be used to obtain matching funds from GSO.

Note: DOH lab sheets are carefully collected over the sampling season as they are sent by the lab. Each sheet is proof of a substantial contribution to the Salt Pond Watcher program by the State. At the end of each year they are turned into the GSO Budget Office for matching funds from the University.

Directions to DOH:

DOH Providence, Orms Street-- Take 95 N to 146 (exit 23) "State Offices" Exit. Get into left lane of exit ramp and turn left on to Orms Street. Get into right lane immediately. The DOH building is the first building on your right. Pull into the driveway. There are usually vacant 15min. parking slots on the right. You can park here without fear of being towed for your visit to DOH.

PROCEDURES FOR THE FIA NUTRIENT ANALYZER, JUNE, 1989

START-UP PROCEDURE

1. check levels of artificial seawater (ASW) and reagents, waste
2. switch on computer master switch (includes printer, monitor=screen, drive)
3. then, switch on machine master switch (includes sampler, pump, manifolds, colorimeters) and allow to warm up. Green light on valve controller should come on, push button and check that red light comes on. Colorimeter controls should read "coarse gain = x10", "display select = V out", "chemistry = direct"
4. turn on heater (check water level)
5. make standards (all channels being run plus NO2 for column effiency check)
6. decide which nutrient channel(s) to hook up to the recorder, move and attach if necessary. (If running NO2+3, need to hook that channel up in order to do column efficiency).
7. snap modules onto pump rollers (~~only those being used~~)
8. run deionized water (DI) through all lines, check for leaks
9. degas NH4 and PO4 reagents and carrier (ASW) with helium
10. check that green valve light is on, then place reagent and carrier lines in proper bottle, cover openings with parafilm
11. after bubbles from reagent line attachment has gone through, attach cadmium column, with pump on minimum
12. put pump back on regular [illegible] computer
13. set baseline(s) as seen on computer screen; turn on recorder and check baseline(s), particularly for drift and noise
14. put through high mixed standard and high NO2 standard

    manually: with green light on valve, put sampler needle in beaker of high standard; press valve light to red on all four channels for about 20 seconds, this puts sample in the sample loop. Then press valve light to green on all four channels, this puts carrier in sample loop to push sample through to manifold. Repeat as needed to set fine gain(s) (see #15). With green light on valve, put sampler needle in beaker of high NO2 standard. Proceed as above and watch NO2 peak come out on NO2+3 and NO2 (if run) channels (see #16).

    automatically: ?fill three standard cups (A,B,C) with the high mixed standard, and fill the next three (D,E,F) with the high NO2 standard, and fill the next three (G,H,I) with high mixed standard. Then, starting with green light on valve, run program "column efficiency" which puts through the nine aliquots of the chosen standard.
15. with high standard coming through, set "fine gain" to put high standard on scale for each channel
16. If running NO2 channel then with high NO2 standard coming through, set "fine gain" on NO2 channel to put standard on scale. If running NO2+3 channel, watch NO2 peak coming through on NO3+2 channel on screen and on recorder for cadmium column efficiency. Measure peak heights and calculate column efficiency. ?Enter efficiency in pseudo-channel. [illegible], check paper feed; recorder on, check paper feed
17. If all set, fill standard cups and run standard curve. Then put pump on minimum, check calibration and adjust, if needed
18. Put pump back on regular, leave standard cups filled and in place, run samples and the "check" standards!!!

THINGS TO WATCH FOR WHEN RUNNING

1. reagents going dry, particularly NO2+3 reagents
2. wash water overflowing, or going dry from feed container
3. air in cadmium column
4. pump on appropriate speed

WHEN BREAKING FOR LUNCH OR OTHER EXTENDED PERIODS

1. pump on minimum
2. cadmium column unhooked
3. reagents in DI
4. heater off

SHUT DOWN PROCEDURE

1. with pump on minimum (optional), unhook cadmium column
2. heater off
3. with pump on regular (or maximum), put carrier lines, NO2+3/NO2 lines, and NH4 lines in DI, PO4 lines in EDTA
4. after 2-5 minutes put NH4 lines in 1N HCl and put PO4 lines in DI
5. after another 5 minutes put NH4 lines in DI
6. after another 5 minutes put all lines in air and pump dry
7. shut off machine master switch
8. pop up pump modules
9. shut off computer master switch
10. shut off recorder