VIRGINIA COAST RESERVE

LONG-TERM ECOLOGICAL RESEARCH PROGRAM

WATER QUALITY MONITORING METHODS MANUAL

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FIELD PROTOCOL

At each sampling station, three replicate samples should be collected. For routine VCR LTER water quality monitoring, sampling should be conducted at approximately monthly intervals on or as to close to slack low water as is logistically possible. This protocol assumes that samples will be collected from a boat by personnel adequately trained for the procedures.

At each sampling station, the following measurements will be made from the boat:

I. Physico-chemical measurements

a) water temperature, ° C

b) salinity, ppt

c) conductivity, mmhos cm^{-1}

d) water depth, m

c) Secchi depth transparency, cm

f) air temperature, °C

g) wind speed and direction, mph; compass heading

h) photosynthetically active, mmoles m^{-2} sec^{-1}

II. Water sample collections for:

a) nutrients

b) chlorophyll

c) total suspended solids and particulate organic matter

The boat operator should approach each sampling station slowly to prevent stirring up the bottom and resuspending sediments that will cloud up the water column and contaminate the water samples.

Upon arriving at each sampling station,

1) collect a 5 gallon bucket of water.

2) submerge the probes for the S-C-T and dissolved oxygen meters in the bucket to start equilibrating with the sample. After two minutes, the oxygen probe should be gently agitated to prevent building up gradients across the membrane. The S-C-T meter probe need not be agitated.

3) If the S-C-T meter is not available, take the temperature and salinity with a thermometer and refractometer. Salinity may also be measured in the lab from water samples using an S-C-T meter or by titration.

4) Drop the Secchi disk over on the shaded side of the boat and take the depth at which the disk just disappears and reappears. Measure the depth to bottom.

5) Fill out the data sheet with the following information: sampling station (code), date, (MM/DD/YY), time (HHMM), station depth, tidal stage, weather conditions, etc.

6) Measure wind direction and speed with hand-held wind meter or estimate.

7. Rinse clean 1 L cubitainers with water from the station 3X (over the side of the boat). Collect the sample by filling to the top and store the bottle in a cooler.

8. For BOD samples, rinse a 300 mL bottle with water from the station (2X). Collect the sample by gently filling the bottle to the top without bubbling. Close the bottle with the ground glass stopper with the bottle top below the surface of the water. Cap the bottle with the plastic cap cover and put the bottle in the carrying rack. Keep the bottle in the dark during transport to the lab. Record the bottle label number, time, dissolved oxygen, and temperature. Collect three replicate bottles at each station.

9) Move to the next station after insuring all station information has been properly recorded, and equipment and materials are stowed.

10) Return the samples to the lab within 3 hours of sampling.

11) Begin filtering the water samples as soon after returning to the lab as possible. See filtering procedure.

12) Retain at least 100 mL in the sample bottles to determine pH of the samples if this has not been done in the field. Measure pH of the water samples according to procedure outlined by manufacturer of the meter.

13) Rinse and dry all meters and sampling equipment to minimize corrosion. Soiled/used sampling and filtering materials should be cleaned and acid-washed if necessary.

PROTOCOL FOR MEASUREMENT OF BOD IN WATER SAMPLES

(by electrometric and chemical titration methods)

Outline of the Method. Water samples are incubated at 20 °C for 5 days in 300 mL B.O.D. bottles after measurement of the initial oxygen content. The final oxygen content is determined and Biological Oxygen Demand is reported as the difference between initial and final oxygen contents.

Materials needed: BOD bottles, bottle rack, D.O. meter, Winkler reagents, pipettes, bucket, salinometer

Preparation:

1. Pack enough clean (detergent washed) BOD bottles for the number of samples needed.

2. Turn D.O. meter on and allow meter to stabilize electronically and the probe to polarize for 15 minutes prior to using the meter and probe. Check red line on the meter

and read temperature. If the meter has a salinity correction knob, make sure it is set to 'Fresh' or zero before calibrating the instrument. Calibrate the instrument according to ambient temperature.

Field Procedure:

1. Check that all materials and equipment needed are on board vehicle or vessel before leaving for the field. Proceed to sampling areas.

2. At each sample site, fill the bucket with water and allow D.O. meter probe to equilibrate with the sample for 2-3 minutes.

3. Agitate the sample gently or keep probe in motion during each reading.

4. Fill three bottles with the sample without bubbling and fix with Winkler reagents (add 2 mL MnSO4, then 2 mL I-KI) then shake. Store bottles in dark cool container until they are titrated.

5. Fill six more bottles and store in cool dark container until they are placed in an incubator.

6. Repeat Steps 2-4 for each designated sampling site and time

Laboratory Procedure:

1. Place unfixed BOD bottles in incubator for five days at 20 °C in the dark. Check bottles periodically to ensure a water seal is maintained.

2. Prepare materials for Winkler titration.

3. Allow flocculent to settle in each fixed BOD bottle before proceeding,

4. Add 2 mL conc. H_2SO_4 to each sample immediately before titration. Shake bottle

thoroughly to dissolve floc. The sample should be clear after this procedure.

5. Measure 100.0 mL into a titration flask and mix sample with magnetic stirrer while

titrating. Titrate with 0.0375 N sodium thiosulfate to a pale straw color.

6. Add 1 mL starch indicator and titrate to a clear end point.

7. Determine volume of titrant used and calculate D.O. in sample.

8. Standardize each batch of working titrant according to procedure.

9. After five days, titrate three bottles of each set according to the Winkler method. Measure final D.O. in the remaining of each set with the D.O. meter. Stir sample with magnetic stirrer during measurement.

