MEAP QAPP, modified for nutrient analysis, January 2007

Quality Assurance Project Plan

MASSACHUSETTS ESTUARINE ASSESSMENT PROJECT

OCTOBER 2000

Project No: MMRP00-03

Massachusetts Coastal Zone Management

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1.0 DISTRIBUTION LIST

Following is a list of key individuals and their organization to which copies of the approved Massachusetts Estuarine Assessment Program (MEAP) QAPP and subsequent revisions will be provided:

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2.0 PROJECT DESCRIPTION

2.1 Objective

The primary objective of the Massachusetts Estuarine Assessment Project (MEAP) is to collect and analyze sediment and waters samples at selected stations identified by EPA and CZM in order to evaluate the ecological conditions of Massachusetts estuaries. This project defines the partnership of MEAP within the larger National Coastal Assessment (also referred to as Coastal 2000) of the U.S. Environmental Protection Agency.

This QAPP is to serve as a supplement to the EPA/EMAP – National Coastal Assessment – Coastal 2000 (August 2000).

2.2 Project Background

The Environmental Protection Agency (EPA) is partnering with the Commonwealth of Massachusetts to conduct one of the most comprehensive assessments of the Commonwealth's coastal sediments, waters and biota as part of the nationwide EPA Coastal 2000 Monitoring effort. The monitoring will be coordinated by the Commonwealth's Office of Coastal Zone Management (CZM) in the Executive Office of Environmental Affairs. Sampling and some of the analyses (see below) will be conducted by the University of Massachusetts at Boston (UMB) and Dartmouth (UMD) in cooperation with CZM. Surface sediments from 40 stations will be sampled for a variety of contaminants of environmental concern, both inorganic (metals) and organic (PAHs, PCBs, and pesticides). Water column samples will be taken at each site to provide a "snapshot" of hydrographic variables, nutrients, pigments and suspended matter at the time of sampling. Fish sampling will be conducted by the Commonwealth's Division of Marine Fisheries (DMF). In addition the University of Massachusetts at Boston has agreed to sample the water column at each site for selected metals in "dissolved" form (operationally defined as metals in the filtrate after 0.4 µm polycarbonate membrane filtration). Cores will be taken for the analysis of metal profiles after dating using appropriate geochronological tracers (^{210}Pb, ^{137}Cs, ^{7}Be) at a subset of the sites sampled. Analysis of the cores will however be dependent on acquisition of additional funds to support this effort.

3.0 PROJECT ORGANIZATION

The team consists of the following groups

- **Project Management (State):**

The Project Officer, Mr. Christian Krahforst, will oversee the project. Mr. Krahforst will be responsible for fish, sediment and water sample delivery to EPA for those analyses not conducted by the University of Massachusetts.

- **Project Management (UMass Teams):**

Dr. Gordon Wallace and Dr. Jefferson Turner, from the University of Massachusetts will oversee the field sampling efforts, the timely completion of sample analyses, and compilation of laboratory data. Ms. Melissa Farraro will be the field crew chief. Dr. Bernie Gardner will oversee the collection of physical parameters (temperature, salinity, etc.). Dr. Wallace, Dr. Turner and Mr. Krahforst will be responsible for the completion and quality of the final report.

- **Field Sampling Team:**

Field sampling will be conducted by staff from UMB (Environmental, Coastal and Ocean Sciences Program), UMass Dartmouth (School of Marine Science and Technology), and

CZM in accordance with training provided by EPA. Dr. Wallace and Dr. Turner will coordinate sampling schedules and logistics. Dr. Wallace will contact Ms. Fararro of UMass Boston to cover logistical considerations (scheduling of boat and crew, field sampling preparation) before initiating sampling. Except for fish, samples will be collected by the University of Massachusetts for all parameters listed in Table A7-1 of EPA's QAPP. Samples will be returned to UMB and UMass Dartmouth within the holding times of the respective parameters. Dr. Wallace will oversee this effort. The Massachusetts Division of Marine Fisheries (DMF) will collect and measure all fish related aspects of EPA's Coastal 2000 Program during their fall trawl surveys. Arnie Howe is the primary contact at DMF.

Results of laboratory analyses will be delivered to Mr. Krahforst for review of data and submission to EPA. Data reports will be submitted to MCZM for QA/QC review and then forwarded to EPA's National Health and Environmental Effects Laboratory's Atlantic Ecology Division in Narragansett, RI.

