

**Quality Assurance
Project Plan (QAPP)**

for

**Water Quality Monitoring
in Cape Cod Bay
2010-2011**

Massachusetts Water Resources Authority
Environmental Quality Department
Report 2010-20



Citation:

Costa A, Larson E, Stamieszkin K. 2010. **Quality Assurance Project Plan (QAPP) for Water Quality Monitoring in Cape Cod Bay 2010-2011**. Boston: Massachusetts Water Resources Authority. Report 2010-20. 82 p.

Quality Assurance Project Plan (QAPP)
for
Water Quality Monitoring in Cape Cod Bay
2010 – 2011

Prepared by

Amy Costa
Elizabeth Larson
Karen Stamieszkin

Provincetown Center for Coastal Studies
Hiebert Marine Laboratory
5 Holway Avenue
Provincetown, MA 02657
(508) 487-3623

October 6, 2010

Quality Assurance Project Plan (QAPP)

for

Water Quality Monitoring in Cape Cod Bay

Prepared by: Provincetown Center for Coastal Studies, 5 Holway Ave, Provincetown, MA
02657

Program, Laboratory, and Database Manager:

Dr. Amy Costa, Director, Cape Cod Bay Monitoring Program
Provincetown Center for Coastal Studies
(508) 487-3623

Date

Field Coordinator:

Capt. Marc Costa, Marine Operations
Provincetown Center for Coastal Studies
(508) 246-1387

Date

Laboratory Quality Assurance:

Elizabeth Larson, QA Coordinator
Provincetown Center for Coastal Studies
(508) 487-3623

Date

Distribution List

Amy Costa, PCCS (Program Manager)

Richard Delaney, PCCS (Executive Director)

Charles Mayo, PCCS (Plankton Studies)

Elizabeth Larson, PCCS (QA Coordinator)

David Taylor, MWRA (Project Manager, Cape Cod Bay Monitoring, Environmental Quality)

TABLE OF CONTENTS

1.0 PROJECT MANAGEMENT	1
1.1 PROJECT ORGANIZATION	1
1.2 COMMUNICATION PLAN	2
1.3 PROJECT DEFINITION AND BACKGROUND	2
1.4 PROJECT DESCRIPTION AND SCHEDULE	2
1.5 QUALITY OBJECTIVES AND CRITERIA FOR MEASUREMENT DATA	6
1.6 SPECIAL TRAINING REQUIREMENTS AND CERTIFICATION	9
1.7 DOCUMENTATION AND RECORDS	9
2.0 MEASUREMENT/DATA ACQUISITION	10
2.1 SAMPLING PROCESS DESIGN (EXPERIMENTAL DESIGN)	10
2.2 SAMPLING METHODS REQUIREMENTS	11
2.3 SAMPLE HANDLING AND CUSTODY REQUIREMENTS	14
2.4 ANALYTICAL METHODS REQUIREMENTS	15
2.5 QUALITY CONTROL REQUIREMENTS	17
2.6 PREVENTIVE MAINTENANCE PROCEDURES AND SCHEDULES	20
2.7 CORRECTIVE ACTION CONTINGENCIES	22
2.8 INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES	22
2.9 DATA ACQUISITION REQUIREMENTS (NON-DIRECT MEASUREMENTS)	23
2.10 DATA MANAGEMENT	23
3.0 ASSESSMENT / OVERSIGHT	27
3.1 ASSESSMENTS AND RESPONSE ACTIONS	27
3.2 REPORTS TO MANAGEMENT	27
4.0 DATA VALIDATION AND USABILITY	27
4.1 DATA REVIEW, VALIDATION AND VERIFICATION REQUIREMENTS	27
4.2 VALIDATION AND VERIFICATION METHODS	28
4.3 RECONCILIATION WITH USER REQUIREMENTS	28
5.0 REFERENCES	29
APPENDIX B	68
LAB DATA SHEET: PHYTOPLANKTON	72
ZOOPLANKTON COUNT SHEET	77
APPENDIX C	80

LIST OF TABLES

TABLE 1-1. CONTACT INFORMATION FOR WATER QUALITY MONITORING PROGRAM IN CAPE COD BAY	2
TABLE 1-2. SAMPLING LOCATIONS OF THE WATER QUALITY MONITORING STATIONS	3
TABLE 1-3. ROUTINE MEASUREMENTS TO BE CONDUCTED AT THE THREE STATIONS	3
TABLE 1-4. SAMPLING SCHEDULE FOR CCB AND SBNMS WATER QUALITY MONITORING	4
TABLE 1-5. ACCURACY AND PRECISION OF INSTRUMENT SENSORS	6
TABLE 1-6. DESIRED PRECISION, ACCURACY AND MDL FOR EACH PARAMETER BASED ON QUALITY OBJECTIVES	9
TABLE 2-1. SAMPLE COLLECTION AND STORAGE.....	13
TABLE 2-2. METHODS OF DETECTION FOR ANALYTES	15
TABLE 2-3. DATA QUALITY OBJECTIVES.....	18
TABLE 2-4. LABORATORY ANALYTICAL QC: NUTRIENTS (NITRATE+NITRITE, ORTHO-PHOSPHATE, AMMONIA, TN, AND TP).....	19
TABLE 2-5. LABORATORY ANALYTICAL QC: CHLOROPHYLL A	19
TABLE 2-6. SPECIFICATIONS FOR DATA SETS	24

LIST OF FIGURES

FIGURE 1-1. ORGANIZATIONAL CHART FOR WATER QUALITY MONITORING IN CAPE COD BAY1
FIGURE 1-2. SAMPLING LOCATIONS IN CCB AND SBNMS5

1.0 Project Management

1.1 Project Organization

Figure 1-1 presents the project management structure for the Water Quality Monitoring Program in Cape Cod Bay for MWRA. This plan details the project organization, sample handling, sample analysis, and data loading for this program.

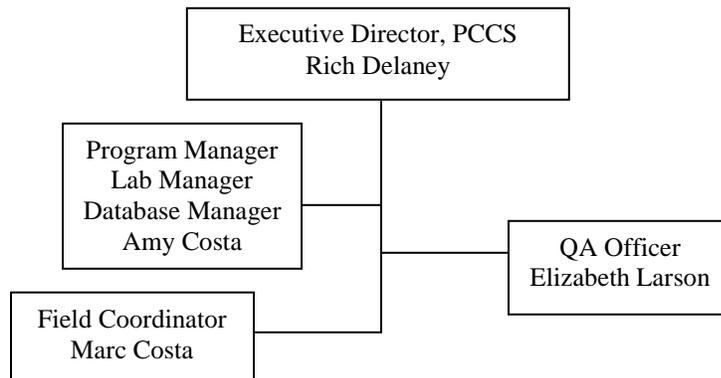


Figure 1-1. Organizational Chart for Water Quality Monitoring in Cape Cod Bay

Dr. Amy Costa is the PCCS Director of the Cape Cod Bay Monitoring Program and will fill a number of roles including Program Manager, Laboratory Manager, and Database Manager. As Program Manager, she will oversee all aspects of the project that incorporate the monitoring program including: fiscal management, project objectives, data uses, and program changes. As Laboratory Manager, she will perform lab analyses according to QAPP and ensure correct procedures are used, holding times are met, and adequate documentation is provided. As Database Manager, she will maintain the data systems for the program, perform/oversee data entry, and check entries for accuracy against field and lab forms. Capt. Marc Costa is the Field Coordinator for this project. He is responsible for the general coordination of monitoring activities on the water. Ms. Elizabeth Larson is the QA Officer. She is responsible for developing, directing, and coordinating the quality assurance/quality control (QA/QC) program regarding the collection and analyzing of water samples. She is also responsible for reviewing laboratory practices and procedures to ensure compliance with quality assurance and safety standards.

Contact information is provided in Table 1-1.

Table 1-1. Contact Information for Water Quality Monitoring Program in Cape Cod Bay

Name	Title/Role	Location	Email Address	Phone
Richard Delaney	Executive Director	PCCS	delaney@coastalstudies.org	508.487.3622
Amy Costa	Program Manager	PCCS	acosta@coastalstudies.org	508.247.7743
Marc Costa	Field Coordinator	PCCS	mcosta@capecod.com	508.246.1387
Elizabeth Larson	QA Coordinator	PCCS	ejl41067@gmail.com	310.916.6634
David Taylor	Project Manager, Harbor and Outfall Monitoring	MWRA	david.taylor@mwra.state.ma.us	617.788.4952

1.2 Communication Plan

Amy Costa will be the primary contact for this project. Email or telephone calls will be the day-to-day method of communication. Significant technical issues should be documented in email or memoranda, summarizing the key discussions and actions taken. Dave Taylor will be notified immediately regarding any issues or deviations from the project plan including if project surveys are not carried out as planned or samples are missing,

Annual project meetings are held in the spring of each year to review and update the project plan, including updating information on sample scheduling, sampling locations and frequency, analytical methods, and staffing.

1.3 Project Definition and Background

The public and regulators have recently raised concerns that MWRA’s outfall in Massachusetts Bay poses a long-term threat to the health of CCB and SBNMS. This project will continue ambient water column monitoring of three farfield stations, instituted by MWRA in 1992 by adding these stations to PCCS’s ongoing Cape Cod Bay monitoring program. Two of these stations are located in Cape Cod Bay (CCB) and one in Stellwagen Bank National Marine Sanctuary (SBNMS).

PCCS has been conducting marine mammal and habitat research in CCB and SBNMS for over 30 years. The monitoring to be conducted as part of this project will allow PCCS to better understand and protect the ecology of CCB and SBNMS, and the whales that use the two areas. It will also provide water quality data at the same three locations that MWRA needs to monitor in CCB and SBNMS, to meet its NPDES permit requirements.