The above procedure can be done using the D.O. meter alone if the meter is initially calibrated against the Winkler procedure and if there is enough BOD in each sample to bring the D.O. down in each bottle at least 10 - IOO times greater than the sensitivity of the oxygen meter used (YSI metcrs have a sensitivity of 0.05 mg 0_2/L at 30 °C).

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PROTOCOL FOR MEASUREMENT OF CHLOROPHYLL-*A* CONTENT

OF WATER SAMPLES

Outline of the Method: 500-1000 mL samples of water are filtered through glass fiber filters. Chlorophyll is extracted from the filters using 90% acetone and the absorbances are read spectrophotomecrically.

Materials Needed: 500-1000 mL polyethylene bottles, Whatman GF/C or Gelman Type A/E glass fiber filters, filter tower, filter forceps, 500 mL graduated cylinder, 25-50 mL centrifuge tubes, vacuum apparatus.

Solutions:

1. Prepare a 3:2 90% acetone:DMSO solution with spectrophotometer garde acetone. Store in a dark bottle under refrigeration.

2. Saturated Magnesium carbonate: to 100 mL of deionized water add enough magnesium

carbonate to make a saturated solution.

3. 1 N HCl: prepare from concentrated hydrochloric acid (12N HCl).

Preparation: Prepare detergent-washed polyethylene bottles and assemble filtration apparatus as needed.

Field Procedure:

1. Collect water samples in 500-1000 mL polyethylene bottles. Store on ice until filtered, Samples must be filtered within 5 hours of collection.

2. If time and resources permit, samples should be filtered in the field as described below.

Laboratory Procedure:

1. Assemble filtration equipment and materials as needed.

2. Place glass fiber filter on filter support with forceps. Assemble filter tower and wet filter with a few drops of saturated magnesium carbonate solution.

3. Filter 250 mL sample (or greater volume dependent upon time of year) under 1/2 atm. vacuum to dryness. Store filters with samples at -20 °C in desiccator. **AVOID EXPOSURE TO LIGHT throughout remaining procedure!**

Tear filter into small pieces and place the filter in a 25-50 rnl tissue homgenation tube. Add 5.0 to 10.0 mL of acetone:DMSO solution. Place sample in ice bath and homogenize. Mix virgorously using Vortex mixer and grind again.

5. Transfer ground sample to a 25-50 mL centrifuge tube. Extract overnight under refrigeration.

6. Centrifuge the contents of each tube at room temperature for 5-10 minutes at 2/3 speed (the exact time will depend on the centrifuge but the optical density at 750 nm should be less than 0.05 in a 10 cm cuvette or 0.005 on a 1 cm cuvette)

6. Zero the spectrophotometer with acetone:DMSO solution. Carefully pipette 1 mL of the supernatant into the cuvette and rinse thoroughly with the sample and discard. **Avoid contamination of the supernatant with filter particles.** Pipette 4.0 mL more sample into the cuvette (about 2/3s full) and measure the extinction at the following wavelengths without delay after zeroing the spectrophotometcr at each wavelength with acetone:DMSO (sample should be at room temperature to prevent condensation of the cuvette while in the cell).

Wavelengths: 750, 664, 647, 630, 510 and 480 nm.

7. Correct each extinction for a small turbidity blank by subtracting the 750 nm from the 664, 665 and 630 nm absorptions. (The 510 nm absorbance is corrected by subtracting 2x

the 750 nm absorbance and the 480 nm absorbance is corrected by subtracting 3x the 750 nm absorbance).

8. Add 2-3 drops of 1N HCl, shake the cuvette while covering the top and then read the absorbance of the acidified samples immediately at 665 and 750 nm.

9. Discard the sample and rinse the cuvette thoroughly with deionized water and then acetone:DMSO. The retention of any acid in the cuvette carried over into the next sample can cause erroneous readings due to the breakdown of chlorophyll under acid conditions.

10. Calculate the amount pigment in the original seawater sample using the equations given below.

For Chlorophylls

Chlorophyll *a* = $11.85E_{664}$-$1.54E_{647}$-$0.08E_{630}$

Chlorophyll *b* = $21.03E_{647}$-$5.43E_{664}$-$2.66E_{630}$

Chlorophyll *c* = $24.52E_{630}$-$1.67E_{664}$-$7.60E_{647}$

where E stands for the absorbance at different wavelengths obtained above (after correction by the 750 nm reading) and C*a*, C*b* and C*c* are the amounts of chlorophyll in mg mL^{-1} if a 1-cm light path cuvette is used); then

mg Chl-*a* m^{-3} = 

where *C* is concentration of chlorophyll in the extract (Chl *a, b* or *c*); *v* is the volume of the extract in mL, *V* is the volume of sample that was filtered in liters.

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PROTOCOL TO DETERMINE AMMONIA CONTENT OF WATER

Outline of Method: This method is based on the formation of the blue color of indophenol by phenol and hypochlorite in the presence of ammonia. Scrupulous cleanliness in glassware and work areas is

required because of the ease by which samples and standards can be contaminated from the air and from surfaces. Smoking should not be allowed in work areas and all glassware used in the procedure should be acid-washed and rinsed in deionized water immediately before use. **Glassware stored for some period before should not be used.**

Materials Needed:

20 x 150 mm test tubes with caps, 10 mL automatic pipettes, 0.3 mL automatic pipettes, 100 mL volumetric flasks, droppers, Vortex mixer, parafilm, 100 mL capped plastic bottles, beakers, spectrophommeter, 1 cm cuvettes.

Solutions:

1. 0.5 N Sodium Hydroxide: Dissolve 20 g NaOH in deionized water and dilute to 1.0 L.

2. REAGENT A. Tri-sodium Citrate Reagent: Dissolve 30 g trisodium citrate dehydrate ($C_6H_5Na_3O_7 \cdot 2H_20$) in approximately 60 mL deionized water. Add 2.5 mL of 0.5 N NaOH. Store in glass container, refrigerated with plastic or glass stopper.