4.0 MONITORING PARAMETERS, COLLECTION FREQUENCY AND STATION LOCATIONS

4.1 Sampling and Parameters

Water column and sediment sampling will be conducted as described in the Coastal 2000 Field Sampling Manual (currently in review by EPA) and in accordance with EPA's Coastal 2000 QAPP (August, 2000). Variables determined for each site will include:

STATION VARIABLES

- Location (GPS)
- Depth
- Weather: Current conditions as well as those 3 days prior to sampling
- Tidal stage
- Submerged aquatic vegetation/macroalgae (presence or absence)
- Secchi depth

WATER QUALITY

- Nutrients (NO_3+NO_2, NH_3,, o-phosphate, and silicate)
- Total Suspended Solids (TSS)
- Chlorophyll *a*

IN SITU PARAMETERS (SEABIRD ELECTRONICS, INC. CTD, YSI 6000UPG)

- Conductivity
- Temperature
- Oxygen
- Chl *a* Fluorescence
- pH
- Light attenuation

SEDIMENT VARIABLES

- Grain size
- Organic carbon
- Inorganic and organic contaminants
- Toxicity (amphipod bioassay)
- Community structure (macrobenthos)

FISH TRAWLS (collected by Division Marine Fisheries and shipped by CZM)

- Community composition
- Pathology
- Parasites
- Inorganic and organic contaminants

4.2 Station Locations

Sampling will be taken at each of the sites indicated by EPA that were randomly chosen inside 80 different sections of the coast. Forty stations will be sampled during the period of July-September in 2000 and 2001. Some of the sample sites may not be appropriate due to bottom sediment type or other logistical considerations (accessibility, navigational concerns, etc.) When such difficulties are encountered, every effort will be taken to occupy one of the two alternate locations indicated by EPA for each sector. Slight variations in location may be necessary to acquire soft bottom sediments amenable to sampling. In some cases these difficulties may be anticipated and, with prior discussion and guidance from EPA (contact: John Paul), the location of the sampling site may be relocated.

Collection of samples will be accomplished using either the UMB vessel RV Neritic or the UMD vessel RV Lucky Lady. The Neritic will be used primarily to sample stations in Massachusetts Bay and north while the Lady Luck will be used to sample Cape Cod and Buzzards Bay and Nantucket and Martha's Vineyard stations. Either a CZM vessel at the Waquoit Bay National Estuarine Research Reserve's or the Neritic will be used to access the shallow stations along the southern and western coastline of Cape Cod. Depending on transit times and on-site conditions it is anticipated that 2- 4 stations will be sampled during a field day. Sampling will begin in July and continue through September as needed.

4.3 Sample Collection and University of Massachusetts Analyses

Samples for water column variables, including those for trace metal analyses, will be collected using a peristaltic pump or Teflon® coated Go-Flo bottles suspended from Kevlar® line. Water column samples for total suspended matter (TSM), particulate organic carbon and nitrogen (POC and PON), chlorophyll a and phaeopigments, and nutrients (NH_3, NO_3 + NO_2, Si and P) will be analyzed by UMB and UMD. UMD will be responsible for the chlorophyll and phaeopigment measurements, UMB will be responsible for the remaining parameters. Collection and analytical protocols for TSM, POC/N, chlorophyll and nutrients are provided in the appendix of this QAPP. All analyses will meet or exceed parameters established in the EPA Coastal 2000 Quality Assurance Plan for all analytes. Any deviations will be submitted in writing to the Project Officer prior to initiation of field activities. In addition samples for selected water-column metals will be analyzed as described in Wallace et al. (1983) using well-established trace metal clean techniques in the laboratory of Dr. Wallace. A complete description of the procedure and all QA/QC data for the collection, processing and analysis of the metals will be provided by Dr. Wallace.

4.4 Sample Delivery to EPA/Narragansett

Those samples not analyzed by UMB or UMD will be delivered to the EPA's National Health and Environmental Effects Research Laboratory's Atlantic Ecology Division in Narragansett, Rhode Island by the Massachusetts Coastal Zone Management Office (contact: Christian Krahforst).

4.5 Data Quality Objectives for Measurement Data

Measurement quality objectives for the Massachusetts Estuarine Assessment Program are shown in Table 4-1. Accuracy (bias) goals are expressed either as absolute difference (± value) or percent deviation from the "true" value; precision goals are expressed as relative percent difference (RPD) or relative standard deviation (RSD) between two or more replicate measurements. Completeness goal is the percentage of expected results that are obtained successfully. Table 4-2 displays quality assurance sample types.

