Standard Operating Procedures

Phytoplankton Monitoring Program

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Great Bay Coast Watch

Mission Statement:

The Great Bay Coast Watch is citizen volunteers working within the UNH Cooperative Extension/NH Sea Grant Program, protecting the long-term health and natural resources of New Hampshire's coastal waters and estuarine systems through monitoring and education projects.

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1.0 INTRODUCTION

1.1 Purpose

Thank you for taking part in this volunteer-based phytoplankton monitoring project. With your help, Great Bay Coast Watch (GBCW) and the University of New Hampshire Cooperative Extension hope to obtain baseline and continuing information on the toxic phytoplankton present in Gulf of Maine waters. This information will be used to provide data on algae blooms and, in turn, further aid the New Hampshire Department of Environmental Services (NHDES) in their management of shellfish resources.

You will be collecting and monitoring water samples to determine whether any of the two toxic or potentially toxic phytoplankton species (*i.e., Alexandrium spp., and Pseudonitzschia spp.*) are present and in what quantity. *Alexandrium spp.* causes Paralytic Shellfish Poisoning (PSP) and *Pseudonitzschia spp.* is associated with Amnesic Shellfish Poisoning (ASP). These toxic or potentially toxic cells will be referred to as target cells throughout the procedures contained in this manual.

In addition to monitoring for the presence of the two potentially toxic species, you will be observing and recording the occurrence of other phytoplankton species in your sample. These data will be used to build a long term record noting species presence and abundance in our region.

1.2 Getting to the Sampling Sites

Phytoplankton monitoring is conducted at five sites in coastal New Hampshire:

- Hampton Harbor
- Parson's Creek, Rye
- Coastal Lab, New Castle
- Rte. 1A, Seacoast Science Center
- Star Island, Isles of Shoals

These sites are primarily near shellfish growing areas or sampling stations currently used weekly by the NHDES Shellfish Monitoring program, and have historically been good locations for indicating early the presence of initial, low-level toxins when they exist. We will provide you with maps and/or instructions on how to locate these stations. Monitoring should be conducted at the same sites weekly March through October at or near high slack tide. You may be asked by GBCW to change your designated site if a more representative site is discovered.

1.3 Safety

Although collection of samples at scheduled times is important, your safety and health are more important. If weather conditions are such that collecting the samples might cause injury or illness, reschedule your sampling run for later in the week. Remember to dress

appropriately for the current weather and be prepared for unexpected bad weather. On wet, cold days avoid wearing cotton clothing as cotton offers little thermal protection when wet. On hot, sunny days be sure to have plenty of fluids to avoid dehydration and sunscreen to prevent burning. Always have a first aid kit either with you or in the car.

If sampling from a dock be sure to wear appropriate footwear to reduce risk of falling and always be sure to steady yourself when working near the edge of the dock. If sampling from a boat, be sure to follow all federal and state safety procedures. Additionally, be careful when reaching over the side of the boat to collect the sample. Although we want you to collect the samples and have fun, the most important thing is your safety.

2.0 GETTING READY TO SAMPLE

2.1 Data Sheets

Data sheets have been provided for recording your observations and sampling results. A sample data sheet is included in Appendix A for you to refer to as you review the procedures in this manual. Note that various environmental factors are listed on the data sheets (e.g., recent weather events, current, wind speed and direction, water temperature, transparency, salinity, dissolved oxygen). These observations will help indicate what conditions existed at sampling time that may have enhanced or deterred the development of a bloom.

Note: It is very important that you completely fill in the data sheet

2.2 Preparation

Before beginning the observation and sampling procedures described below, first enter the Site Name, assigned Site Number, and Date on the first line of the Data Sheet, followed by the names of the Samplers participating in the testing. Then enter the following parameters on your data sheet:

- Recent Weather Events – Note any recent weather events (i.e., storms, wind, heavy runoff) or unusual environmental factors.
- Water Current – Observe the water current at the location where you will be collecting your phytoplankton sample (If this is difficult to observe, it may be easier to estimate the current when using the Secchi disk.) Enter 0 for no current, L for a current of one to two knots, M for a current of three to four knots, or H for a current of five knots or greater.
- Wind Speed – Enter wind speed in knots (i.e., use Beaufort scale to estimate speed as explained in Appendix B) and wind direction.
- Time – Enter current time in military format (i.e., 24 hour format).