3. REAGENT B. Phenol Reagent: Dissolve 9.5 g C_6H_50H (phenol) or 10.8 mL of 88% phenol stock solution and 100 mg of disodium nitroprusside dehydrate ($Na_2Fe(CN)_5NO \cdot 2H_20$) in deionized water and dilute to 250 mL. Store in refrigerator in amber glassware; stable for months.

4. REAGENT C. Hypochlorite Reagent: Dissolve 0.05 g of Trione (dichloroisocyanuric acid) in 20 mL of 0.5 NaOH. Stable for only a few days - **best practice to make fresh each day.**

5. Standard Stock Solution: Dissolve 100 mg ammonium sulfate, $(NH_4)_2SO_4$, in deionizcd water and dilute to 1.0 L. Preserve with 1 mL chloroform. Store in glass container, refrigerated. Stable for several months if well-stoppered.

1.0 mL of the stock solution gives 1.5 mg at $N\text{-}NH_4^+$.

Prepare dilutions with deionized water as follows:

mL stock	dilute to:	mM concentration

1.00	100	15.0
0.50	100	7.5
0.20	100	3.0
0.10	100	1.5
0.05	100	0.75

Procedure:

1. Rinse test tubes and caps once with 15% HCl and four times with deionized water immediately before starring procedure. Shake out excess drops of water and keep tubes inverted and capped until used.

2. To avoid contamination of samples and reagents, all containers should remain capped until needed for use and gloves should be worn. Any dilutions done should utilize the same stock or batch of synthetic seawater or ammonia-free seawater.

a) Add 10.0 mL of sample into the tube.

b) Add 0.3 mL of Reagent A and shake/vortex.

c) Immediately add 0.3 mL of Reagent B and shake/vortex.

d) Immediately add 0.3 mL of Reagent C and shake/vortex.

3. Cap the tubes tightly with parafilm. Let the reaction proceed in the dark for at least six hours, preferably overnight.

4. Prepare ammonia blanks and standards by adding reagents as above to 10.0 mL volumes of deionized water blanks and standards. Allow color development to proceed for at least 6 hours.

5. When samples and standards are ready for reading of absorbance, prepare reagent blanks using 10 mL of deionized water or synthetic seawater. Add reagents as above. Measure absorbance immediately.

The spectrophotometer is adjusted to 630 nm wavelength and adjusted to read zero with deionized water or synthetic seawater in a 1 cm pathlength cuvctte.

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PROTOCOL TO DETERMINE NITRATE AND NITRITE CONTENT OF WATER SAMPLES

Outline of Method: Nitrite is determined by diazotizing with sulfanilamide and coupling with N-(l-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye, Nitrate is reduced to nitrite with a Cu-Cd column and analyzed for nitrite. Nitrate concentration is calculated as the difference between the reduced sample and the unreduced sample.

Nitrite Analysis

Materials: test tubes. 10 mL automatic pipettes, 100 mL automatic pipettes, parafilm, beakers, Vortex stirrer, spectrophotometer, 1-cm cuvettes

Reagents:

1. REAGENT A. Sulfanilamide solution - - Dissolve 10.0 g sulfanilamide in 200 mL conc. HCl and dilute to 1 L. Solution is stable for several months.

2. REAGENT B. NNED solution - Dissolve 1.0 g NNED, dilute with deionized water to 1 L. Renew solution once a month or a strong brown coloration will develop.

Standards:

A stock solution is prepared by dissolving 8.511 g of oven-dried (100 °C for 1 hour) anhydrous potassium nitrite in 1000 mL of deionized water. Add 1 mL chloroform as preservative and store in a brown glass bottle in cold. Discard if brown coloration develops.

1.0 mL of this stock solution contains 100 mg at $N\text{-}NO_2^-$

Prepare a working stock solution by diluting 5 mL of standard stock solution to 500 mL of deionized water.

1.0 mL of this working solution contains 1 mg at $N\text{-}NO_2^-$

Prepare dilutions with deionized water or synthetic seawater as follows:

mL of stock	dilute to:	mM concentration
2.5	100	25.0
1.0	100	10.0
0.5	100	5.0

Procedure:

a) Add 10.0 mL of sample into a tube.

b) Add 0.2 mL of sulfanilamide reagent. Vortex and allow reagent to react for 2-8 minutes.

c) Add 0.2 mL of NNED reagent and vortex. Allow a minimum of 10 rninutes for color development.

Prepare a reagent blank using deionized water and add reagents as above.

If necessary prepare a seawater blank using synthetic seawater and add reagents as above.

Adjust spectrophotometer to 543 nm wavelength and adjust to zero using distilled water in a 1 cm pathlength cuvette. Take all absorbance readings within 2 hours of adding reagents.

Nitrate Reduction and Analysis

Materials: nitrate reduction apparatus, test tubes, 10 mL automatic pipettes, 0.2 mL automatic pipettes, beakers, flasks, 100 mL volumetric flasks, droppers, Vortex mixer, parafilm, spectrophotometer, cuvettes, glass wool, copper shavings

Reagents:

1. Concentrated ammonium chloride- Dissolve 125 g ammonium chloride (NH_4Cl) in 500 mL deionized water. Store in plastic or glass bottle.

2. Diluce ammonium chloride- Dilute 50 mL of conc. ammonium chloride to 2000 mL deionized water. Store in plastic or glass bottle.

3. 1 N HCl - dilute concentrated HCl

4. 2% copper sulfate- Dissolve 20 g $CuSO_4$ in 250 mL of deionized water and dilute to 1000 mL.