TABLE 4-1

Indicator/Data Type	**Maximum Allowable Accuracy (bias) Goal**	**Maximum Allowable Precision Goal**	**Completeness Goal**
Water Column Characteristics			
Salinity	±1.0 ppt	10%	100%
Dissolved Oxygen	± 0.5 mg L^{-1}	10%	100%
Temperature	± 1.0 ^{o}C	10%	100%
Depth	± 0.2 m	10%	100%
Water Quality Parameters			
Total Suspended Matter (TSM)	NA	30%	100%
Chlorophyll *a*	10%	30%	100%
Nutrients (DON, nitrates, nitrites, ammonia, and phosphate)	10%	30%	100%
Particulate Organic Nitrogen/Carbon	5%	20%/10%	100%

TABLE 4-2. Quality assurance sample types, frequency of use, and types of data generated for MEAP.

Variable	QA Sample Type or Measurement Procedure	Frequency of Use	Data Generated for Measurement Quality Definition
Water Quality Parameters			
CTD (Seabird/YSI):			
Salinity	Certified Standard (~50 µS cm^{-2})	Each survey	Difference between probe value and Standard level
Dissolved Oxygen	Water-saturated air calibration	Each survey	Difference between probe value and Saturation level
Temperature	QC check with St'd Thermometer	Each survey	Difference between probe and thermometer
Depth	QC check at 1 m mark on line	Each survey	Difference between probe and 1 m mark
pH	QC check with St'd buffers (7&10)	Each survey	Difference between probe and Standards
CTD (Seabird)			
Salinity, DO, pH, Temperature, Depth	Performance verification at certified calibration center	Annually	Difference between instrument response and

	(manufacturer)		calibration standards
Salinity, DO, pH, Temperature, Depth	Comparison with YSI (2nd) instrument	Each survey	Difference between instruments readings
Nutrients	Standards and Duplicates	Per batch	Relative accuracy and precision
Chlorophyll *a*	Standards and Duplicates	Per batch	Relative accuracy and precision
TSM	Duplicates	Per batch	Precision
PON/C	Duplicates and standards	Per batch	Duplicate results and standard recoveries

5. ANALYTICAL PROCEDURES

5.1 Overview

Analytical methods employed in the nutrient assays are the standard methods of research-level environmental laboratories and comparable to the Massachusetts Water Resources Authority's investigations of the Massachusetts Bays. Since marine systems are typically much lower in ambient nutrient concentrations than freshwater systems, greater detection sensitivity than standard methods are needed to discern nutrient concentration and variability. This study will utilize the University of Massachusetts Boston (UMB) and Dartmouth (UMD) laboratory for its analytical component. These laboratories have provided analytical support for nutrient work on the Massachusetts Water Resource Authority monitoring efforts, NOAA and National Science Foundation funded research programs, and many programs involving process-oriented marine monitoring and research.

Samples are logged in field notebooks upon collection and stored appropriately for transfer to the appropriate laboratory. Samples will be logged in upon arrival to respective university laboratories. During log-in, sample integrity is checked upon arrival, and any unusual sample characteristics (identified by visual inspection) are noted in the field notebooks. All frozen and/or archived samples are stored in an ultra-freezer (-40°C) accessible only to authorized laboratory personnel. The laboratory analysts are responsible for sample collection, sample analysis and data entry.

Techniques used by this laboratory are methods generally used by state-of-the-art research laboratories and those used and reviewed by EPA & DEP. As with marine nutrient assays, ambient TSM concentrations are much lower than in fresh water systems. TSM techniques developed at UMB will be employed for this study because of the greater sensitivity and reproducibility in characterizing total suspended matter in the water column. Details of laboratory procedures for Nutrient, Elemental analysis (POC and PON), total dissolved organic nitrogen, Chlorophyll *a* , total suspended solids are provided in the Appendix.

5.2 Analytical Methods Quality Requirements and Assessments

Analytical methods, desired method accuracy and detection thresholds for monitoring parameters are listed in Table 5-1. Details of sample handling and storage are provided in Table 5-2. Complete standard curves for nutrient measurements are generated for each analytical run to assure both accuracy and precision are at or below stated levels. Samples falling outside the upper range of standard curves are diluted appropriately with artificial (nitrogen-free) seawater (620 g NaCl (ultrapure) + 200 g $MgSO_4$ heptahydrate

and 5 g of $NaHCO_3$) and re-analyzed. Because the amount of nitrogen associated with artificial seawater may be too great to be neglected, a blank run on the artificial seawater is run prior to its use as a diluent. In all cases, standards are prepared new each day and selected to give five points over the sample concentration range. Standards well above the sample concentration range are not used. Nitrate+Nitrite and particulate C & N by autoanalysis have additional standards (run as samples) before and after every five (5) samples. Failure of these standards to agree within 10% of their known value halts the analytical run and complete re-calibration and reanalyzing of the last five (5)-bracketed sample set is preformed. After completion of analyses, remaining samples are frozen, and stored for possible reanalysis. For the particulate elemental analyses (PON, POC) and Chl *a*, only field duplicates and laboratory standard duplicates are run as the analysis consumes the entire sample.