3.0 MEASURING THE WATER AND AIR TEMPERATURE

3.1 Required Equipment:

- Air Temperature Thermometer
- Armored Thermometer
- Water Sampling Bucket

3.2 Step-by-Step Procedure:

1. Check both thermometers for continuous fluid - no breaks.
2. Hang the air thermometer in a location above ground and out of the sun for at least three (3) minutes.
3. Rinse sampling bucket twice by filling it halfway and disposing of contents in an area **downstream** and **away from the sampling spot**. Let water flow through the tube in order to rinse it out and then clamp tube shut.
4. Take water sample with bucket at a depth of one to two feet, hang armored thermometer in bucket, and note the temperature reading after waiting at least three but no more than five minutes. Record the temperature value **to the nearest half degree using one decimal place** (e.g., 12.0 and 13.5 are OK – 12 is not). When reading the armored thermometer make sure the bottom of the thermometer remains in the water.
5. Record air temperature reading on the data sheet **to the nearest degree using no decimal points** (e.g., 16 or 18 is OK – 16.5 is not). Make sure to use the Celsius scale. If you have a thermometer that reads in Fahrenheit, you must convert Fahrenheit to Centigrade. To do this, first subtract 32 degrees from the Fahrenheit reading; then divide this result by 9 and multiply by 5.

4.0 MEASURING THE WATER TRANSPARENCY

4.1 Required Equipment:

- Secchi disk with line marked every five centimeters

4.2 Step-by-Step Procedure:

1. Take transparency readings at the same spot each time. If possible, stand with your back to the sun to shade the sampling spot. Do not wear a hat or sunglasses when taking these readings.
2. Lower the Secchi disk into the water until it just goes out of sight. Note depth to the closest five centimeters. Then raise the Secchi disk until it just reappears. Again, note the depth to the closest five centimeters. Record the average of the two depths to the nearest centimeter under Water Transparency on the data sheet. If the disk hits bottom and is still visible, record the water depth to the nearest five centimeters under Water Transparency. (In this case Water Depth and Water Transparency will be the same value.)
3. Lower the Secchi disk into the water until it hits bottom (i.e., the rope will go slack at this point) and note the water depth to the nearest five centimeters. Record this value under Water Depth on the data sheet.

5.0 MEASURING THE SALINITY

5.1 Required Equipment:

- Armored thermometer
- Hydrometer
- Hydrometer jar (500 mL cylinder)
- Hydrometer case with cork stopper

5.2 Step-by-Step Procedure:

1. Using water from the sampling bucket, fill the 500 mL cylinder to approximately one inch below the rim.
2. Hang the armored thermometer in the jar.
3. **Gently** insert the hydrometer with a twisting motion. This removes any air bubbles. Be sure not to drop the hygrometer into the jar because it could hit the bottom of the jar too hard and break.

> Note: The water temperature measured in the "Measuring Water and Air Temperature" procedure cannot be used for this test as it may have changed. You must measure the water temperature in the cylinder just before you read the

4. Level the cylinder so that the hydrometer is vertical and not touching the sides. Try to keep it out of the wind.
5. After three minutes, read the thermometer to the nearest 0.5º C and record on the data sheet using one decimal place (e.g., 12.5 and 14.0 are OK – 12 or 14 are not). Make sure the bottom of the thermometer remains in the water.
6. Remove the thermometer.
7. Read the density using the scale on the hydrometer, taking care to read at the bottom of the curve formed where the water rises slightly as it touches the sides of the hydrometer. (A magnifying glass may be helpful.) This curve is called the meniscus.
8. On your data sheet, show where the meniscus is by marking the "Reading the Hydrometer" diagram. Record the density reading on your field data sheet.
9. To determine the salinity use the five-page salinity table (see Appendix C). Locate your density reading in the left hand column and your recorded temperature across the top of the appropriate page. Then read down to the appropriate salinity value and record the result on your field data sheet using one decimal place.

> Note: If you find the density or temperature reading to be a value ending in five, you will need to interpolate the result on the table. This is done by taking the average of the values immediately above and below the reading. For example, if the hydrometer read 1.0135, you would then take the salinity values for 1.0130 and 1.0140 and average them. Record the average using one decimal place and round the average value as necessary.