1.4 Project Description and Schedule

PCCS will continue to monitor three farfield stations selected by MWRA as part of their on-going program to monitor for possible impacts of the MWRA outfall on areas downstream. These stations have been monitored since 1992, giving both 9 years of baseline data and 9 years of post-outfall data. Although no statistically significant changes in water quality at these stations can be linked directly to

the outfall, it is necessary to continue to monitor these stations. Furthermore, continued monitoring of CCB and SBNMS is important because these stations, being in the “farfield”, place the contribution (or lack thereof) of the outfall to such trends in a larger spatial context. The continued monitoring and assessment of conditions in these areas is important to the conservation of right whales and to our understanding of the influential patterns of change to which the outfall may be contributing.

This study includes 3 sampling locations in the farfield (Figure 1-2), sampled nine times a year. Water quality monitoring at these stations includes measurements of temperature, salinity, dissolved oxygen, fluorescence, PAR, nutrient concentrations (dissolved and total nitrogen and phosphorous), phytoplankton biomass (chlorophyll a and phaeophytin), and phytoplankton and zooplankton identification and enumeration. Sampling locations are listed in Table 1-2; samples collected at each location are listed in Table 1-3; and proposed sampling schedule is outlined in Table 1-4.

Table 1-2. Sampling locations of the water quality monitoring stations

Station Id	Description	Target latitude	Target longitude	Average water depth (m)
F01	East CCB	41.85083	-70.4533	26.2
F02	West CCB	41.90817	-70.2283	32.8
F29	South SBNMS	42.11667	-70.29	64.7

Table 1-3. Routine measurements to be conducted at the three stations

Type of measurement	Depth	Parameter
Hydro profile	From near surface (approximately 0.5-1.5 m) to near-bottom (3-5 m from bottom). Profiling at 0.5 m intervals	Temperature Salinity Dissolved oxygen Depth of sensor Chl fluorescence PAR
Water chemistry	Two depths: Near- surface Near- bottom	Nitrate + nitrite Ammonia Ortho-phosphate Total nitrogen Total phosphorus Extracted chl
Phytoplankton	Near-surface	Enumeration + identification

Zooplankton	Oblique net tow	Enumeration + Identification
-------------	-----------------	---------------------------------

Table 1-4. Sampling schedule for CCB and SBNMS water quality monitoring

Month	Week of year	Proposed sampling
Early February	6	2011
March	12	2011
Early April	15	2011
Mid-May	20	2011
Mid-June	25	2011
Mid-July	30	2011
Mid-August	34	2011
September	36	2011
October	Week of 10/18	2010
November	Week of 11/9	2010

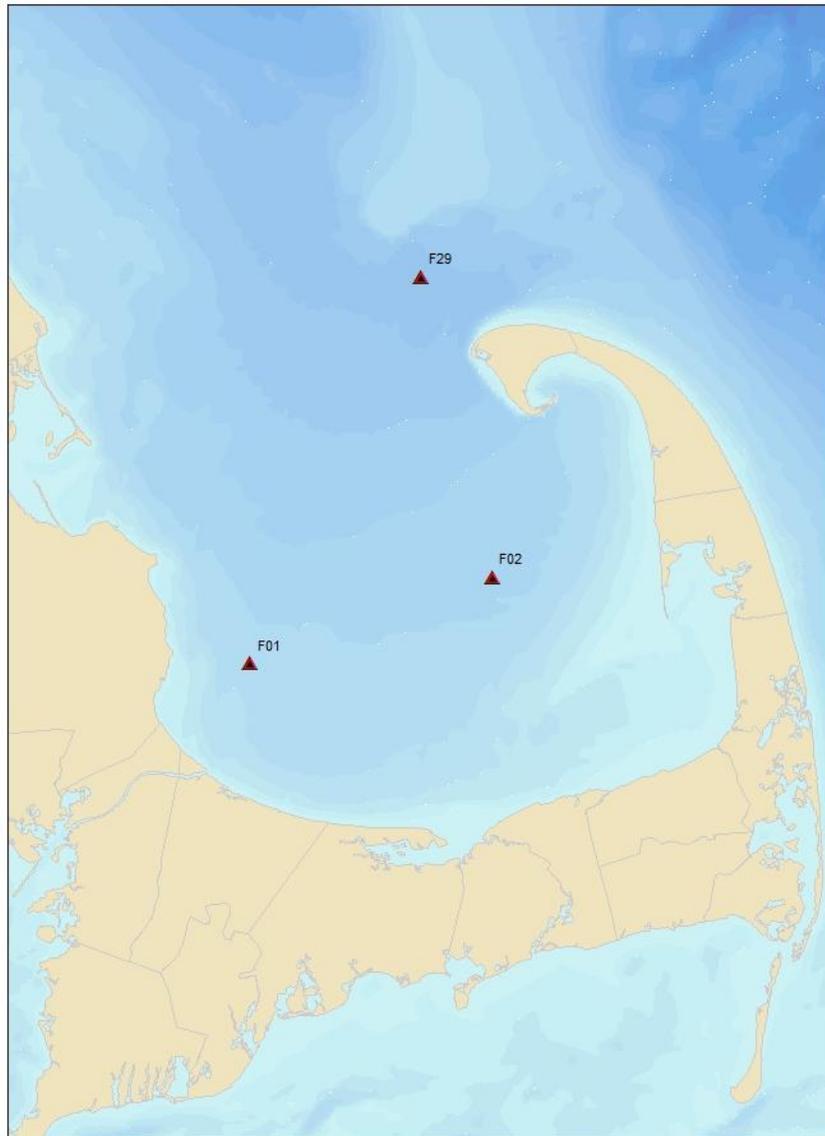


Figure 1-2. Sampling locations in CCB and SBNMS

1.5 Quality Objectives and Criteria for Measurement Data

The parameters measured and the concentration reporting units are listed in Table 2-3.

1.5.1 Quality Objectives

Data quality objectives are as follows:

- To determine if the eutrophication status of Cape Cod Bay has a long-term response to nutrients from the MWRA outfall and/or to other regional forcing
- To ensure that the sample results are representative of the location sampled
- To ensure that the sample results are accurate

1.5.2 Measurement Performance Criteria

The first objective will be met by examining data collected during these 9 surveys to measure water column parameters, quantify nutrients and chlorophyll, and document changes in phytoplankton and zooplankton community structure. The second objective will be met by repeated measurements collected at the same locations over time to quantify the variability of results at each station. The third objective will be met by analyzing laboratory replicates to ensure reproducibility of results. Definitions of quality control samples are provided in Section 2.4.2.

1.5.2.1 Navigational and Hydrographic Data

1.5.2.1.1 Precision and Accuracy

Manufacturer precision and accuracy objectives for navigation and hydrographic sampling are presented in Table 1-5. Navigational accuracy of 10 m is required for this program.

1.5.2.1.2 Comparability

All sampling positions will be comparable to positions obtained by previous MWRA monitoring activities. The station locations are targets and sampling will be conducted within 300 m of the targets as visualized on the Northstar 952XD navigation display. The electronic measurement instruments that will be used during the water quality monitoring surveys are similar to the instruments that have been used by MWRA contractors since 1992.

Table 1-5. Accuracy and precision of instrument sensors

Sensor	Model	Units	Range	Accuracy	Precision
Navigation	Northstar 952XD	Degree	World	4 m	4 m
Pressure	Seabird SBE 19plus V2	Decibars	0 to 1000	0.1%	0.1
Temperature	Seabird SBE 19plus V2	°C	-5 to 35	0.001	0.01

Sensor	Model	Units	Range	Accuracy	Precision
Conductivity	Seabird SBE 19plus V2	mS/cm	0 to 70	0.03	0.01
Dissolved Oxygen	Seabird SBE-43	mg/L	0 to 15	0.50	0.05
Fluorometer (Chl a)	WET Labs ECO-FL	µg/L	0.01 to 125	0.01	0.01
PAR	Biospherical QSP-2300L	µE/(m ² ·sec)	0.14 to 5000	10	1
Temperature	YSI 556 Sensor	°C	-5 to 45	± 0.15	0.1
Conductivity	YSI 556 Sensor	mS/cm	0 to 200	±1% of reading or 0.001 mS/cm, whichever is greater	0.001 mS/cm to 0.1 mS/cm (range dependent)
Salinity (calculated from conductivity and temperature)	YSI 556 Sensor	ppt	0 to 70	±1.0% of reading or 0.1 ppt, whichever is greater	0.01
Dissolved Oxygen	YSI 556 Sensor	mg/L	0 to 50	0 to 20 mg/L, ± 0.2 mg/L or ± 2% of reading, whichever is greater; 20 to 50 mg/L, ± 6% of the reading	0.01 mg/L
pH	YSI 556 Sensor	pH units	0 to 14	±0.2	0.01

1.5.2.1.3 Representativeness

The representativeness of the sampling program design is detailed in the Outfall Monitoring Plan (MWRA 1997) and defined by the results collected since 1992. Representativeness will also be ensured by proper handling, storage, and analysis of calibration samples so that the materials analyzed reflect the collected material. Deviations from the data collection procedures described in this QAPP will be documented in the survey logbook and described in the survey report.

1.5.2.1.4 Completeness

The navigation software system outputs navigation positions at an interval of 1-second. The software system will display all position fixes and save these fixes in an electronic file during hydrocasts and sampling operations. The project time interval requirement for obtaining positions during sampling is one (1) minute. Thus, even if a few bad data streams from the dGPS navigation system to the computer are experienced, the software will provide enough position fixes within each 1-minute period for 100% data collection.

Because hydrographic data are acquired electronically and monitored in real time, no loss of data is expected. Stations will not be occupied if CTD measurements and navigation coordinates (at a minimum) cannot be obtained. If instrument malfunctions occur and operations are modified or suspended during any survey day, a decision on modification of activities for that survey will be made with consultation and agreement of MWRA, whenever possible. A 10% loss of hydrographic and navigation data over the entire program is not expected to compromise the objectives of the program.