Reagent blanks are prepared and analyzed on each new reagent mixture; additional blanks are then prepared and analyzed simultaneously with the creation of the standard curve. Spiked samples are periodically analyzed as analytical checks in ammonium and nitrate+nitrite assays. Spiked samples are sometimes run as "unknowns" similar to field duplicates. Recovery of spikes must be within 20% of expected values to meet QA. Standards are analyzed daily and checked for linearity ($r^2 > 0.98$) and acceptability of blanks.

5.3 Sample Holding Times

Assays will be within the recommended holding times specified in Table 5-2. All chemical assays for dissolved constituents are filtered upon collection in the field. If stations are within close proximity to the UMB laboratory, initial sample processing will be undertaken immediately upon reaching the laboratory. Suspended particulates are collected on 0.4 µm polycarbonate (Poretics) filters after vigorous shaking of the sample bottles.

5.4 Inspection and Maintenance of Analytical Instrumentation

Analytical equipment (Perkin-Elmer Model 2400 CHN Elemental Analyzer, TrAACs 800 Autoanalyzer, Bausch and Lomb Spectrophotometer, and Turner Model 10 Fluorometer) are calibrated through the processing of standards and blanks in the normal analytical procedures. The Seabird CTD sensors and laboratory analytical balances are under annual manufactures service and calibration.

5.5 Documentation, Data Reduction and Reporting

Raw data is maintained in electronic format and archived on separate media for storage. Data reduction involves the process of converting raw data numbers into concentration values based upon the regression equation calculated from chemical standards. The results will be reported in terms of concentration, as means and standard errors. All data are open for review at all stages. All data reported are reviewed to check for errors in transcription, calculation or computer input. If data points are found to violate QA criteria, the archived sample will be reanalyzed. Data are also reviewed for adherence to analytical protocols and to pre-established criteria (e.g., holding times, surrogate recoveries, initial and continuing calibration, matrix spikes, laboratory duplicates, and blank contamination). At a minimum, Students *t*-test for paired samples and analysis of variances are used for interpretation. Data is transcribed only for statistical analysis and each point is checked for accuracy.

Table 5-1
Target Accuracy and Detection Limits for Laboratory Measurements

Parameter	Method	Reference	Accuracy*	Lower Detection limits
Laboratory Parameters				($\mu g\ L^{-1}$)
· ammonia	Phenol/hypochlorite (autoanalyzer)	a	5%	1
· nitrite+nitrate	Cd-Cu reduction/sulfanilamide/N-ED HCl_2	a	5%	1
· total particulate carbon/nitrogen	Elemental analyzer	b	5%**	6
· Total dissolved nitrogen (DON)	Persulfate oxidation/NO_3 analysis	c	5%	3
· orthophosphate	Molybdenum Blue (autoanalyzer	a	5%	3
· Chlorophyll a	Acetone extraction fluorometric	d	10%	0.01
· Total Suspended Solids (TSS)	Dessicator, gravimetric microbalance	e	5%	0.5 mg L^{-1}

a Guffy, J.D., M.A. Spears, and D.C. Biggs, 1988: Automated analyses of nutrients in seawater with the Technicon TRAACS-800 Autoanalyzer System.
b Hedges, J.I., and J.H. Stern, 1983: Carbon and nitrogen determinations of carbonate-containing solids. Modified for the Perkin-Elmer Model 2400 CHN Elemental Analyzer.
c D'Elia, C.F., P.A. Stuedler and N. Corwin. 1977. Determination of total nitrogen in aqueous samples using persulfate digestion. Limnol. Oceanogr. **22**: 760-764.
d Strickland, J.D.H., and T.R. Parsons (1972), A Practical Handbook of Seawater Analysis, § IV.3.IV, 201-203.
e UMass Boston protocol using 0.4 µm polycarbonate (Poretics) membrane filtration.

* Accuracy based on results of laboratory control standards and spiked samples
** Precision based on relative percent difference of subsample analysis. No spikes are available for POC/PON analysis.