6.0 MEASURING THE DISSOLVED OXYGEN

6.1 Required Equipment:

- 2 graduated burettes
- 2 glass rods
- 2 glass marbles
- 2 glass Wheaton DO/BOD bottles with stoppers
- 1 100 mL graduated cylinder
- 1 box manganese sulfate pillows (pillow #1)
- 1 box iodide-azide pillows (pillow #2)
- 1 bag sulfamic acid pillows (pillow #3)
- 1 pair scissors or clippers
- 1 bottle starch solution
- 1 dropper bottle sodium thiosulfate
- 1 plastic beaker
- 1 transfer pipette (optional)

6.2 Step-by-Step Procedure:

1. Insert flow tube from sample bucket into the BOD bottle, all the way to the bottom of bottle. Then let the water flow into the bottle by opening the clamp on the sampling bucket.
2. Keep track of the amount of time it takes to fill the bottle to the point of overflow (counting as it fills will be fine), and allow enough time for the bottle to have filled three times. Remove the flow tube from the bottle slowly before stopping the flow of water from the bucket. This ensures the bottle is full to the brim.
3. Examine sample to make sure no bubbles are trapped inside. Don't splash water out. Repeat step 2 until there are no bubbles. Temporarily replace glass stopper if carrying sample away from water's edge to do the procedure. Once a satisfactory sample has been collected, proceed to steps 4, 5, and 6.
4. Cut open the manganese sulfate powder (pillow # 1) and add to sample.

> Note: Should powder stick to the neck of the bottle as a result of step 4 or 5 below, use the stopper to wet the neck and gently mix the powder in. Once you insert the stopper (some liquid will overflow), do not remove it until step 7.

5. Cut open the alkaline iodide-azide powder **(**pillow #2**)** and add to sample.
6. Carefully add a small marble to the bottle. Replace the stopper while twisting it 1/4 turn to get a good seal. Place finger on top to hold the stopper on the bottle. Swirl bottle gently several times, using a circular wrist motion, to mix the reagents with water. A precipitate will form. Place sample aside and allow precipitate to settle to bottom half of bottle. Once precipitate has settled, repeat the mixing and settling process once to ensure the chemical reaction is complete.

Note: Addition of the marble in step 6 has two benefits. It tops off the level of the liquid in the bottle, preventing formation of the air bubble can sometimes form between the liquid and the stopper. Also, the marble helps to mix the powered reagents when the bottle is swirled. The marble should be clean and added gently to prevent the possibility of introducing air into the bottle.

After finishing step 6, go on to your other tests while the precipitate is settling. Now that step 6 is complete, contact between the water sample and the atmosphere will not affect the test result. Once the sample has been "fixed" in this manner, it is not necessary to perform the actual test procedure immediately. Thus, several samples can be collected and "fixed" in the field, and then carried back to a testing station or laboratory where the titration procedure (steps 7 through 16 below) is to be performed. Make certain samples are kept cool if titrating later; however, the titration procedure must be completed within eight hours.

7. Cut open the sulfamic acid **(**pillow #3**)** and add to sample. Replace stopper and swirl to mix until all powdered reagents have dissolved. Look at the bottom of the bottle to see if there are crystals that look like grains of sugar. Continue to mix until all crystals are dissolved A clear yellow to brown-orange color will develop, depending on the oxygen content of the sample.
8. Pour 100 mL of the sample carefully into the clean 100 mL graduated cylinder. Tilt the cylinder and pour the sample carefully down the inside wall to avoid mixing bubbles into the sample. (The bubbles will not add dissolved oxygen to the water at this point, but can displace water and give you an incorrect measurement.) Tap the cylinder to remove any bubbles and make sure the meniscus is at 100 mL. If necessary, you may use a transfer pipette to obtain an exact volume of 100 mL. Then pour the sample from the cylinder into the test beaker by carefully pouring down the inside wall of the beaker.
9. Fill burette to above the zero mark with sodium thiosulfate titrant and clear bubbles out of burette. Tap the burette to get rid of bubbles above the bead valve. Point the tip over your waste container and tap or squeeze the bead valve to remove the bubbles below the bead valve. Make sure liquid fills burette from tip to the zero mark. Refill to zero mark if necessary.
10. Slowly add sodium thiosulfate titrant to the test beaker containing the 100 mL sample, stirring as titrant is added. Stop titrating when the amber-colored solution in beaker begins to lighten to a light hay color. Place white paper under beaker to watch for the test color change in step 12.
11. Add eight drops of starch solution to beaker. Sample will turn a medium blue color.
12. Continue the titration process with the sodium thiosulfate remaining in the burette until test sample becomes colorless. Do not add any more titrant than is necessary to produce the color change. Be sure to stir sample after each drop is added.
13. Using the scale on the side of the burette, count the total number of mL of sodium thiosulfate used in the titration. Enter this number in the space provided on your data sheet.
14. Rinse out the beaker and repeat steps 8 through 13 on a second sample.