1.5.2.2 Water Sampling and Analysis

1.5.2.2.1 Precision and Accuracy

Precision and accuracy of laboratory procedures are ensured by the analysis of quality control (QC) samples including procedural/filter blanks, prepared standards, standard reference samples (SRS), where available, laboratory control samples, laboratory replicates and field replicates, as applicable. Table 1-6 lists the desired precision, accuracy, and detection limit goals for each parameter to be measured. QC samples to be analyzed in the laboratory to assess precision and accuracy are listed in Table 2-4 and Table 2-5. Method procedural blanks for parameters that use blank correction are the batch-average uncorrected method procedural blanks.

1.5.2.2.2 Comparability

Data will be directly comparable to results obtained previously at the same or similar sites in Massachusetts and Cape Cod Bay by PCCS and/or MWRA because field program design and analytical procedures are similar or identical. In addition, use of written standardized procedures ensures that sample preparation and analyses will be comparable throughout the project and with other projects.

1.5.2.2.3 Representativeness

Representativeness is addressed in sampling design. The sampling practices and laboratory measurements that will be performed during the water quality monitoring have already been used in many systems to characterize eutrophication and/or microbiological effects on the water column and are, therefore, expected to yield data representative of the study area. Representativeness will also be ensured by proper handling, storage (including appropriate preservation and holding times), and analysis of samples so that the material analyzed reflects the material collected as accurately as possible.

Deviations from the analytical scheme described in this QAPP will be noted in the laboratory records associated with analytical batches in the QA statements.

1.5.2.2.4 Sensitivity

Sensitivity is the capability of methodology or instrumentation to discriminate among measurement responses for quantitative differences of a parameter of interest. The method detection limits (MDLs) provide the sensitivity goals for the procedures as outlined in Table 1-6.

Data users should be aware that precision and accuracy generally degrade as analyte concentrations decrease. While numerical results are being reported down to the MDL, results below the lowest calibration standard will often have precision and accuracy that don't meet the data quality objectives for the project.

Table 1-6. Desired precision, accuracy and MDL for each parameter based on quality objectives

Parameter	Field Precision	Lab Precision	Accuracy	Blank Cleanliness	MDL ¹
Nitrate+Nitrite	<30% RPD ² for field duplicates	<10% RPD for instrument duplicates	± 15% PD ³ based on recovery of standards	Method procedural blank ≤5 x MDL Field Blank ≤5 x MDL	0.05 uM
Ammonia	<30% RPD for field duplicates	<10% RPD for instrument duplicates	± 15% PD ³ based on recovery of standards	Method procedural blank ≤5 x MDL Field Blank ≤5 x MDL	0.1 uM
Ortho-phosphate	<30% RPD for field duplicates	<10% RPD for instrument duplicates	± 15% PD based on recovery of standards	Method procedural blank ≤5 x MDL Field Blank ≤5 x MDL	0.02 uM
Total nitrogen	<30% RPD for field duplicates	<10% RPD for laboratory duplicates	± 15% PD based on recovery of standards	Field Blank ≤5 x MDL	1.07 uM
Total phosphorus	<30% RPD for field duplicates	<10% RPD for laboratory duplicates	± 15% PD based on recovery of standards	Field Blank ≤5 x MDL	0.23 uM
Chlorophyll <i>a</i> and Phaeophytin	<50% RPD for field duplicates	<15% RPD for laboratory (instrument) duplicates	± 15% PD based on recovery of standards	Filter Blank ≤5 x MDL	0.02 ug/L

¹ MDL = method detection limit. The actual MDL may be updated periodically. MDLs are based on the target sample volumes shown in Table 2-1

² Relative Percent Difference (RPD)% = $\left| \frac{\text{replicate 1} - \text{replicate 2}}{\text{replicate 1} + \text{replicate 2}} \right| \times 100$.

³ Percent Difference (PD) % = $\left[\frac{\text{true concentration} - \text{measured concentration}}{\text{true concentration}} \right] \times 100$.

1.5.2.2.5 Completeness

It is expected that 100% of samples collected for analysis will in fact be analyzed. However, a sample loss of <10% will not compromise the objectives of the project.

1.6 Special Training Requirements and Certification

Field Monitoring. Sample collection requires no non-routine field sampling techniques, field analyses, laboratory analyses, or data validation. Specialized training is therefore not required. Field personnel are experienced in using the equipment identified within this QAPP.

Laboratory Analyses. Nutrient and chlorophyll measurements use routine laboratory analyses, or data validation, therefore specialized training is not required. Lab personnel are experienced in standard protocols specified in PCCS's Laboratory Quality Assurance Plan for handling, storing, and preparing samples for analysis. Laboratory personnel are also experienced in using the equipment identified within this QAPP.

1.7 Documentation and Records

PCCS will maintain all documents relevant to sampling, laboratory analysis, and data analysis activities. All data will be archived electronically and backed up online. Hard copies of field and lab notebooks will be archived in the vault located in the basement of the Hiebert Marine Laboratory. Hard copies of data will be kept for at least one year following the termination of the contract.

1.7.1 Document Control

PCCS will maintain documents relevant to laboratory analysis activities and data entry.

A copy of the most current analyses SOP is kept in the lab area where the analysis is being performed. This document references the SOP number without the revision number. All members of the project team will inform the PCCS QA Officer of the need for SOP revisions.

Document Control is the responsibility of the Program Manager.

1.7.2 Laboratory Analyses Records

All data will be recorded initially into bound laboratory logbooks, onto established data forms (Appendix B) or onto electronic file, where applicable.

1.7.3 Records Retention and Storage

All data will be archived electronically and backed up online. Recent hard copies of field and lab notebooks will be kept in the designated file cabinet in the Coastal Ecology Lab in the Hiebert Marine Laboratory. Archived records will be stored in the data vault located in the basement of the Hiebert Marine Laboratory for at least one year following the termination of the contract.

1.7.4 Technical Workshop

The results of the year's monitoring will be presented at the MWRA's annual technical workshop conducted in the spring. The technical workshops are typically scheduled for March or April, and conducted in Duxbury, Woods Hole or Boston. Following the completion of the workshop, MWRA will be provided with a digital copy of the Power Point slides, and a two page abstract describing the major results of the year.

2.0 Measurement/Data Acquisition

2.1 Sampling Process Design (Experimental Design)

2.1.1 Scheduled Project Activities, Including Measurement Activities

The CCB and SBNMS surveys will be performed on an ongoing basis as specified in this QAPP. These stations have been sampled since 1992 with only slight changes in sampling frequency. It is anticipated that this project will include 9 surveys per year between February and November.

2.1.2 Design Rationale

The objective of this project is to continue to monitor for changes in water quality in CCB and SBNMS since the transfer of wastewater discharges offshore to Massachusetts Bay. The evaluation of water quality changes due to the transfer of discharges offshore will be assessed through the measurement of nutrient and chlorophyll concentration, and changes in plankton assemblages, among others.

2.1.3 Design Assumptions

Since Cape Cod Bay is generally well-mixed in cold months and has a well defined pycnocline throughout the warmer months, samples collected near surface and near bottom will accurately characterize the vertical variation. It is assumed that the spatial scales of variation are large enough that the sampling locations selected for this region are representative of water quality for this region. It is also assumed that, since surveys are conducted independent of tidal influence and weather, that the annual survey frequency is high enough that fluctuations in conditions due to weather or tide will not result in biased results.

2.1.4 Procedures for Locating and Selecting Environmental Samples

All sample locations are identified using GPS coordinates. Error of +/- 10 m is considered acceptable to allow for error in the GPS readout.

2.1.5 Classification of Measurements as Critical or Non-critical

All measurements collected as part of this survey are considered critical due to the requirement in MWRA's discharge permit to conduct the measurements described in the Ambient Monitoring Plan.

2.2 Sampling Methods Requirements

2.2.1 Sample Collection, Preparation, Decontamination Procedures

2.2.1.1 Hydrocasts and Sensor Measurements

At each station, a hydrocast will be conducted with a SBE 19*plus* V2 conductivity-temperature-depth (CTD) system equipped with various sensors (dissolved oxygen, chlorophyll fluorescence, PAR). Sensor measurements will be collected during the downcast from near surface (approximately 0.5-1.5 m) to near bottom (3-5 m from seafloor). Salinity and density ($\sigma\text{-t}$) will be calculated from the conductivity, temperature, and depth data. YSI 556 Handheld Multiparameter Instrument will also be used to measure temperature, salinity, and dissolved oxygen from water samples collected from near surface and near bottom in the Niskin bottle once it is on deck.

2.2.1.2 Water Collection and Plankton Net Tows

Near surface (0.5-1.5 m from surface) and near-bottom (3-5 m from seafloor) samples for each suite of analytes are collected in PVC Niskin bottles. The Niskin bottle will be lowered by hand to the designated depth. On deck, water from the Niskin bottle will be subsampled for analysis of dissolved inorganic nutrients, total nutrients, chlorophyll. All sample bottles are acid washed prior to use in the field and rinsed three times with sample water before filling. The sample bottles and analytes are shown in Table 2-1. Surface water will be collected for phytoplankton identification and enumeration (whole water) and an oblique net tow will be conducted to collect zooplankton for identification and enumeration. Because we will be following similar protocols as carried out in previous years for phytoplankton identification and enumeration, all information in this QAPP pertaining to phytoplankton

collection, analysis and quality control was taken from Libby et al. (2010) with only slight modifications.

The following describes the optimal order of operations for water and plankton collections.

- YSI sonde and CTD will be secured at the surface to let sensors equilibrate.
- Niskin will be lowered to the near-surface depth, targeting 1 m with a window of 0.5 to 1.5 m depending on sea conditions, and triggered to collect the water sample.
- YSI near-surface reading will be logged.
- CTD will be lowered at a velocity of approximately 0.5 m per second to within 3-5 m of the seafloor where it will remain for 5-10 seconds and then retrieved.
- Niskin will be lowered to within 3-5 m of seafloor and triggered to collect water sample. Once brought back on deck, YSI readings will be immediately logged.
- Zooplankton tow will be initiated
- Water samples from Niskin bottles will be processed.