5. REAGENT A. Sulfanilamide reagent- as above

6. REAGENT B. NNED reagent - as above

Standards:

A stock solution of potassium nitrate is prepared by dissolving 10.106 g of anhydrous KNO_3 in about 500 mL deionized water and diluted to 1 L. The solution is preserved with 1 mL chloroform and is stable for 6 months. Store cold.

1.0 mL of this stock solution contains 100 mg at $N\text{-}NO_3$

Dilute 5 mL of standard stock solution to 500 mL deionized water

1.0 mL of this working stock solution contains 1mg at $N\text{-}NO_3$

Prepare standards with deionized water or synthetic seawater as follows:

mL of stock	dilute to:	mM concentration
2.5	100	25.0
1.0	100	10.0

0.5	100	5.0
0.1	100	1.0

Process standards solutions by passing 50 mL volumes of each standard through the reduction columns after adding 1.0 mL of concentrated ammonium chloride solution.

Procedure:

The Cu-Cd reduction column is prepared by treating enough cadmium granules for the number of columns needed with the following solutions:

a) 1 N HCl - new cadmium granules will appear silvery while used cadmium will show a dull gray color.

b) deionized water- wash the cadmium granules in several changes of ionized water until the wash is no longer acidic.

c) 2% copper sulfate - add enough solution to the cadmium slurry until there is just a suggestion of blue color indicating there is now excess copper sulfate. At this point the granules should appear black.

d) deionized water- wash the slurry with deionized water until all colloidal material has been removed. Gently swirl the mixture while washing.

e) dilute ammonium chloride- Wash the slurry with 3 changes of dilute ammonium chloride. After this point, the cadmium slurry should always be kept under a solution and away from air. Exposure to air will ruin the copper-cadmium slurry.

Set up the columns on their stands and insert copper shavings into the bottom of each column. Fill the columns with dilute ammonium chloride. Carefully fill the column with the copperized cadmium granules. Continuously tap the sides of die column to avoid dead spaces. Leave about 1 inch clearance from the top of the cadmium to the base of the upper vessel. Fill the top with a glass wool plug.

Care MUST be taken to avoid exposing the reduction column to air. Keep the tip of the drip tub above the top of the cadmium column to prevent accidentally exposing the column to air.

The column is conditioned for use by passing 100 mL of dilute ammonium chloride solution and activated by passing 500 mL of 50 mg at $N\text{-}NO_3$ standard solution buffered with 10 mL of concentrated NH_4Cl solution.

a) Pipette 50.0 mL of sample or standards in acid-washed centrifuge tubes and 1 mL of concentrated ammonium chloride solution. Mix thoroughly.

b) Immediately introduce into the cadmium column. Let 25 mL of the treated sample pass through the reduction column with the flow s@ adjusted to I mL per niinute.

c) Discard the first 25 mL of reduced sample.

d) Pass the remaining sample through the reduction column at the same speed and collect the last 25 mL in a centrifuge tube. Pipette 10 mL for nitrite analysis.

To each 10 mL sample,

f) Add 0.2 mL of sulfanilamide reagent. Vortex and allow the reaction to proceed for 2-8 minutes.

g) Add 0.2 mL of NNED reagent and vortex. Allow 20 minutes for color development.

Prepare a blank using 50 mL of synthetic seawater or dilute ammonium chloride. Pass the blank through the reduction column as above. Add reagents as above. Adjust spectrophotometer to 543 nm wavelength and adjust to read zero using deionized water or synthetic seawater.

Read samples, standards and blanks in 1-cm cells with the spectrophotometer set at 543 nm wavelength. Take all readings within 2 hours of adding reagents.

Cadmium Column Storage:

After using the reduction column, it is prepared for storage by passing 500 mL of 50 mM nitrate standard and then 100 mL of dilute ammonium chloride solution through the column. Cap the top of the column with parafilm and make sure the tip of the drip tube is completely sealed.

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PROTOCOL FOR FILTERING WAT'ER SAMPLES FOR NUTRIENT ANALYSIS

Outline of the Method: Filtration of water samples prior to nutrient analysis is necessary for several reasons: a) to minimize microbial activity in the sample that may affect nutrient concentrations (by uptake or release); b) to remove particulates that may contain adsorbed nutrients; c) to remove microbial cells which may lyse, releasing nutrient, upon addition of reagents; and d) to remove turbidity that may interfere with colorimetric measurements of the samples. About 100 mL of filtered sample are required for the current protocols (10 mL for ammonia, nitrite, and phosphate analyses, and 50 mL for nitrate analysis). Samples are filtered through 0.45 mm membrane filters (some microbiological applications may require filtration through a 0.2 mm filter) and collected in acid-washed centrifuge/test tubes for splitting.

Materials Needed: 47 mm dia. filtration tower, filter flask 250 mL polyethylene bottles, vacuum pump, filter forceps, 45/47 mm dia. 0.45 mm (or 0.2 mm) Supor membrane filters

Preparation:

Acid-wash all glassware to be used in the procedure. Store filters in air-tight containers when not in use.

Field Procedure:

1. Collect samples in acid-washed 250 mL sample bottles after rinsing each bottle with some sample prior to filling up the bottle. Keep the samples in ice if filtering cannot not proceed immediately. Sample bottles should not be submerged in meltwater while being held prior to filtration.

2. Using filter forceps, place the filter on the filter tower platform and clamp the filter as needed. Never handle clean filters with your fingers. Fill the tower with sample water, noting the volume added. The volume of water that can be filtered using one filter will depend on how turbid the samples are. If the filter is clogged replace with a new filter. The tower need not be acid-washed. Filter 150 mls of samples and retain the balance for measurement of pH, salinity, etc.