15. Record results of the second titration in the space provided on your data sheet.
16. Add the results of the two titrations (mL = mg/l) and enter the value on your data sheet.

> Note: These duplicate titrations are run to guard against analysis errors. If the DO result in the second titration is greater than 0.3 mL different from the first titration, a third titration must be performed. If less than 100 mL of the sample remains for the third titration, use 50 mL of the sample and double the result. Record all three results; however, add only the two results that are within 0.3 mL.

17. Once the DO testing has been completed, rinse the BOD bottle thoroughly. Also make sure glass marbles are cleaned and stored to prevent loss.

7.0 COLLECTING THE PHYTOPLANKTON SAMPLE

7.1 Required Equipment:

- Blue ten liter bucket with rope
- Small collection container
- 20 micron PVC sieve
- 100 mL graduated cylinder
- Funnel
- 8 oz. plastic spray bottle
- Sample storage bottle

7.2 Step-by-Step Procedure:

Note: You will be filtering a total of ten liters of water in collecting your sample. Do this in two five liter increments

1. **Filter the first five liters.** Using the blue bucket, collect the first five liters of surface seawater. (Be certain you are using the liter markings.) Place the 20 micron PVC sieve in the small collection container and pour one liter of the collected seawater through the sieve saving the filtered water in the small container. This filtered seawater will be used in step 3 below to wash the sample from the 20 micron mesh. You can now set the sieve on the deck or ground and continue to pour the water through until the blue bucket is emptied. If by this point the sieve is so clogged it will no longer drain easily, move on to step three. (IMPORTANT: if you move directly to step three, remember to record on the phytoplankton data sheet that you only filtered five liters.)
2. **Filter the second five liters:** Refill the blue bucket again with five liters of seawater and continue to pour through the sieve. Once the full ten liters of seawater have been poured through the sieve, the phytoplankton sample has been retained on the mesh in the 20 micron PVC sieve and will need to be backwashed into the 100 mL graduated cylinder.
3. **Backwash the sample**: Fill the spray bottle using the filtered sea water collected in step 1. Put the sieve upside down into the funnel and the funnel end into the 100 mL graduated cylinder. Spray the 20 micron mesh at close range for several pumps until the collected volume in the graduated cylinder equals 15 mL. This is your sample!

NOTE: The collected sample should be examined immediately, if at all possible. If you transport the sample elsewhere for analysis, pour it from the 100 mL graduated cylinder into the small sample storage bottle ensuring that the top is screwed on tightly. Keep the sample cool during transport by placing it in a cooler with ice.

8.0 ANALYZING THE PHYTOPLANKTON SAMPLE

8.1 Required Equipment:

- Field microscope with penlight
- Flat capillary tubes marked to indicate the counting field with container for storage
- Collected 15 mL sample (from procedure 7.0 above)
- Phytoplankton counting sheets (see Appendix D)
- Phytoplankton data sheets (see Appendix A)
- Color identification sheets (see Appendix E)
- Identification book
- Bottle of Lugol's preservative
- Glass storage bottle for transporting Lugol's preserved sample
- Small cooler for transporting sample

8.2 Step-by-Step Procedure:

1. **Prepare the field microscope for use**. You will find instructions for the use and care of your microscope both in the microscope box and in your notebooks. Briefly, when setting up your scope you should:

   - Pull out eyepiece tube flange fully and twist it to the right to lock it into position.
   - If you need illumination, install the illuminator bracket as illustrated and insert the "Mini Mag-Lite". (You will certainly need illumination when working at 400X.)
   - Be sure that all screws are tightened securely, but not too securely.

   The filters have been positioned for you. In many of the scopes, there will be a small piece of paper visible near the filter. Please do not remove it, as this has been used to prevent the filter from falling out.

2. **Prepare a capillary tube for examination.** The collected 15 mL sample should first be gently swirled to resuspend any material that has settled out. Submerge the end of a capillary tube in the sample until it fills with sample material. Check to be certain the tube is filled between the marked lines. Then clip the tube to the microscope stage for examination.

3. **Using 100X magnification, examine the capillary tube and record results on the phytoplankton counting sheet.** First scan between the lines to identify and record the actual number of *Alexandrium* spp. and *Pseudonitzschia spp.* cells observed. Then scan the tube again for non-target cells and record for each non-target species observed either (1) the actual number of cells observed OR (3) the relative abundance observed using the following abundance index:

   - R (rare): < 1%
   - P (present): ≥ 1% but < 10 %
   - C (common): ≥ 10% but < 50%

- A (abundant): ≥ 50%

Note: To increase the accuracy of observations, at least two samplers should collaborate and record a consensus of opinion on the phytoplankton counting sheet after each makes his or her own observations. To identify particular species, use the color identification sheets in Appendix E and/or the key sheet on the back of the phytoplankton data sheet. It will take some time to become familiar with the different species and you will certainly see some not shown on the identification sheets. Certain species will be more prevalent than others, depending on the time of year. You will probably switch to 400X to help you in identifying certain species. Don't forget to switch back to 100X to do your counting.