2.2.1.2.1 Dissolved Inorganic Nutrients

Water will be sucked up from a transfer bottle (1 L polypropylene container) using a 60-mL syringe. The syringe will then be used to push the sample water through an in-line filter (Nuclepore 47-mm-diameter, 0.4- μ m-membrane filter) and into a 100-mL pre-labeled Whirl Pak. At the start of each survey day the 60-ml syringe is rinsed with 10% HCl solution then with Milli-Q. Additionally, the syringe is rinsed with Milli-Q between each station. The sample processing begins with the syringe receiving a triple rinse with site water. The sample will be stored in a cooler until it can be transferred to the lab and frozen within 8 hours.

2.2.1.2.2 Total Nutrients

Water from a transfer jar will be decanted into a sterile, pre-labeled 30 ml polypropylene container. This container will receive a triple rinse with site water before being filled with sample water. The container will be stored in a cooler until it can be transferred to the lab and frozen within 8 hours.

2.2.1.2.3 Chlorophyll a and Phaeophytin

Samples for chlorophyll *a*/phaeophytin determination will be processed according to PCCS SOP for chlorophyll *a*/phaeophytin. 960 ml sample water will be filtered through Whatman 4.7-cm-diameter GF/F using a 60 ml syringe. The filter will be held in a Swinlok filter holder. The final volume should result in a light green/brown residue on the filter. Using forceps, the filter will be removed from the filter holder, folded in half, and blotted on acid-free blotting paper to remove excess moisture. The folded filter will then be placed in a foil packet, and stored in a cooler in a pre-labeled whirl pak until it can be transferred to the lab and frozen within 8 hours.

2.2.1.2.4 Whole-Water Phytoplankton

Water from the near surface Niskin sampling bottle will be poured into a graduated cylinder that has been cut at the 850 mL mark. Before filling the cylinder to 800 ml, it is rinsed three times with water

from the Niskin sampling bottle. The filled cylinder is then poured into a 1-L bottle containing 8 mL of Utermöhl's solution preservative. The preserved samples are stored at ambient temperature and in the dark until analysis. The Utermöhl's solution is prepared as described in Guillard (1973): 100 g potassium iodide, 50 g iodine, and 50 g sodium acetate each are dissolved incrementally in distilled water to a final volume of 1 L.

2.2.1.2.5 Zooplankton

Zooplankton samples are collected using a standard 60-cm diameter, 333- μ m mesh conical net fitted with a General Oceanics helical flow meter. A vertical-oblique net tow will be conducted at each station to sample for zooplankton. Collections will be initiated by vertically dropping the net on-station. When the net has dropped the full 19 meters, the net will be pulled obliquely through the water column by the boat until a mark on the rope reaches the surface, indicating that the net is now horizontal at the surface of the water column. At this point, the net will be retrieved. Once on board, the samples will be washed from the nets carefully with a sea-water hose, concentrating the sample into the bottom of the net to the collection bucket. From there it will be concentrated further into a 333- μ m mesh fluorette. This concentrated sample will be rinsed into a sample jar and preserved with 10% buffered formalin. Samples will be placed in a cooler until transferred to the lab.

Table 2-1. Sample collection and storage

Parameter	Sample Container	Analytical Sample Volume per analyte	Sample Processing	Maximum Holding Time to Analysis
Total Nitrogen Total Phosphorus	1-L wide-mouth HDPE bottle	500 mL	Decant into 30 mL polypropylene bottle and freeze until analysis.	28 days
Nitrate/Nitrite Ammonia Total phosphorous Ortho-phosphate		500 mL	Pass through Nucleopore filter, freeze filtrate in 100 ml whirl pak until analysis	28 days
Chlorophyll <i>a</i> Phaeophytin		960 mL	Pass sample through Whatman GF/F. Wrap filter in foil and freeze until analysis.	28 days
Phytoplankton (Whole Water)		800 mL	Preserve with Utermöhl's solution	6 months
Zooplankton (Net Tow)	Wide-mouth HDPE bottle (volume dependent on zooplankton density)	Dependent on zooplankton density	Wash into jar. Fix with formalin to 10% solution	6 months

2.2.2 Sampling/Measurement System Failure Response and Corrective Action Process

Corrective action in the field may be necessary when the sampling schedule is disrupted due to weather or other logistical difficulties, or when sampling procedures or field analytical procedures require modification due to unforeseen circumstances. Any corrective measures taken must be approved by the Program Manager. It is the responsibility of the Program Manager to ensure that the corrective measure has been implemented. Corrective actions will be documented in the field logbook.

Corrective action in the laboratory may occur at one of several phases of the analytical process. Conditions such as broken or contaminated sample containers may be identified during sample login or prior to analysis. The Laboratory Manager will identify the need for corrective action and consult with the Laboratory QA/QC officer. These corrective actions are performed prior to the release of the data from the laboratory. The action will be documented in the laboratory notebook.

The occurrence of a practice or incident that is inconsistent with the established quality assurance and quality control procedures of the laboratory must be formally addressed with a corrective action response. Examples of situations requiring initiation of the corrective action process include mishandling of a sample or its documentation or use of unapproved modifications to an analytical method.

Upon the initiation of a corrective action, the problem is documented, and a corrective action plan is developed and then approved by the Laboratory Manager and QA manager. After required corrective action has been taken, the information is documented and verified to be effective and sufficient by the Laboratory Manager and QA Manager. All information is maintained in the Corrective Action Logbook.

2.3 Sample Handling and Custody Requirements

2.3.1 Sample Custody Procedure

Field logbooks will be used to record field activities performed during the survey. Upon arriving at each station, date and time of sampling, sample depth, Secchi depth, wind conditions, sampler's initials and any other relevant information will be documented (such as site-specific environmental conditions including presence of wildlife, floatables, algae, etc.). When not in use, logbooks will be stored in the laboratory. Each logbook cover must be labeled with the project name and the range of survey dates included in the logbook.

Information will be entered in the logbook in pencil or waterproof ink, initialed and dated with no erasures made. If corrections are required, the information will be crossed out with a single line and initialed by the sampler.

Sample containers will be pre-labeled with the following information: Survey number (vessel designation and trip number), site, and depth (S for near surface, D for near bottom).

SW765 [F01] S

All other information relevant to the sample (time, date, depth, etc.) can be cross-referenced in the field logbook.

All samples will be collected and analyzed by the PCCS Hiebert Marine Laboratory. Since the same individual will be doing both the sample collection and sample analysis, no chain of custody form is required. Samples are hand delivered from the boat via cooler to the laboratory for processing. All information specific to the samples or errors made during sample collection or delivery (e.g. sample spilled, flowmeter broken, etc.) will be written in the field logbook.

All samples covered by this QAPP will be analyzed by PCCS following its SOPs (Appendix A).

2.4 Analytical Methods Requirements

2.4.1 Preparation of Samples

All analytes are pre-processed (filtered and/or frozen in subsample containers, depending on the analyte) for storage until analysis. Plankton samples are preserved on board vessel. See Table 2-1 for a summary of storage methods and holding times.

2.4.2 Analytical Methods

Table 2-2 summarizes the methods used for sample analysis. The analyses will be conducted as described in the SOPs listed, with are based on literature references or EPA methods.

Table 2-2. Methods of detection for analytes

Parameter	Units	Instrument	SOP/Analysis Method
Total nitrogen	µmol/l	Astoria 2 Autoanalyzer	SOP 004/USGS 03-4174
Nitrate/Nitrite			SOP 001/EPA 353.4
Ammonia			SOP 003/EPA 350.1
Total phosphorous			SOP 004/USGS 03-4174
Ortho-phosphate			SOP 002/EPA 365.5
Chlorophyll <i>a</i> Phaeophytin	µg/L	Turner Trilogy	SOP 005/Modified EPA 445.0

Phytoplankton (Whole Water)	cells/L	Olympus BH-2 compound microscope with phase contrast optics	Borkman (1994), Borkman et al. (1993), Turner et al. (1995)
Zooplankton	Organisms/m ³	Leica L2 Stereomicroscope	Leeney et al. 2008

2.4.2.1 Dissolved and Total Inorganic Nutrients

The analysis of dissolved inorganic nutrients is based on the cited EPA and/or USGS methods. Dissolved inorganic nutrient concentrations are determined for samples that have been passed through a 0.4- μ m pore size membrane filter in the field. The concentrations of nitrate/nitrite, ortho-phosphate, ammonia, total nitrogen and total phosphorous are measured colorimetrically on an Astoria 2 Autoanalyzer. This instrument automates standard manual techniques for analysis of nutrients.

- For nitrate/nitrite analysis, nitrate in the sample is reduced quantitatively to nitrite by cadmium metal in the form of an open tubular cadmium reactor (OTCR). The nitrite thus formed plus any originally present in the sample is determined as an azo dye at 540 nm following its diazotization with sulfanilamide and subsequent coupling with N-1-naphthylethylenediamine. These reactions take place in acidic solution.
- For analysis of ortho-phosphate, the ortho-phosphate in the sample reacts with molybdenum (VI) and antimony (III) in an acidic medium to form a phosphoantimonymolybdenum complex. This complex is subsequently reduced by ascorbic acid to a heteropolyblue with an absorbance maximum at 880 nm.
- For analysis of ammonia, the ammonia in the sample reacts with alkaline phenol and hypochlorite to form indophenol blue. Sodium nitroferricyanide intensifies the blue color formed which is measured at 640 nm.
- For analysis of total nitrogen and total phosphorous, an alkaline persulfate digestion oxidizes all forms of inorganic and organic nitrogen to nitrate and hydrolyzes all forms of inorganic and organic phosphorous to ortho-phosphate. After digestion, samples are analyzed as described for nitrate/nitrite) and ortho-phosphate.