3. Transfer the filtrate into acid-washed sample bottles.

4. After filtering samples, remove and discard the filter, Wash the filter tower and flask with 15% HCI and rinse with 3 changes of deionized water. Between replicate samples. The tower and filter flask may be rinsed with deionized alone. Through washing with acid and rinsing with copious amounts of deionized water is required between different

samples.

5. After completing the filtration, store the filtered samples in the freezer is nutrient samples cannot be conducted immediately.

Clean all used materials with phosphate-free detergent, acid-wash and rinse with deionized water.

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PROTOCOL TO DETERMINE PHOSPHATE CONTENT OF WATER SAMPLES

Outline of Method: Samples are allowed to react with a composite reagent containing molybdic acid, ascorbic acid and trivalent antimony resulting in a blue colored solution which can be measured at 885 nm. Since phosphate may be bound onto inorganic and organic particulate, filtration is required to avoid release of this phosphorus fraction when acid reagents are added to sample. Removal of the particulate fraction can be done by centrifugation (1200 rpm for 20 min.) or filtration (with well-rinsed 0.2 mm or 0.45 mm membrane filters). For highly turbidsamples, pre-filtration through a pre-ashed glass fibre filter (e.g, OF/C) may be done. There is no salt effect so dilutions and blanks may be made with deionized water. Care should be taken to avoid contamination from glassware washed with phosphate-containing detergents. Acid-washed glassware should be used at all times in this procedure.

Materials Needed: vacuum filtration apparatus, GF/C glass fibre filters, 0.45 gm membranefilters, 20 x 150 mm test tubes with caps, 10 iffl automatic pipettes, I mL automatic pipettes, 100 mL volumetric flasks, 100mL stoppered bottles, droppers, Vortex mixer, parafilm, beakers, spectrophotometer. 1-cm or 5-cm cuvettes

Solutions:

1. Ammonium molybdate solution - Dissolve 15.0 g ammonium molybdate ($(NH_4)_6Mo_7O_{24})\cdot 4H_2O$) in 500 mL deionized water. Store in plastic in dark. Stable indefinitely.

2. 5 N Sulfuric acid solution - Add 140 mL concentrated sulfuric acid (H_2SO_4) to 900deionized water, cool and store in glass bottle, stable indefinitely when sealed.

3. Ascorbic acid solution - Dissolve 27.0 g ascorbic acid in 500 mL deionized water, store in plastic. Stable for months if frozen, weeks if cold, days at room temperature.

4. Potassium Antimonyl Tartrate - Dissolve 0.34 g potassium antimony tartrate ($K(SbO)C_4H_4O_6 \cdot 1/2\ H_20$) in 250 deionized water, warm if necessary, Store in glass or plastic. Stability in months.

5. Combined reagent - Mix in the strict order the following solutions in an acid-washed container that can be capped.

25.0 mL ammonium molybdate solution

62.5 mL sulfuric acid solution

25.0 mL ascorbic acid solution

12.5 mL potassium antimonyl tartrate solution.

Stability of this reagent is 6 hours or less. The solution should be a light straw-color. A shade of green suggests the presence of phosphate in the deionized water or contamination from the environment.

6. Phenolpthalein Indicator Solution: Dissolve 0.5 g phenolphthalein in a solution of 50 mL ethyl alcohol and 50 mL deionized water,

7, 6 N NaOH Solution: Dissolve 240 g sodium hydroxide in approximately 500 mL and

dilute to 1L with deionized water.

8. 1 N H_2SO_4 Solution: Mix 28.0 mL of concentrated H_2SO_4 with 900 mL of distilled water and dilute to 1 liter.

Standards:

A stock solution is prepared by dissolving 136.1 mg of anhydrous potassium dihydrogen phosphate in deionized water, and making up to 1000 mL after adding 0.2 mL of the sulfuric acid solution. Store in glass in cold. Stable for months.

1mL of this stock solution contains 1 mM at. P-PO_4^{3-}

Prepare standard dilutions as follows:

mL of stock	dilute to:	mM concentration
1.50	100	15.0
1.00	100	10.0
0.50	100	5.0
0.20	100	2.0
0.10	100	1.0
0.05	100	0.5

Procedure:

Unless previously done, rinse test tubes and caps once with 15% HCl and four times with deionized water before starting procedure. Shake out excess drops of water and keep tubes inverted and capped until used.

a) Adjust the pH of each sample as follows:

-add one drop of phenolphthalein indicator to each sample

-add 6 N NaOH dropwise until a pink color develops

-add 1 N H_2SO_4 until pink color disappears

b) Add 10.0 mL of filtered or centrifuged sample into a tube.

c) Add 1.0 mL of combined reagent and shake immediately. Allow color development to proceed for 20 minutes.

Prepare phosphate standards by adding the combined reagent as above to 10 mL volumes of standards.

Prepare a reagent blank using 10.0 mL of acidified (with 0.1 mL 1N HCl) deionized water. Add reagents as above.

The spectrophotometer is adjusted to 885 nm wavelength and adjusted to read zero absorbance with deionized water in a 1-cm or 5-cm pathlength cuvette.

Take readings within 1 hour of adding reagents.

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PROTOCOL FOR NMASUREMENT OF pH OF WATER SAMPLES

Outline of the Method: Water samples are collected and measured for pH as soon as possible. As much as possible, measurements should be done.

Materials Needed: 250 mL sampling bottles, 150 nil beakers, dionized. water squeeze bottles, magnetic stirrer bars, magnetic stirrer

Solutions:

1. Electrode filling solution

2. pH buffer solutions (pH 4,7 and 10)

3. Electrode storage solution

Preparation:

Check the pH meter for proper operation: power/ba@ level and proper operation of the mode and calibration knobs/switches.

Check that the electrode is clean, free of cracks and properly filled with filling solution. Ensure that the seal over the filling hole is open during the measurements.