Table 5-2
Sample Storage

Parameter	Matrix	Total # of Samples	# of Duplicates per round	Sample Volume needed (mL)	Sample Container	Preserv. Method	Max. Allowable Hold Time
ammonia	water	80	3	5	30 mL plastic vial= (HCl-Acid washed)	Filtration/ Filtrate Frozen Dark	30 days
nitrite+nitrate	water	80	3	5	30 mL plastic vial= (HCl-Acid washed)	Filtration/ Filtrate Frozen Dark	30 days
total particulate nitrogen	water	80	3	300	2 Liter plastic bottle== ; Glass Fiber Filter	Filtration/Filtrate at 4°C Dark	60 days
Total dissolved nitrogen	water	80	3	60-125	Polyethylene (HCl-Acid washed)	Filtration/ Filtrate Frozen Dark	30 days
Chlorophyll *a*	water	80	3	100	100 mL==; glass fiber filter	Filtration/ Frozen	21 days
Total Suspended Matter (TSM)	water	80	3	200-800	2 Liter plastic bottle== ; 0.45:m nuclepore filter	No Presev.	7 days
Dissolved oxygen	water		N/A	*in situ*	NA	N/A	N/A
temperature	water		N/A	*in situ*	N/A	N/A	N/A
conductivity	water		N/A	*in situ*	N/A	N/A	N/A

= Nitrate-Nitrite and ammonia will be analyzed from a single 30 mL scintillation vial (HCl-acid cleaned).
== Total particulate nitrogen and carbon, Chl *a*, and TSS will be analyzed from a single 2 liter plastic bottle.

6.0 AUDITS

The U.S. EPA may conduct field and laboratory audits of this project for adherence to this and EPA's Coastal 2000 QAPP. EPA may also conduct audits of field notebooks and other project documents at any point during the study.

7.0 REPORTS

All data will be sent to CZM after undergoing reduction and QA/QC processing at UMB and UMD. Data will be sent in both hard copy and electronic formats designated by CZM (contact: Christian Krahforst). All QA/QC data will also be provided including that for the trace metal measurements made in the water column.

8.0 PROJECT FISCAL INFORMATION

This project is funded, in part, by the US Environmental Protection Agency through the National Coastal Assessment Program. Additional in kind support is provided the University of Massachusetts, Massachusetts Coastal Zone Management and the Massachusetts Division of Marine Fisheries. The total annual budget from EPA for the program is $90,000.

APPENDIX

ANALYTICAL PROCEDURES

FOR:

I. TOTAL SUSPENDED SOLIDS

II. CHLOROPHYLL AND PHEAOPIGMENTS

III. PARTICULATE CARBON AND NITROGEN

IV. NUTRIENTS

I. Total Suspended Matter (TSM) Analytical Protocol

Field Collection

Aliquots of TSM samples are obtained from the 2L polycarbonate bottles filled in the field. See field sampling protocol outlined in the body of this QAPP (§6.1).

Processing Equipment

Nalgene® polysulfone filtration apparatus (Fisher Cat. # 09-740-23D)
Top reservoir = 500 mL
Filtrate receiver can be either 500 mL or 1000 mL

Filters (47 mm, 0.4 μm Poretics® polycarbonate membranes), pre-weighed
[Filters for TSM are pre-weighed by placing filters in a desiccator for a minimum of three (3) days prior to weighing on the Perkin-Elmer microbalance next to the CHN analyzer. Tared weights (M_t) are recorded to the nearest 1/1000th milligram. Typical tared weights are between 13-17 mg. Tared filters are stored in disposable Falcon® petri dishes (Fisher Cat. # 08-757-105) with their tared weight recorded on the petri dish cap in permanent marker.]

Forceps

Filter apparati holder

Vacuum pump with vacuum gauge

Q HOH in squirt bottle.

Graduated Cylinders, 250 mL, 500 mL, and 1000 mL

Procedure:

A. Set up filtration apparatus/apparati on lab bench in holder. Connect Teflon fitting, for vacuum, to one of the ports on the top of the filtrate receiver. Using forceps, remove a tared polycarbonate filter from petri dish and place onto the analytical plate (the two-piece white/off-white support bed) of the filtration apparatus. Save petri dish with tared weight recorded on the cap for later storage of processed sample. Place top of filtration apparatus on and screw down finger tight. *Make sure at least one of the black (sometimes clear) silicone plugs (there are three) from the screw cap on the top reservoir is removed before filtering* -This allows for air to enter, preventing the formation of a vacuum and potentially rupturing the filter.