2.4.2.2 Chlorophyll a and Pheophytin

Samples for chlorophyll a/phaeophytin are processed according to EPA method 445.0 using a Turner Trilogy Fluorometer. Samples are filtered in the field as soon as possible after collection and the filters stored at -10°C. All handling steps are performed in subdued light. The chlorophyll a/phaeophytin is extracted from the cells retained on the GF/F filter by a 16-24 hour steep in 90% acetone at 4°C. The extract is analyzed using a fluorometer. 150 μ L of 0.1 N HCl is added to the extract and the extract is remeasured after 90 seconds to determine phaeophytin concentrations.

2.4.2.3 Whole-Water Phytoplankton

At the laboratory, Utermöhl's-preserved whole seawater samples will be prepared for analysis by concentrating the sample by gravitational settling as described by Borkman (1994), Borkman *et al.* (1993), and Turner *et al.* (1995). Samples will be settled in graduated cylinders with no more than a 5-

to-1 height-to-width ratio. Phytoplankton abundance is calculated by dividing the number of cells counted by the volume examined in a gridded Sedgwick-Rafter chamber. The theoretical maximum possible volume that would be examined would be an entire Sedgwick-Rafter chamber (1 ml). The grid subdivides the chamber into μl divisions so that if an entire chamber is not counted, an exact volume can still be determined. Typical volumes counted are one row of the chamber (50 1- μl cells or 1/20 of 1 ml). The volume of sample examined is dependent on number of cells encountered and how long it takes to reach cut-offs of 75 entities (unicellular forms, colonies, or chains) of each of the top 3 taxa, and 400 entities total. Calculation of abundance also accounts for the concentration factor used in the settling process. Normally, the volume processed is 800 ml of whole-water sample, settled to 50 ml of concentrate, for a 16:1 ratio.

The following equation results in the abundance estimate for cells counted:

$$C * [V_S / V_C] [1000 / V_{TOT}] = \text{cells/ L.}$$

where C = cells counted

V_S = Volume of concentrated sample

V_C = Volume of sample examined

V_{TOT} = Original volume

2.4.2.4 Zooplankton

The zooplankton are identified and counted by trained individuals. Sub-samples to be counted are taken by 1) suspending the sample in a known volume of water, and taking a subsample of at least 250 organisms to count and identify using a Wildco Hensen-Stemple pipette with plunger, 2) first splitting the sample with a Folsom plankton splitter and then continuing with the steps in (1), or 3) counting and identifying all organisms in the sample, should sub-sampling be impossible. The results of the counts are expressed in organisms per cubic meter (organisms/ m^3), derived from the flow meter correction constant, flow meter change during the tow, the area of the mouth of the net, volume of the sample, and volume of the sub-sample that was counted.

2.5 Quality Control Requirements

2.5.1 Calibration Procedures

2.5.1.1 Hydrographic Instruments

All hydrographic instruments and sensors are sent to the respective manufacturers (Seabird, WET Labs, Biospherical, or YSI) annually for calibration.

2.5.1.2 Nutrients (nitrate+nitrite, ortho-phosphate, ammonia, TN, and TP)

At least 6 working calibrants for each chemistry will be prepared from certified standards to cover the concentration range of the samples to be analyzed. The calibrants are run at the beginning of the analyses, and a calibration curve is fitted. If the correlation <0.995 , new calibrants will be prepared, and calibration will be re-done. See SOPs for more detail. Standards are supplied from Astoria Pacific.

Each standard is labeled with concentration and expiration date. Standards are stored at room temperature. Working calibrants of concentrations >100 µM are prepared weekly and stored at 4°C. Working calibrants of concentrations <100 µM are prepared daily.

2.5.1.3 Chlorophyll a and Pheophytin

The laboratory fluorometer is calibrated at the beginning of each monitoring season with 2 liquid pure chlorophyll a standards and reagent. At the time of calibration a solid secondary standard is also analyzed and the formula for calculating chlorophyll a in samples is determined. The solid secondary standard is analyzed with each batch of samples. Blanks of 90% acetone, and an unused filter extracted with 90% acetone are set up with each rack of samples.

2.5.1.4 Net and Flowmeter

The net used for zooplankton collection and the flowmeter will be rinsed with fresh water and inspected for damage following each survey. Additionally, the flowmeter will be calibrated annually to attain the most accurate correction constant possible.

2.5.2 Data validation, reporting and verification

2.5.2.1 Analytical Methods

Data Evaluation: Both the Laboratory Manager and the QA Officer will review data to determine if it meets the quality assurance objectives (Table 2-3). Decisions to qualify or reject the data will be made by the Laboratory Manager and the QA Officer and if required, corrective actions will be implemented as outlined in Table 2-4 and Table 2-5 .

Table 2-3. Data quality objectives

Parameter	Units	MDL	Expected Range	Accuracy (+/-)	Precision
Nitrite/Nitrate	µM	0.05	0 – 10	80-120 % recovery for QC std. and lab fortified matrix	± 0.1 µM if less than 0.5 µM or 20% RPD if more than 0.5 µM
Ortho-Phosphate	µM	0.02	0 – 3	80-120 % recovery for QC std. and lab fortified matrix	± 0.05 µM if less than 0.1 µM or 20% RPD if more than 0.1 µM
Ammonia	µM	0.1	0 – 5	80-120 % recovery for QC std. and lab fortified matrix	± 0.1 µM if less than 0.5 µM or 20% RPD if more than 0.5 µM
Total Nitrogen	µM	0.05	0 – 30	80-120 % recovery for QC std. and lab fortified matrix	± 0.1 µM if less than 0.5 µM or 20% RPD if more than 0.5 µM
Total Phosphorous	µM	0.02	0 – 6	80-120 % recovery for QC std. and lab fortified matrix	± 0.05 µM if less than 0.1 µM or 20% RPD if more than 0.1 µM
Chlorophyll a	µg/l	0.02	0 – 50	75-125% recovery for QC std.	± 2.0 µM if less than 15 µg/l or 25% RPD if more than 15 µg/l

Table 2-4. Laboratory Analytical QC: Nutrients (Nitrate+Nitrite, Ortho-Phosphate, Ammonia, TN, and TP)

QC	Frequency/ Number	Method/SOP QC Acceptance Limits	Corrective Action	Person Responsible for Corrective Action	Measurement Performance Criteria
Method Blank	1 per set of 20	< MDL	Re-run	Lab Manager	< MDL
Reagent Blank	1 per set of 20	< MDL	Re-run	Lab Manager	< MDL
Laboratory Duplicate	10% of samples	<20%RPD	Re-run	Lab Manager	<20%RPD
Internal Standards*	1 per set of 20	90-110% recovery	Re-run	Lab Manager	90-110% recovery
External Standards**	1 per set of 20	90-110% recovery	Re-run	Lab Manager	90-110% recovery

***Internal standard:** a known amount of a standard added to a test portion of a sample and carried through the entire determination procedure as a reference for calibrating and controlling the precision and bias of the applied analytical method.

****External standard:** USGS Standard Reference Nutrient Samples

Table 2-5. Laboratory Analytical QC: Chlorophyll a

QC	Frequency/ Number	Method/SOP QC Acceptance Limits	Corrective Action	Person Responsible for Corrective Action	Measurement Performance Criteria
Method Blank	1 per set of 20	< MDL	Re-clean, re-run	Lab Manager	< MDL
Instrument Blank	1 per set of 20	< MDL	Re-clean, re-run	Lab Manager	< MDL
Laboratory Duplicate	10% of samples	<20%RPD	Qualify	Lab Manager	<20%RPD
External Standards**	1 per set of 20	90-110% recovery	Qualify	Lab Manager	90-110% recovery

***Internal standard:** a known amount of a standard added to a test portion of a sample and carried through the entire determination procedure as a reference for calibrating and controlling the precision and bias of the applied analytical method.

****External standard:** either a liquid primary chlorophyll a standard provided by Turner Designs or a solid secondary standard.

2.5.2.2 Plankton Analyses

2.5.2.2.1 Whole-Water Phytoplankton

Counts of 400 phytoplankton cells will provide a precision of $\pm 10\%$ of the mean (Guillard 1973). Therefore, a minimum of 400 entities (solitary single cells, chains, or colonies) will be tallied for each sample. Unicellular forms (*e.g.*, *Cryptomonas* spp., microflagellates), aggregate forms (*e.g.*, *Phaeocystis pouchetii*), and chained forms (*e.g.*, *Skeletonema* spp.) will each count as one entity towards the 400-entities-counted-per-sample minimum tally. To increase precision of the abundance estimates for the

most abundant taxa, when practical at least 75 entities of each of the three most abundant taxa will be counted in each sample. The overall goal then is to enumerate a minimum of 400 entities total and the 3 most abundant taxa to at least 75 entities each. An additional data quality procedure will be performed on the whole water phytoplankton samples. A subset of samples will be counted in duplicate by a different taxonomist or as a blind recount by the same taxonomist to provide an estimate of the variability in the analysis and ensure the accuracy and comparability of the results. One whole water sample from the surveys in February, April, June, August and October will be analyzed in duplicate. This range of samples should cover the major taxonomic groupings and various levels of abundance. The results, as relative percent difference (RPD), will be included in the data submission as an estimate of the variability in the analysis. The RPD for total and the most dominant species should be $\leq 20\%$. If the RPD is greater than 20 a second aliquot will be counted and the three results used to calculate the relative standard deviation (RSD), which should be $\leq 20\%$.