Calibrate the pH meter using fresh buffer solutions. Use a two point calibration. If the expected sample pH is above 7 pH units, calibrate the meter with pH 7 and pH 10 buffer solutions, otherwise use pH 4 and pH 7 solutions. Bring the buffer solutions to room temperature before using. As much as possible the calibrating buffer solutions and samples should be at the same temperature. Calibrate the meter with the probe tips immersed in the buffer solutions with moderate stirring speed. Check and record the slope.

Field/Laboratory Procedure:

Collect the samples in clean 250 mL amber bottles. If any holding time is necessary, a maximum of 3 hours can be allowed. Note that as soon as a sample is collected in the bottle the dissolved $C0_2$ can will exchange with the atmosphere and thus affect the pH of the sample. Because the calibration value of the buffer solutions is affected by temperature, measure the temperature of the buffers and samples prior to making pH measurements. Do not store the used calibrating solutions. Always use fresh solutions.

Rinse the beaker with a small volume of the sample and fill to the halfway mark. Place the clean stir bar in the sample.

Assuming the pH meter has been properly calibrated, immerse the tip of the probe into the sample and stir the sample at moderate speed. Switch the mode knob to pH. Allow at least 1 minute for the meter to equilibrate before reading the value. Record the time and date of collection, time of measurement, temperature, and pH.

Cleaning Procedure:

Stop the stirrer, retrieve the stir bar and discard the sample. Wash the tip of the probe with DI water from a squeeze bottle. Do not wipe the tip of the probe.

Short-term Storage:

Rinse the electrode with deionized water. Cover the filling hole with sleeve and store in electrode storage solution at room temperature.

Long-term Storage:

If the electrode is not to be used for more than a month, drain the filling solution from the electrode, rinse the chamber thoroughly with deionized water and allow the electrode to dry before storing it in it original storage box.

Electrode Rejuvenation:

If the electrode tip has been dried out or has been in dry storage, the electrode may be rejuvenated by immersing the electrode in 0.1 N HCl solution for several hours. Refill the electrode with fresh filling solution and do a 2 point calibration. Check the slope. It should be greater than 95% for proper meter operation.

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PROTOCOL FOR MEASURMENT OF TOTAL SUSPENDED SOLIDS IN WATER SAMPLES

Outline of the Method: 250-500 mL water samples collected in polyethylene bottles are filtered through tared glass-fiber filters.

Materials Needed: Whatman GF/C, Gelman Type A/E, or GF/F filters, filtration tower, filter flask, 500 mL polyethylene bottles, vacuum pump. filter forceps

Preparation:

1. Pre-ash 47 mm dia GF/C filters placed in an aluminum foil packet in a muffle furnace at 500 °C for 6 hours.

2. Weigh each filter to nearest 0.000 g. Record tare weights in a log with appropriate filter codes.

Field Procedure:

1. Collect samples in clean 500 sample bottles.

2. If time and resources permit, filtration may be done in the field. Proceed as described below.

Laboratory Procedure:

1. Assemble filtration equipment and materials as needed.

2. Using filter forceps, place a filter on filter tower and clamp filter tower as needed. Fill tower with sample water.

3. After filtering samples, rinse the tower and filter with 100 mL of deionized water.

4. Remove the filter with forceps and fold in half, keeping sample within folded filter.

5. Dry filters in envelopes in drying oven at 105 °C overnight.

6. Allow filters to cool in dessicator. Weigh filters to 0.000 g accuracy.

7. Ash each filter in a muffle furnace for 6 hours at 500 °C.

8. Allow filters to cool to room temperature in dessicator. Weigh each filter to nearest 0.000 g.

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PROTOCOL FOR TOTAL NITROGEN CONTENT OF WATER SAMPLES

Outline of the Method: Total nitrogen (inorganic and organic fixed nitrogen) is determined by subjecting a sample to a potassium persulfate digestion. After digestion an HCI and borate buffer solution are added; the sample may now be analyzed as in the nitrate procedure.

Materials Needed: vacuum filtration apparatus, Whatman GF/C glass fibre filters, test tubes, 10 mL automatic pipettes, 1 5-mL adjustable automatic pipettes, parafilm, beakers, Vortex mixer, spectrophotometer, 1-cm cuvettes

Solutions:

1. Oxidizing Reagent: 3.0 g of NaOH and 6.7 g of low N (<O.001%) potassium persulfate ($K_2S_2O_8$) are dissolved in 1 L of distilled water just prior to use.

2. 0.3 N HCl: Dilute 25 mL of concentrated HCl with 975 mL of distilled water. Tnis solution is stable for months.

3. Borate Buffer Solution: 30.9 g of H_3BO_3 are dissolved in distilled water, 101 mL of 1 M NaOH are added, and the solution is diluted to 1 L. This solution is stable for months.

4. 1 M NaOH: Dissolve 40 g of low N NaOH in 1 L of distilled water.

Standards:

Urea Standard Stock Solution.- Dissolved 2.1437 g of urea in 1 L of deionized water. 1.0 mL of this

solution contains 1 mg of N.

Working Standard Solution: Dilute 5 mL of urea stock solution to 500 mL with deionized water. 1.0 mL of this solution contains 0.01 mg of N.

Using working standard stock solution make the following standard solutions. Make up standards in 100.0 mL volumetric flasks with deionized water.

mL stock solution	Concentration (mM)
1.0	7.14
2	14.28
5	35.38
10	71.38
15	107.07

Total Nitrogen Analytical Procedures

1) Add 15 mL of oxidizing reagent to 10.0 mL of filtered sample and cap the test tube.

2) Autoclave the standards and samples at 100-110 °C for 30 minutes and allow to slowly return to atmospheric pressure.