B. Now you are prepared to process samples. Add a "reasonable" amount of sample to be filtered for TSM measurement. For example:

Boston Harbor water -- ~200 mL (sometimes more depending on season, i.e. winter)
Gulf of Maine – up to 2 L on occasion
Coastal waters – between 500-1000 mL

The goal is to load the filters sufficiently to measure the mass of particulates (~0.3-0.6 mg) without causing artifacts due to clogging (pore size changes) during processing. Add additional sample in 25 – 50 mL increments until filtration slows down. This will be noticed by a substantial slowing in the rate of filtration as the filter begins to clog. During last incremental filling, swirl sample to prevent loss of particulate material to the side of

the apparatus. After sample has been completely filtered, remove vacuum and decant filtrate through one of the ports on the filtration receiver into an appropriate graduate cylinder (500 mL for coastal waters, 1-2L for open ocean samples) and measure the amount of filtrate. Record this volume (V_f). After obtaining the filtrate volume, close port, re-apply vacuum and rinse filter with 10 – 20 mLs of QHOH, using the squirt bottle and rinsing all of the filter and sides of the apparatus, to ensure complete particle collection and to remove residual saltwater from filter. Residual saltwater trapped in the filter pore spaces may add a substantial amount of mass if allowed to dry with captured particulates. Following the QHOH rinse, remove top portion of apparatus away from receiver, taking care not to disturb filter. This is easiest if the vacuum is left on while removing the top portion of filtration apparatus. Turn off vacuum now, and remove filter by using forceps and return processed filter to original petri dish. Place filter with particulate matter open with cap underneath base, in a desiccator and allow to dry for a minimum of three (3) days prior to weighing. After filters have been desiccated, weigh filters on the CHN microbalance, recording to the nearest 1/1000th mg (M_s).

Blanks are processed in the same manner, using 50 mLs of QHOH. Sometimes QHOH may be contaminated with particles and a better measure of "blanks" are obtained from re-weighing "control" filters-those that were not subjected to any filtration processing. It is important to re-weigh at least three (3) "control" filters since changes in relative humidity can cause a significant artifact in the determination of TSM. As a control of relative humidity, three (3) pre-weighed filters are reserved for re-weighing at the time of sample weighing. On occasion, these filters will be consistently higher or lower (~0.01 mg) than when originally weighed. Use the mean value (M_b) to correct sample weights for effects of relative humidity.

Clean filtration apparatus (just the top, with a small amount of laboratory soap and gently wiping with Kimwipes® (UNLESS you are using the filtrate for dissolved constituents – like nutrients-BUT NOT METALS-see trace metal protocol for dissolved metals), followed by substantial rinses of Q-HOH. Use either QHOH or deionized water for rinses.

C. TSM are calculated as follows

$$[(M_s - M_t) - M_b]/(V_f)$$

where:

M_s is the mass of filter *plus* sample in mg
M_t is the mass of filter *(tared wt.)* in mg
M_b is mean blank value in mg
V_f is the filtrate volume (in liters)

Note:

When processing large batches of samples (10-20) guestimate which ones are the "cleanest". (i.e., have the lowest TSM content). DO THESE FIRST. Dirtiest last. One strategy is to process the blanks after processing a few of the very "clean" samples (like Mass. Bay, Gulf of Maine); or run them at the very beginning. A minimum of 3 blanks should be processed, and 10% of the samples should be replicated.

Past Observations:

Blanks:	Ftr Tare (mg)	Ftr Mass (mg)	Delta (mg)
1	15.604	15.609	0.005
2	15.783	15.797	0.014
3	15.913	15.929	0.016

5	15.69	15.702	0.012	LOD:	0.07mg L-1
6	15.808	15.834	0.026	(3 * STD, based on 350 mL sample vol.)	

Replicates

Sample	mg/L
MB16-1	2.68
MB16-1R	2.97
WE6	2.18
WE6 (Rep)	2.28
28.2 A	1.00
28.2 B	1.02

II. Fluorometric Determination of Chl *a* and Pheo-pigments

(UMB ver. 2.3b)

(The procedure, detailed below, is adapted mainly from Strickland and Parsons – A Practical Handbook of Seawater Analysis, pp185-194 and pp. 201-203.)

I. Sample collection:

Water samples are collected employing standard sampling techniques. Typically, discrete seawater samples are collected in Niskin or Go-Flo bottles at depth over the water column. A subsample is collected from the Niskin bottle and stored in a 2L polycarbonate bottle. For field filtration, a sample of about 50-100 mL is syringe-filtered through a glass-fiber filter (2.4 cm Whatman GF/F). The filter is removed, carefully folded in aluminum foil and frozen for later analysis. Samples that are not filtered in the field are stored in a cool dark place and processed within 8 hours of collection.