2.5.2.2.2 Zooplankton

Zooplankton samples will be either sub-sampled with a Wildco Henson-Stemple pipette or split with a Folsom plankton splitter, and an aliquot of at least 250 animals will be counted. If the total count in an aliquot is less than 250 animals, additional aliquots will be counted until either the targeted number of organisms is reached or the entire sample is counted. One sample from each survey in February, April, June, August and October will be analyzed in duplicate. The results, as RPD, will be included in the data submission. The RPD for total and the most dominant species should be $\leq 20\%$. If the RPD is greater than 20 a second aliquot will be counted and the three results used to calculate the relative standard deviation (RSD), which should be $\leq 20\%$.

2.6 Preventive maintenance procedures and schedules

2.6.1 Maintenance for Astoria 2 Autoanalyzer

The Astoria 2 Autoanalyzer will be cleaned and maintained no less than once a month as described in the following procedure:

1. Place all lines (including autosampler wash line) in DI water and pump for 10 minutes.
2. Place all lines in CHEMWASH* (or equivalent) for 5 to 10 minutes.
3. Place all lines in a clean beaker of DI water for 5 to 10 minutes.
4. Place all lines in a 5 to 10% Bleach solution for 5 to 10 minutes.
5. Place all lines in a clean beaker of DI water for 5 to 10 minutes.
6. Place all lines in a 2% Neutrad** solution for 5 to 10 minutes.
7. Place all lines in a clean beaker of DI water for 5 to 10 minutes.
8. Place all lines in 1N HCl for 5 to 10 minutes.
9. Place all lines in a clean beaker of DI water for 10 minutes.
10. Place reagent lines in Startup/Shutdown solution, sample probe in sample wash pot and wash line into DI water and allow to pump for at least 20 minutes.
11. Change all pump tubes and polyflow tubing.
12. Inspect all injection fittings, sample splitter(s), debubblers, sample probe and reagent lines on the cartridge for debris. If necessary, clean appropriately or replace with clean parts.

13. Clean all reagent bottles with the bleach solution and rinse thoroughly followed by 1N HCl and rinse thoroughly before adding reagents. This step is only needed if the same bottle is continuously used for the reagent.
14. Clean all platens using a tissue moistened with isopropyl alcohol or methanol.
15. Wipe the pump rollers using a tissue moistened with isopropyl alcohol or methanol. Try to remove any debris or particulates around the pump rollers and bushings.
16. Pump Startup/Shutdown solution for 5 to 10 minutes until you have obtained good flow through the system.

The Open Tubular Cadmium Reactor (OTCR) will be cleaned with 1 N HCl followed by a DI rinse every 2 weeks. Auxiliary pump tubing will be replaced every 6 months.

2.6.2 Maintenance for Turner Trilogy Fluorometer (1/month or more frequently as needed)

At least once a month, or more frequently as needed, the sample chamber and cuvette holder will be cleaned with a water dampened cotton swab or soft cloth. All components will be dried thoroughly before reassembling.

2.6.3 Maintenance for Direct Q3

The Direct Q3 is used to produce Milli-Q water (referred to in Section 2.2.1.2.1 and recommended in section H of the SOP for Ammonia).

At least once a year, or more frequently as needed, the SmartPak filter cartridge and final filter will be replaced and the system and tank will be sanitized with 30% Hydrogen Peroxide solution.

2.6.4 Maintenance for Plankton Collection and Analysis Equipment

2.6.4.1 Plankton Collection Equipment

The chamber inside the General Oceanographic Environmental flowmeters are filled with fresh water before every research cruise, so that minimal air is left inside the chamber; they are also checked for ease of rotation and corrosion. The nets used for zooplankton collection are checked for holes before each research cruise. Small holes are filled using a glue product called Zap-a-gap®. The cod ends on the zooplankton collection nets are checked for holes in the mesh before every research cruise. Should a hole be found, the mesh is replaced; if the mesh starts to separate from the plastic, it is glued back down using PVC cement.

2.6.4.2 Plankton Analysis Equipment

Fluorettes are checked for holes in the mesh, or separation of the mesh from the plastic, before every research cruise. If holes are found, the mesh is replaced; if the mesh is separating from the plastic, it is glued back down using PVC cement.

The Hensen-Stempel pipettes are cleaned once per year, or more frequently if needed. They are soaked in warm soapy (dish soap) water, and then soaked in fresh hot water. They are then thoroughly rinsed to remove all soap, and set aside to dry.

The Folsom plankton splitter is cleaned once per year, or more if necessary. It is scrubbed with dish soap, and then thoroughly rinsed with hot water until all soap is removed. It is then set aside to dry.

The microscopes are cleaned once per year. The lenses in the eyepieces and eyepiece tubes are wiped clear of dust using Kimwipes®. The lens over the light condenser is also wiped clean. After every use the microscope is unplugged and covered with a dust cover.

The multi-channel digital counter used in the enumeration of zooplankton is regularly cleaned using compressed air. The area under the buttons is flushed of dust and dirt.

2.7 Corrective action contingencies

If results from any analyses of QC checks are unacceptable, corrective actions will be taken as described for each SOP above. Whenever possible, analyses will be re-run with new QC checks. If results are still unacceptable, the instrument will be re-calibrated according to manufacturer's instructions. The Lab manager is responsible for all corrective actions. The QA Officer must also be consulted. All corrective actions will be documented in the lab notebook.

2.8 Inspection/Acceptance of Supplies and Consumables

Prior to use, supplies and consumables will be inspected and tested to ensure that they conform to the required level of quality. Any defective material will be replaced before the sampling event or before analysis begins. Supplies and consumables consist of: sample containers (whirl paks, polypropylene sample vial), filters (Whatman GF/F, Nucleopore 0.4 µm), filtration apparatus (syringe and Swinlok filter holder), preservation solutions (formalin, Utermohls solution), distilled water, laboratory reagents, and standards (chlorophyll and nutrient).

- Sample containers are either cleaned by the laboratory or purchased new. Containers must be cleaned according to SOPs prior to use and must be rinsed three times with station water prior to being filled with sample. Field blanks assess potential contamination of containers and sampling equipment.
- All filtering equipment (the syringe and filter holder) are cleaned prior to use. The equipment gets a 10% HCl rinse in the followed by a triple rinse of distilled water. Between stations the equipment gets rinsed with distilled water and a triple rinse with station water.
- Filters for chlorophyll and dissolved nutrients are used directly from the manufacturer and are not cleaned or treated.
- Preservation solutions must be prepared using at least reagent grade chemicals / HPLC grade solvents. Solutions must be assigned an expiration date of 1 year.
- Distilled water must be collected into cleaned containers and refreshed prior to each survey.

- Laboratory reagents must be at least reagent grade. Dry reagents must be assigned an expiration date of no more than 5 years; be stored in a clean, dry environment, away from light, and be traceable to receipt and certificate of analysis. Reagent solutions must be assigned an expiration date of no more than 1 year and be stored appropriately. The laboratory must maintain a chemical tracking inventory.
- Laboratory standards must be certified as at least 96% pure or the lot-specific analysis purity must be incorporated into calculation of the standard concentration. Standards must be assigned an expiration date “as received” based on the manufacturer’s expiration date, or a date consistent with laboratory SOPs and stored as recommended by the manufacturer.

All supplies and consumables are purchased from Fisher Scientific, Sigma Aldrich, or Astoria Pacific. Chlorophyll standards are purchased once a year from Turner Designs. Nutrient standards are purchased yearly from Astoria Pacific. Nutrient samples for inter-laboratory comparison and internal quality control are purchased twice a year (spring and fall) from USGS as part of the Standard Reference Sample Program (<http://bqs.usgs.gov/srs/>).

2.9 Data Acquisition Requirements (non-direct measurements)

Data from previous and on-going monitoring conducted by MWRA and PCCS will be utilized to assess the state of CCB and SBNMS. Other possible data from non-direct measurements that may be used include satellite imagery and mooring data. These secondary data are used “as received” and not censored.

2.10 Data Management

2.10.1 Hydrographic Data

The hydrographic data generated during the survey consists of rapidly sampled, high-resolution measurements of conductivity, temperature, depth, DO, fluorescence, and PAR. Data will be logged internally and downloaded using Seabird SeaTerm software.

SBE Data Processing Software from Seabird will be used to process the raw data from the instrument. Using this software, data are first converted from the raw data to scientific units (i.e. conductivity, temperature). Only data from the downcast are used. In order to match the response of the temperature and conductivity sensors, the data have to be filtered. A low pass filter of 0.5 seconds is used to match the time constants of temperature and salinity. A second low-pass filter of 2 seconds is applied to pressure readings to separate data that are collected at a less than minimum descent rate. Temperature and conductivity are advanced 0.5 seconds to account for misalignment of sensors and a loop edit function is used to remove data collected during a cast in which the CTD either decelerates or travels backwards (loops) due to rolling of the vessel. Finally depth, potential temperature, and salinity are derived from pressure, temperature and conductivity data, respectively, and density (sigma-t) is calculated based on temperature, salinity and pressure.

2.10.2 Field Data

All data from field notebooks will be manually entered into the appropriate database format.

2.10.3 Laboratory Data

All laboratory data will either be electronically transferred from the instrument or manually read from the instrument display (or optical field of a microscope) and entered onto a standard data form. Forms used for data entry are included in Appendix B. Data in laboratory notebooks will be manually entered when necessary. All data reduction will be performed electronically either by the instrument software or in a spreadsheet and will be validated according to procedures described in Section 4.0. Laboratory replicates will be reported as mean sample values. All field replicates will be reported as individual sample values. The format for final data submission to MWRA is shown in Table 2-6 . Data will be submitted electronically as Microsoft Excel spreadsheets.