3) After test tubes cool to room temperature add 1.5 mL of 0.3 N HCl and mix.

4) Add 2.0 mL of borate buffer and dilute with distilled water to a mark on the tube indicating 50 mL and mix. The pH of the buffered digest should be about 8.

5) Analyze for nitrate

6) Prepare a blank by mixing 15 mL of oxidizing reagent and 10 mL of distilled water.

Source(s):

Parsons, Maita and Lalli, 1984

C. F. D'Elia, et al. WoWshotc Oceanographic Institute Contribution # 3954

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PROTOCOL FOR TOTAL PHOSPHORUS CONTENT OF WATER SAMPLES

Outline of the Method: Filtered samples are subject to an ammonium persulfate digestion. This hydrolyzes phosphates bound to organic substances and polyphosphates. The pH of the digested samples are adjusted and mixed with a combined color reagent which allows samples to be read spectrophotometrically.

Materials Needed: vacuum filtration apparatus, GF/C glass fiber filters, 0.45 mm membrane filters, 20 x 150 mm test tubes with caps, autoclave, screw-cap test tubes, 10 mL automatic pipettes, 0.2 mL automatic pipettes, 1 mL automatic pipettes, 100 mL volumetric flasks, 100 mL stoppered bottles, droppers, Vortex mixer, parafilm, beakers, spectrophotometer, 1-cm or 5-cm cuvettes

Solutions:

1) 11 N Sulfuric Acid Solution: Slowly add 310 mL of concentrated sulfuric acid (H_2SO_4) to 600 mL of distilled water. When cool, dilute to 1L. **Caution: Always add ACID to WATER.**

2) Ammonium Persulfate Solution: Dissolve 400 g of ammonium persulfate ($K_2S_20_8$) in about 600 mL of distilled water. Dilute to 1 L.

3) Ammonium Molybdate Solution: Dissolve 15 g ammonium molybdate ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$) in 500 mL deionized water. Store in plastic in dark. Stable indefinitely.

4. 5N Sulfuric Acid Solution: Add 140 mL concentrated sulfuric acid (H_2SO_4) to 900 mL deionized water. Cool and store in glass bottle. Stable indefinitely when sealed.

5. Ascorbic Acid Solution: Dissolve 27 g ascorbic acid in 500 mL deionized water. Store in plastic. Stable for months if frozen, 1 week if cold, 1 day at room temperature.

6. Potassium Antimonyl Tartrate Solution: Dissolve 0.34 g potassium antimonyl tartrate ($K(SbO)C_4H_4O_6\cdot 1/2\ H_2O$) in 250 mL deionized water, warm if necessary. Store in glass

or plastic. Stability in months.

7. Combined Reagent Solution: Mix in the strict order the following solutions in an acid-washed container that can be capped:

25 mL ammonium molybdate solution

62.5 mL 5 N sulfuric acid solution

25 mL ascorbic acid solution

12.5 mL potassium antimonyl tartrate solution.

Stability of this reagent is 6 hours or less. The solution should be a light straw-color. Ashade of green suggests the presence of phosphate in the 'deionized' water or contamination from the environment.

8. Phenolpthalein Indicator Solution: Dissolve 0.5 g phenolphthalein in a solution of 50mL ethyl alcohol and 50 rTd deionized water.

9. 1 N H_2SO_4 Solution: Mix 28 mL of concentrated H_2SO_4 with 900 mL of deionized water and dilute to1L.

10. 6 N NaOH Solution: Dissolve 240 g sodium hydroxide in approximately 500 mL and dilute to 1 liter with deionized water.

Standards:

A stock solution is prepared by dissolving 136.1 mg of anhydrous potassium dihydrogen phosphate (KH_2PO_4) in deionized water, and making up to 1000 mL after adding 0.2 mL of the sulfuric acid solution. Store in glass in cold. Stable for months.

1.0 mL of this stock solution contains 1 mM P-PO_4^{3-}.

Prepare standard dilutions as follows:

mL of stock	dilute to:	mM concentration
1.50	100	15.0
1.00	100	10.0
0.50	100	5.0
0.20	100	2.0

0.10	100	1.0
0.05	100	0.5

Procedure:

Unless previously done, rinse test tubes and caps once with 15% HCl and four times with deionized water before starting procedure. Shake out excess drops of water and keep tubes inverted and capped until used.

1) Take 10.0 mL of filtered sample (or standard) and add to a prepped test tube. Test tubes are prepped by adding 0.2 mL of 11 N H_2SO_4 and 0.2 mL ammonium persulfate solution.

2) Heat for 30 minutes in the autoclave at 121 °C (15-20 psi). Remove tubes when cool.

3) Adjust the pH as follows:

-add one drop of phenolphthalein indicator to each sample

-add 6 N NaOH dropwise until a pink color develops

-add 1 N H_2SO_4 until pink color disappears

4) Dilute sample to 13 mL mark on test tube with deionized water.

5) Add 2.1 mL of combined reagent and vortex.

6) After a minimum of 10 minutes but no more than 30 minutes, measure the absorbance of each sample at 885 nm.

7) Prepare phosphate standards by adding the combined reagent as above to 10.0 mL volumes of standards.

8) Prepare a reagent blank using 10.0 mL of acidified (with 0.1 mL 1 N HCl) deionized water. Add reagents as above.

The spectrophotometer is adjusted to 885 nm wavelength and adjusted to read zero absorbance with deionized water in a 1-cm or 5-cm pathlength cuvette.

Take readings within 1 hour of adding reagents.

Reference: U.S. EPA. 1974. Methods for Chemical Analysis of Water and Wastes pp. 249-263.