II. Lab Filtration (performed under low light conditions to minimize artifacts):

A. The water samples may be passed through a 300 :m mesh sieve (in order to remove large material/zooplankton) into a 200 mL filtration funnel loaded with a 2.4 cm Whatman GF/F glass fiber filter.
B. Tue sample is filtered through the glass fiber filter, taking care not to exert too much pressure. (Pressure above 55 mm Hg may rupture cells and allow material to pass through the filter).
C. Using forceps, remove filter and associated retained material and place in a extraction vial. (A capped 20 mL KIMAX test tube serves this purpose well.) Record sample and test tube #. If samples are to be extracted at a later date, wrap test tube/filter in aluminum foil and store at or below 0°C (in freezer).

III. Extraction:

Under a low-light regime, add 10 mL of 90% acetone (10% DI HOH[2]) v/v to the test tube/filter and shake vigorously. Save an aliquot of the 90% acetone for a reagent blank. Sample procedural blanks are performed by filtering a representative "sample" volume (50-200 mL, estuarine-Mass. Bays samples) using DI HOH, through two filters loaded in the filtration apparatus. Procedural blanks are performed on the most downstream filter. (In the past, DI HOH have given high blanks due to the microbial buildup in our DI system. Obviously, this would be an inappropriate procedural blank. Typical blank values should read between 0.0-0.1 units on the most sensitive setting.) Store test tubes with the extracting sample in the dark at 0°C (in freezer) for 12 – 24 hrs.

[2] Test DI water for Fluorescence blank readings before preparing the 90% acetone solution

IV. Fluorometry:

A. Under a low light regime, remove extraction samples from the freezer , keeping them covered under an aluminum tent. Allow samples to equilibrate to near room temperature. (Most fluorescent materials have significant temperature coefficients. Since standards are prepared at room temperature, it is recommended that samples and blanks all be determined at room temperature.)

B. Turn instrument[3] on and allow and allow 5-10 minutes for warm-up. Check instrument zero with a fresh aliquot of 90% acetone. (Probably now is a good time to prepare fluorometric standards while the instrument is warming up and samples are equilibrating to room temperature.)

C. For coastal samples, the following settings are recommended:
   1. 1X-100X sensitivity knob should be set to 1X.
   2. Range switch set to "auto".

D. Shake sample and fill cuvett (13x100 mm culture tube) with extract.

E. Insert cuvette into the instrument holder and record the relative intensity as $\mathbf{R_b}$ (*read only from the 0-10 scale* regardless of scale factor – this is accounted for in the equations listed in § V.)

F. Add 2 drops of 5% v/v HCl to cuvette and take a 2nd reading approximately 30 s later. Record this value as $\mathbf{R_a}$.

V. Calculations:

Convert the measured fluorescence relative intensities to chlorophyll and pheo-pigments concentrations using the following equations:

$$\text{Chl } a \text{ (ug L}^{-1}) = 10 \times [(F_d \times (t/t-1)] \times [((R_{b\ samp}/Scale_{b\ samp}) - (R_{a\ samp}/Scale_{a\ samp}))/\text{Samp. Vol } (mL)]$$

$$\text{Pheo-pigment (ug L}^{-1}) = 10 \times [(F_d \times (t/t-1)] \times [((R_{a\ samp}/Scale_{a\ samp}) - (R_b/Scale_{b\ samp}))/\text{Samp. Vol } (mL)]$$

where:

$$t = (\text{mean } R_{b\ std}/ \text{ mean } Scale_{b\ std}) / (\text{mean } R_{a\ std}/ \text{ mean } Scale_{a\ std})$$

$$F_d \text{ (door factor)} = \text{Fluorescence Standard Conc. (ug L}^{-1}) / (\text{mean } R_{b\ std}/ \text{ mean } Scale_{b\ std})$$

The fluorescence standard is made by quantitative dilution (typically 1/50) of the spectrophotometric standard. The spectrophotometric standard is determined by reading absorbances at 630, 647, 664, and 750 nm. The concentration of the spectrophotometric standard is calculated by:

$$\text{Chl } a \text{ (mg L}^{-1}) = 11.45\ (Abs_{664} - Abs_{750}) - 1.54(Abs_{647} - Abs_{750}) - 0.008(Abs_{630} - Abs_{750})$$

[3] Turner Designs, Model 10 Series Fluorometer

VI. **Standard Preparation:**

A. Stock Standard

In a 100 mL volumetric flask, add approximately 2 mg of commercially available chlorophyll *a* ($C_{55}H_{72}MgN_4O_5$, available from Sigma, product # C-5753) and dilute to mark with acetone. Wrap volumetric flask with aluminum foil and store at 0^oC.