4. **Repeat steps 2 and 3 for three additional tubes**.
5. **Summarize results on the phytoplankton data sheet:** Once results for all four tubes have been recorded on the phytoplankton counting sheet, enter the target species data for each of the four tubes and the totals on the phytoplankton data sheet. Also record counts and totals OR the abundance index for each of the three most common non-target species. For species names use the two-letter ID from the counting sheet. Table I on the next page shows a sample completed phytoplankton data sheet.
6. **Record additional comments.** Record on the phytoplankton data sheet comments about any unusual observations. If you have seen unidentified species, include a description or drawing.

Important: If you have noted potentially toxic target species or an abrupt increase in the level of previously reported target cells, contact the coordinator (see below for contact info) as soon as possible so that the need for more intense monitoring can be evaluated.

Congratulations! This completes the sampling process. Please note the time and mileage for each sampler at the bottom of the data sheet. As soon as practical, send the data sheet and the counting sheet by mail to Candace Dolan, Kensington Rd, Hampton Falls, NH 03844 or leave at Kingman Farm. If any target cells are observed call 603-828-4701 or e-mail: candace.dolan@unh.edu

Table I. Example data sheet entry for phytoplankton observations.

Sampling Counts:	**Tube 1**	**Tube 2**	**Tube 3**	**Tube 4**	**Total**
Alexandrium spp.	5	8	5	3	21
Pseudonitzschia spp.	6	2	0	4	12
1. TL	A	C	A	C	
2. RH	C	P	C	C	
3. SK	4	3	5	7	19

1. Note that actual counts are always used for the target species (i.e., Alexandrium and Pseudonitzschia).
2. Note that you can use a count for some non-target species and an abundance index for others.
3. Note that if the abundance index is used there is no total specified.

9.0 MAINTAINING THE EQUIPMENT

Capillary tubes, PVC sieves, buckets, bottles and all sample containers should be thoroughly rinsed between sampling events. Capillary tubes can and should be reused. It is much easier to clean the tubes if you keep them wet until they can be washed. If you note a buildup of algae or dirt in one or more tubes, it will help to soak them in a weak bleach solution for a few minutes.

After sampling has been completed for the day, please rinse your PVC sieve thoroughly with fresh water and allow them to dry. Wipe down the microscope to prevent contaminants and salt spray from drying on the exposed surfaces.

Never store the microscope in your kit near wet equipment!!!

Before closing up the kit for future use, inventory the contents so that any necessary replacement parts, fresh batteries, capillary tubes, chemicals, etc. can be obtained prior to the next sampling event.

Appendix A

Phytoplankton Data Sheet

Appendix B

The Beaufort Scale

The Beaufort scale was originally devised in order to estimate wind speeds while at sea without the necessity of instruments. This is done by noting the condition of the sea and using this observation to approximate the wind speed. The following table relates sea state to wind speed.

Beaufort Force No.	Wind Speed (knots)	Description of Sea Conditions
0	<1	Calm, sea like a mirror.
1	1-3	Light air, ripples only.
2	4-6	Light breeze, small wavelets (0.2 m). Crests have a glassy appearance.
3	7-10	Gentle breeze, large wavelets (0.6 m), crests begin to break.
4	11-16	Moderate breeze, small waves (1 m), some white horses.
5	17-21	Fresh breeze, moderate waves (1.8 m), many white horses.
6	22-27	Strong breeze, large waves (3 m), probably some spray.
7	28-33	Near gale, mounting sea (4 m) with foam blown in streaks downwind.
8	34-40	Gale, moderately high waves (5.5 m), crests break into spindrift.
9	41-47	Strong gale, high waves (7 m), dense foam, visibility affected.
10	48-55	Storm, very high waves (9 m), heavy sea roll, visibility impaired. Surface generally white.
11	56-63	Violent storm, exceptionally high waves (11 m), visibility poor.
12	64+	Hurricane, 14 m waves, air filled with foam and spray, visibility bad.

Appendix C

Salinity Table

Appendix D

Phytoplankton Counting Sheet

Appendix E

Color Identification Sheets