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CHECKLIST OF MATERIALS AND EQUIPMENT

Physico-chemical Measurements Number

YSI S-C-T meter and probe 1

YSI/Orbisphere Oxygen meter and probe 1

meter stick 1

Secchi disk 1

5 gal. Bucket 1

refractometer 1

mercury thermometer 1

Water Sampling

250-mL amber HDPE bottle, acid-washed, deionized-water rinsed (3 per station)

500-mL clear HDPE bottle, detergent-washed, deionized-water rinsed (3 per station)

500-mL amber HDPE bottle, detergent-washed, deionized water rinsed (3 per station)

300-mL BOD bottle, detergent-washed (3 per station)

OR

4-L cubitainer, acid-washed, deionized-water rinsed (3 per station)

Sediment Sampling

Ponar Dredge 1

line 1

wash basin 1

centrifuge tubes 3 per sta.

Filtration

hand vacuum pump tubing 1

Filtration flask 3

filtration tower 3

0.45mm (or 0.2 mm) membrane filters 100+

Nutrient Analysis

test tubes 150 x 20

test rube racks

appropriate reagents

Total Suspended Sediments Analysis

Whatman GF/C, 0.47 mm diam. filter papers, ashed and weighed

Drying oven set at 100 °C

Muffle furnace set at 500 °C

Chlorophyll Analysis

glass fibre filters Whatman GF/F or Getman Type AE

centrifuge tubes

3:1 solution of 90%acetone:DMSO

saturated magnesium carbonate solution

Biological Oxygen Demand Analysis

300 mL BOD bottles

BOD bottle racks

oxygen meter

Miscellaneous Supplies

liquinox detergent

kimwipes (paper towels)

15% HCI wash bottle

labeling tape

labeling markers

coolers for BOD bottles/ nutrient and sediment samples

Prior to beginning field work, inspect all meters and equipment for proper operation, adequate battery supply and availability of required accessories and peripherals. Calibrate oxygen meter and salinometer. As leaving the dock, allow electronic circuits to warm-up before use. Typically a 15 minute warm up time is adequate for most field equipment.

CONDENSED FIELD PROTOCOL

Upon arriving at each sampling station, fill out the data sheet with the following information: sampling station (code) date (MMDDYY), time (HHMM), station depth, tidal stage, weather conditions, etc.

I. Physico-chemical Measurements

1) collect a 5 gallon bucket of water.

2) measure temperature, salinity and conductivity of sample in the bucket with S-C-T meter

3) measure temperature and dissolved oxygen of sample in the bucket with the d.o. meter

3a) If the 5-C-T tnecer is not available, take the temperature and @ty with a therrnometer and refractometer. Salinity may also be measured in the lab from water samples using an S-C-T meter or by Titration.

4) measure transparency of the water column with the Socchi disk over on the shaded side of the boat.

5) measure depth Lo the bottom

6) measure wind direction and speed with hand-held wind meter or estimate.

7) Record in appropriate data sheet water temperature (degrees Celsius °C), salinity (parts per thousand ppt or o/oo, conductivity, dissolved oxygen content (mg 0_2 L^{-1} (mg L^{-1}) or parts per million (ppm)), wind direction (e.g. northwest NW) and speed (miles per hour mph).

II. Water and Sediment Sample Collections

1) Nutrient Analysis, Total Suspended Sediments, Chlorophyll-*a*: Rinse 4-L cubitainer with water from the Station (1time) over the side of the boat. Collect the sample by filling to the top and store the cubitainer in a cooler with ice.

OR

Nutrient Analysis: Acid wash the amber 250 mL bottles before collecting a sample. Rinse the acid-washed 250 @ amber bottles with water from the station 3X (over the side of the boat). Collect the sample by filling to the top and store the bottle in a cooler with ice. Record thelabel number.

2) Total Suspended Solids: Rinse the 500 mL bottle with water from the Station (1 time) over the side. Collect the sample by filling to the top and store the bottle in a cooler with ice. Record the label number.

3) Chlorophyll *a*: Rinse the 500 mL amber bottle with water from the station (1 time) over the side or from the bucket. Collect the sample by filling to the top and store the bottle in a cooler with ice. Record the label number.

4) Biological Oxygen Demand-. Rinse the 300 mL BOD bottle with water from the station (1 time). Collect the sample from the bucket by gently filling the bottle to the top without bubbling. Close the bottle with the ground glass stopper beneath the water

surface. Cap the bottle with the plastic cap/cover and replace the bottle in the carrying rack. Keep the bottle in the dark while transporting to the lab. Record the label number and initial time, D.O., and temperature.

5) Trace metals: Rinse the 50 mL bottle with water from the station (1 time). Collect the sample from over the side and fill to the top. Stabilize the sample by adding 0.5 mL of 1:1 aq. nitric acid. Cap the bottle and store in a cooler with ice. Record the label number.

6) Sediment Analysis: Collect a sample of the sediment with the Ponar dredge or corer.

Store a 50-100 mL volume of the sediment in a sample cup, rube or plastic bag and place on ice until processed or preserved.

7) Move to the next station after ensuring all station information have been properly recorded, labels are properly logged and equipment and materials are stowed.

8) Return the samples to the lab within 3 hours of sampling. If necessary and feasible, filtration should be conducted on site. See filtration procedure for nutrients, chlorophyll and total suspended solids (TSS).

III. FIELD/LABORATORY PROCESSING OF SAMPLES

1) Upon returning to the laboratory, place the BOD bottles in the incubator set at 20 C, If it has been done in the field, store the TSS and chlorophyll filters, water and sediments samples in designated freezers.

2) begin filtering the water samples (nutrients, chlorophyll-*a* and TSS) soon after returning to the lab if this has nor been done in the field. See filtering procedure.

3) Retain at least 100 mL in the sample bottles to determine pH of the samples. Measure pH of the water samples according to procedure.

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