Table 2-6. Specifications for data sets

a. Samples

Description	Field	Required Field	Data type & format
Identifier of sampling event (survey)	EVENT_ID	Y	alphanumeric, maximum 10 characters
Identifier for station.	STAT_ID	Y	alphanumeric, maximum 10 characters
Latitude measured at each station visit (decimal degrees)	LATITUDE	Y	number (7 decimal places)
Longitude measured at each station visit (decimal degrees)	LONGITUDE	Y	number (7 decimal places)
Depth to bottom in meters measured by echo-sounder	DEPTH_TO_BOTTOM	Y	number (2 decimal places)
Station arrival date and time (local time)	STAT_ARRIV_LOCAL	Y	date
Sample identifier	SAMPLE_ID	Y	alphanumeric, maximum 15 characters
Code for type of gear used to collect sample.	GEAR_CODE	Y	alphanumeric, maximum 12 characters
Depth of sample, from water surface to bottom of sample, in m.	DEPTH	Y	number (2 decimal places)
Depth of water sample, from water surface to top of sample, in m.	DEPTH_TOP		number (2 decimal places)
Date and time sample was taken (local time)	SAMPLE_DATE_TIME_LOCAL		Date
Sample depth-type code (A=near-surface, E=near-bottom, Z=zooplankton net tow)	SAMPLE_DEPTH_CODE	Y	alphanumeric, maximum 2 characters
Volume of sample as collected (e.g. calculated tow volume for zooplankton tows)	SAMP_VOL		number

Description	Field	Required Field	Data type & format
Unit of volume measurement.	SAMP_VOL_UNIT_CODE		alphanumeric, maximum 3 characters
Comments for a given station visit and sample	COMMENTS		alphanumeric, maximum 150 characters

b. Hydrographic measurement data

Description	Field	Required Field	Data type & format
Identifier of sampling event (survey)	EVENT_ID	Y	alphanumeric, maximum 10 characters
Identifier for station.	STAT_ID	Y	alphanumeric, maximum 10 characters
Station arrival date and time (Local Time).	STAT_ARRIV_LOCAL	Y	Date
Depth (in meters= decibars) at which data were collected.	DEPTH	Y	number (2 decimal places)
Date and time when data were collected (Local Time).	PROF_DATE_TIME_LOCAL		Date
Code for parameter measured.	PARAM_CODE	Y	alphanumeric, maximum 20 characters
Result for parameter.	VALUE		Number
Value qualifier.	VAL_QUAL		alphanumeric, maximum 4 characters
Code for the unit of measurement	UNIT_CODE		alphanumeric, maximum 12 characters
Code for method.	METH_CODE		alphanumeric, maximum 13 characters
Code for instrument used.	INSTR_CODE		alphanumeric, maximum 10 characters
Comments for the sensor measurement	COMMENTS		alphanumeric, maximum 150 characters

c. Nutrient measurement data

Description	Field	Required Field	Data type & format
Identifier of sampling event (survey)	EVENT_ID	Y	alphanumeric, maximum 10 characters
Sample identifier	SAMPLE_ID	Y	alphanumeric, maximum 15 characters
Identifier for bottle	BOTTLE_ID	Y	alphanumeric, maximum 15 characters
Code for parameter measured.	PARAM_CODE	Y	alphanumeric, maximum 20 characters
Result for parameter.	VALUE		number
Value qualifier.	VAL_QUAL		alphanumeric, maximum 4 characters
Code for unit of measurement	UNIT_CODE		alphanumeric, maximum 20 characters
Code for method.	METH_CODE		alphanumeric, maximum 13 characters
Code for instrument used.	INSTR_CODE		alphanumeric, maximum 10 characters
Comments on this result	COMMENTS		alphanumeric, maximum 150 characters

d. Plankton measurement data

Description	Field	Required Field	Data type & format
Identifier of sampling event (survey)	EVENT_ID	Y	alphanumeric, maximum 10 characters
Sample identifier	SAMPLE_ID	Y	alphanumeric, maximum 15 characters
Subsample (bottle) identifier	BOTTLE_ID	Y	alphanumeric, maximum 15 characters
Code for species	SPEC_CODE	Y	alphanumeric, maximum 17 characters
Taxonomic name for species	DESCR	Y	alphanumeric, maximum 80 characters
Qualifier for species code, including sex and/or life stage codes. Default = 'null' for when sex or life stage is unknown or not relevant.	SPEC_QUAL		alphanumeric, maximum 4 characters
Count of cells for that species	VALUE	Y	number
Value qualifier.	VAL_QUAL		alphanumeric, maximum 4 characters
Code for the unit of measurement	UNIT_CODE		alphanumeric, maximum 12 characters

Description	Field	Required Field	Data type & format
Code for method.	METH_CODE		alphanumeric, maximum 13 characters
Number assigned by the laboratory to the sample.	LAB_SAMPLE_ID		alphanumeric, maximum 35 characters
Comments on the record.	COMMENTS		alphanumeric, maximum 150 characters

3.0 Assessment / Oversight

3.1 Assessments and Response Actions

3.1.1 Performance Audit

The QA Officer will conduct an initiation audit and, as needed, laboratory and field inspections to ensure that laboratory analyses and data recording and entry are carried out in accordance with this QAPP. Deviations from the QAPP will be reported directly to the Program Manager and the appropriate corrections will be made. All deviations will be noted in the respective field notebook, lab notebook or electronic file and tracked by the QA Officer.

3.1.2 Corrective Action

All field and laboratory personnel share responsibility for identifying and resolving problems encountered in the routine performance of their duties. The Program Manager will be responsible for identifying and resolving any problems that have not been adequately addressed by technical personnel as well as any problems that require changes in this QAPP and/or require consultation with MWRA. The Program Manager is also accountable to MWRA for overall performance of this monitoring program. Issues regarding scheduling (e.g. synoptic sampling with MWRA's Massachusetts Bay monitoring consultant) will be reported to MWRA and dealt with under their guidance.

3.2 Reports to Management

Two reports per survey will be submitted to MWRA, a pre-survey plan outlining the anticipated sampling, and a post-survey report detailing the sampling that was conducted. Additionally, quarterly reports will be submitted to MWRA which will include the hydrographic data and laboratory data (nutrients, phytoplankton, zooplankton) collected during the surveys conducted in the respective quarter.

All data collected during the year will be presented at the MWRA annual technical workshop held each spring. Following the completion of the workshop, MWRA will be provided with a digital copy of the Power Point slides, and a two page abstract describing the major results of the year.

4.0 Data Validation and Usability

4.1 Data Review, Validation and Verification Requirements

No data will be used until they are validated and verified as described in 4.2. The adherence to the data quality objectives (Table 2-3) and laboratory QC's, (Table 2-4, Table 2-5), RPD's of duplicate plankton counts, notations in field and laboratory notebooks, cross-checks of data entry, and the results of the audits will be used to objectively and consistently determine whether the data are useable for the purposes of this project.

4.2 Validation and Verification Methods

To assess the quality and usability of the data, several methods of data validation and verification are used throughout the data collection, analysis, and reporting process and overseen by the QA Officer.

Sample containers are pre-labeled to ensure completeness and accuracy of sample collection. Manual entry of field sampling data is verified for correctness and completeness by comparing the field survey log book to the post-survey report. Data downloaded directly from instrumentation are date and time stamped and can be cross-referenced to the field survey log book.

Manual data entry of laboratory data (e.g. zooplankton and phytoplankton) will be verified by 100% double keypunching and using the computer to check for differences. All other laboratory data is downloaded directly from the instrument.

Calculations performed on the data (e.g. plankton concentration) will be done in Excel and verified manually at a frequency to ensure that formulas are correct, appropriate, and consistent, and that calculations are accurately reported.

Additional data validation is achieved by following the protocols for holding times, instrument calibration and maintenance, quality control sample results, and other criteria for data quality requirements as outlined in this QAPP.

4.3 Reconciliation with User Requirements

A key step in the evaluation of the validated data to determine if it meets the user requirements is the presentation of the results at the MWRA's annual technical workshop in the spring. This will provide a means for peer review of the data and interpretation of results. Data collected for this project will also be compared with data collected during the on-going Cape Cod Bay Monitoring Program and Right Whale Habitat Program conducted by PCCS. A synthesis of the data collected from these programs will provide a context from which to evaluate nutrient loading and the possible effects on the food chain from phytoplankton to right whales.

5.0 References

- Borkman, D. 1994. Phytoplankton and Nutrients in Buzzards Bay, Massachusetts 1987-1988. M.S. Thesis. University of Massachusetts Dartmouth, Dartmouth, MA. 203 pp.
- Borkman D, Pierce RW, Turner JT. 1993. Dinoflagellate blooms in Buzzards Bay, Massachusetts. Pp. 211-216 in Smayda, T.J., and Y. Shimizu (Eds.), *Proceedings of the Fifth International Conference on Toxic Marine Phytoplankton*, Elsevier.
- Costa AS. 2010. Laboratory quality assurance plan. Provincetown Center for Coastal Studies. Final draft submitted to Massachusetts Department of Environmental Protection.
- Guillard RRL. 1973. Division rates. Pages 289-311 In: J.R. Stein, (Ed.) *Phycological Methods*. Cambridge Univ. Press.
- Leeney RH, Stamieszkin K, Jaquet N, Mayo CA, Osterberg D, Marx MK. 2008. Surveillance, monitoring and management of North Atlantic right whales in Cape Cod Bay and adjacent waters. Final Report submitted to the Division of Marine Fisheries, Commonwealth of Massachusetts.
- Libby PS, Fitzpatrick MR, Buhl RL, Lescarbeau GR, Leo WS, Borkman DG, Turner JT, Oviatt CA. 2010. Quality assurance project plan (QAPP) for water column monitoring 2010: Tasks 4-9 and 13. Boston: Massachusetts Water Resources Authority. Report 2010-02. 105 p.
- Turner JT, Borkman DG, Pierce RW. 1995. Should Red Tide Dinoflagellates be Sampled Using Techniques for Microzooplankton Rather than Phytoplankton? Pp. 737-742 in P. Lassus *et al.* (Eds.), *Harmful Marine Algal Blooms*, Lavoisier, Paris, France

APPENDIX A
Standard Operating Procedures

SOP-001: General Laboratory Safety

SOP-002: General Labware Cleaning Procedure

SOP-003: Nitrate+Nitrite Analysis

SOP-004: Ortho-Phosphate Analysis

SOP-005: Ammonia Analysis

SOP-006: TN and TP Analysis

SOP-007: Chlorophyll a Analysis

SOP-008: Phytoplankton Analysis

SOP-009: Zooplankton Analysis

Standard Operating Procedure 001 General Laboratory Safety

Date: Nov 2007

Revision: 2

Purpose and Description: Provide guidance on appropriate lab safety protocols. Lab complies with most OSHA requirements for worker safety.