B. Spectrophotometric Standard:

In a 100 mL volumetric flask, quantitatively[4] add 5 mL of stack standard and dilute to mark with acetone solution (approximate concentration ~ 1 mg L^{-1}). Prepare fresh daily.

C. Fluorometric Standard:

In a 100 mL volumetric flask, quantitatively add 2 mL of spec. standard and dilute to mark with acetone solution.

Note: All solutions should be at room temp. (ideally at 20^oC) to allow for quantitative dilutions.

Reference:

Strickland, J.D.H., and T.R. Parsons. 1972. A Practical Handbook of Seawater Analysis. Fisheries Research Board of Canada, Bulletin 167 (2nd Edition). 310 pp.

[4] Technically, the only quantitative dilution need be in the preparation of the fluorometric standard since the concentration of the spectrophotometric standard is determined by established empirically-derived equations. (See Strickland and Parsons for details).

III. Method for Particulate Organic Carbon and Nitrogen Analysis

Methods for particulate carbon and nitrogen follow those described by Hedges and Stern, 1984.

Particulate matter collected on pre-combusted glass fiber filter (Whatmann GF/F) is subjected to vapor acidification to remove inorganic carbon ($CaCO_3$).. This is accomplished by placing the "wetted" sample in a 50 x 9 mm tight-fit petri dish (Falcon No. 1006, Fisher Sci. Cat. # 08-757-105). Filters are "wetted" using organic free water (Q HOH: sub-boiled, quartz-distilled, deionized water). A drop of organic-free concentrated HCl is placed in the petri dish, away from the filter and sealed. The filter containing the sample is exposed to HCl vapor for 24-48 h at room temperature.

The acidified sample is air-dried on a Class 100 clean bench and analyzed by high temperature (925° C) combustion. A Perkin-Elmer 2400 CHN Elemental Analyzer is used in this analysis. Carbon, nitrogen, and hydrogen in the particulate material are converted to simple gases (CO_2, H_2O, and N_2). These gases are quantified by the analyzer using gas chromatographic column and thermal conductivity detection. Analytical blanks are carried throughout the procedure from wetting, vapor acidification, air drying, and analysis.

Reference:

Hedges, J.I., and J.H. Stern. 1984. Carbon and nitrogen determinations in carbonate-containing solids. Limnol. Oceanogr. **29** (3), 657-663.

IV. Analytical Methods for the Automated Analysis of Nutrients In Seawater UMB

Methods for nutrient analysis follows those described by Guffy et al., 1988. These methods are an automation of colorimetric techniques developed by Solorzano (1969), Bendschneider and Robinson (1952), Morris and Riley (1963), Murphy and Riley (1962) and Brewer and Riley (1966) for ammonia, nitrite, nitrate, phosphate and silicate, respectively. The concentrations were measured colorimetrically on a Technicon TRAACS-800 Autoanalyzer System.

References:

Bendschneider, K. and R.J. Robinson. 1952. A new spectrophotometic determination of nitrite in seawater. J.Mar. Res. **11**: 87-96.

Brewer, P.G., and J.P. Riley, 1966. The automatic determination of silicate silicon in natural waters with special reference to seawater. Anal. Chim. Acta **35**: 514-519.

Guffy, J.D., M.A. Spears, D.C. Biggs. 1988. Automated analysis of nutrients in seawater with the Technicon TRAACS-800 Autoanalyzer System. Prog. Proceedings, Oceans 88, **4**: p 1173-1177.

Morris, A.W., and J.P. Riley. 1963. The determination of nitrate in seawater. Anal. Chim. Acta **29**: 272-279.

Murphy, J., and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta. **27**: 31.

Solorzano, L. 1969. Determination of ammonia in natural waters by phenol hypochlorite method. Limnol. Oceanogr. **14**: 799-801.

Addendum (1/4/2007) Methods for nutrient analysis follows those described by the procedures listed at: http://www.lachatinstruments.com/products/qcfia/apps.asp for the following:

1 QuikChem® Method 31-107-06-5-A Ammonia in Brackish Water or Seawater 2.5 to 10 µM N/L
2 QuikChem® Method 31-107-04-1-A Nitrate/Nitrite in Brackish Waters or Seawater 1.25 to 5.0 µM N
3 QuikChem® Method 31-115-01-1-G Orthophosphate in Brackish or Seawater 2.0 to 10.0 µM P
4 QuikChem® Method 31-107-04-3-A Total Nitrogen in Brackish Waters with In-line Persulfate Digestion 0.1 to 1.0 mg N/L

The concentrations were measured colorimetrically using a Lachat QuikChem® 8500 Flow Injection Analysis System