Lab Safety Officer: Amy Costa

Emergency Numbers:

Hazardous Materials Hotline 800-319-2783

Provincetown Fire Department 508-487-7023

Provincetown Health Center 508-487-9395

General Health and Safety Information

1. Eye protection, gloves, and lab coats are available and required when working with chemicals
2. Closed-toed shoes are required
3. Locations of accident and safety equipment:
 - a. First-aid kit is located on wall to right of sink
 - b. Eye wash station is located to the right of the sink
 - c. Shower is located downstairs
 - d. Spill pads are located under sink
4. Report any accidents immediately to Amy Costa and/or Rich Delaney
5. Nearest Medical Facility is the Provincetown Health Center located at 49 Harry Kemp Way
6. All liquid chemicals must be stored below eye level
7. All chemicals must be properly labeled and stored at all times. Hazardous labels must indicate what harm the chemical represents.
8. MSDS sheets are located in the file cabinet in folder labeled MSDS
9. Laboratory must comply with local building/fire codes.
10. Wastes must be properly stored in their own container (i.e. acetone waste in container labeled "Acetone Waste," acid waste in container labeled "Acid Waste," etc.)
11. Disposal of hazardous waste will be done through Barnstable County's Cape Cod Cooperative Extension. (Hazardous waste generator status: very small quantity generator, limited primarily to copper sulfate required for nitrate/nitrite analyses and activation of cadmium coil and acetone.)

Hazardous Wastes Requiring Special Disposal:

Acetone

Copper Sulfate

Acid Wash (10% HCl)

Standard Operating Procedure 002 General Labware Cleaning Procedure

Date: Nov 2007

Revision: 2

Purpose and Description: Outline appropriate techniques for cleaning labware and sampling containers. Only glass sample containers will be reused. Whirl paks and disposable polypropylene tubes will be discarded. Clean labware and sampling containers are required to ensure that results for the assays analyzed in the laboratory are accurate.

Method Description: All labware is washed in non-phosphate detergents (i.e. Liqui-Nox), stored under the sink in a 1 gallon container. Glassware is soaked in a bath of dilute hydrochloric acid (10%) kept in labeled glass tub.

Glassware Cleaning Procedure

1. Empty non-hazardous contents of bottles down the drain and hazardous contents into appropriate waste containers
2. Remove labels. This is expedited by soaking bottles in tap water
3. Wash in soapy water (Liqui-Nox)
4. Rinse with tap water at least 3 times
5. Soak glassware for at least 24 hours in tub of 10% HCL. Make sure glassware is completely submerged
6. Rinse with DI water
7. Fill with DI water and allow glassware to soak for at least 1-2 hours
8. Rinse thoroughly – at least 3 times, inside and out – with DI water
9. Air dry, inverted
10. After drying, store with either parafilm or aluminum foil across the top, or with caps loosely attached

Plasticware Cleaning Procedures: Do not soak in acid bath.

1. Empty non-hazardous contents of bottles down the drain and hazardous contents into appropriate waste containers
2. Remove labels. This is expedited by soaking bottles in tap water
3. Wash in soapy water (Liqui-Nox)
4. Rinse with tap water at least 3 times
5. Rinse thoroughly – at least 3 times, inside and out – with DI water
6. Air dry, inverted

Standard Operating Procedure 003 Nitrate+Nitrite Analysis

Date: Nov 2007
Revision: 2
Primary Method: EPA 353.4

A. Scope and Application

This method is used for the determination of nitrite or nitrate plus nitrite in seawater and is applicable to many ranges.

B. Summary of Method

Nitrate is reduced quantitatively to nitrite by cadmium metal in the form of an open tubular cadmium reactor (OTCR). The nitrite thus formed plus any originally present in the sample is determined as an azo dye at 540 nm following its diazotization with sulfanilamide and subsequent coupling with N-1-naphthylethylenediamine.⁽¹⁾ These reactions take place in acidic solution. Nydahl provides a good discussion of nitrate reduction by cadmium metal, while the specific details of OTCR's are given by Patton.^(2,3) The information concerning mechanisms and kinetics of the color forming reactions can be found in References 3 and 4.

C. Sample Handling and Preservation

All samples will be filtered using 0.4 μm Nucleopore filters. Samples must be kept frozen until analysis. Holding time should not exceed 28 days.

D. Raw Materials Required

NOTE: Chemicals should be of ACS grade or equivalent.

Ammonium Chloride NH_4Cl (FW 53.50)
 Chloroform CHCl_3 (FW 119.38)
 Cupric Sulfate, Pentahydrate $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (FW 249.69)
 Deionized Water (ASTM Type I or II)
 Detergent TX-10 (API p/n 90-0760-04)
 Hydrochloric Acid, Concentrated HCl (FW 36.46)
 Imidazole, $\text{C}_3\text{H}_4\text{N}_2$ (FW 68.08)
 Low Nutrient Seawater (LNSW)*
 Magnesium Sulfate $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (FW 246.48)*
 N-1-naphthylethylenediamine Dihydrochloride $\text{C}_{12}\text{H}_{14}\text{N}_2 \cdot 2\text{HCl}$ (FW 259.18)
 Potassium Nitrate KNO_3 (FW 101.11)
 Sodium Bicarbonate NaHCO_3 (FW 84.01) *
 Sodium Chloride NaCl (FW 58.44) *
 Sodium Nitrite NaNO_2 (FW 69.0)
 Sulfanilamide $\text{C}_6\text{H}_8\text{N}_2\text{O}_2\text{S}$ (FW 172.21)

*See Operating Notes for information on matrix choices.

E. Reagent Preparation**1. Copper Sulfate Solution 2% (1 L)**

Cupric Sulfate 20 g
 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (FW 249.69)
 Deionized Water

Dissolve 20 g of cupric sulfate in approximately 900 ml of deionized water contained in a 1 L volumetric flask. Dilute the solution to the mark with deionized water and mix well. Stable at room temperature.

2. Stock Ammonium Chloride-Copper Sulfate (1 L)

Ammonium Chloride250 g
 NH_4Cl (FW 53.50)
 Copper Sulfate Solution 2%..... 2.5 ml
 Deionized Water

Dissolve 250 g of ammonium chloride in 900 ml of deionized water contained in a 1 L beaker. Add 2.5 ml of 2% copper sulfate solution. Transfer the solution to a 1 L volumetric flask and dilute to the mark with deionized water. Store refrigerated at 2-8°C.

3. 10% HCl (2 L)

Hydrochloric Acid Concentrated 200 ml
 HCl (FW 36.46)
 Deionized Water

Carefully add 200 ml of hydrochloric acid to about 1000 ml of deionized water. Cool and dilute to 2000 ml.

4. Stock Imidazole Buffer (2 L)

Imidazole.....6.8 g
 $\text{C}_3\text{H}_4\text{N}_2$ (FW 68.08)
 Stock Ammonium Chloride-Copper Sulfate..... 30 ml
 10% HCL as needed
 Deionized Water

Dissolve 6.8 g imidazole in about 1500 ml deionized water. Add 30 ml of stock ammonium chloride-copper sulfate solution. Adjust the pH to 7.8 - 7.85 with 10% HCl. Dilute to 2000 ml with deionized water and mix well. Filter to 0.45 μm . Stable at room temperature.

5. Working Imidazole Buffer (500 ml)

Stock Buffer	500 ml
Detergent TX-10	40 drops

Add 40 drops TX-10 to each 500 ml of Stock Buffer required. Mix well.

6. NED Reagent (1 L)

N-1-naphthylethylenediamine Dihydrochloride	1.0 g
$C_{12}H_{14}N_2 \bullet 2HCl$ (FW 259.18)	
Deionized Water	

Dissolve 1.0 g N-1-naphthylethylenediamine dihydrochloride in about 800 ml deionized water contained in a 1 liter volumetric flask. Dilute to the mark with deionized water. Filter to 0.45 μ m. Store in a brown bottle and refrigerate when not in use. Reagent is stable for several months. Discard if colored.

7. Stock SAN Reagent (1 L)

Sulfanilamide	10.0 g
$C_6H_8N_2O_2S$ (FW 172.21)	
10% HCl	

Dissolve 10.0 g of sulfanilamide in about 800 ml of 10% HCl contained in a 1 L volumetric flask. Dilute to the mark with 10% HCl and mix well. Filter to 0.45 μ m. Stable at room temperature, but may be refrigerated.

8. Working SAN Reagent

Stock SAN Reagent	250 ml
Detergent TX-10	30 drops

Add 30 drops TX-10 to 250 ml of stock SAN reagent and mix well.

9. Artificial Seawater (ASW) (4L)

Sodium Chloride	128.5 g
NaCl (FW 58.44)	
Magnesium Sulfate	28.5 g
$MgSO_4 \bullet 7H_2O$ (FW 246.48)	
Sodium Bicarbonate	0.672 g
$NaHCO_3$ (FW 84.01)	
Deionized Water	

Dissolve 128.5 g of sodium chloride, 28.5 g of magnesium sulfate and 0.672 g of sodium bicarbonate in about 3 liters of deionized water. Dilute to 4 liters with deionized water. These reagents must be high quality, reagent grade to avoid excessive nutrient or trace metal contamination.

10. Sampler Wash

See Operating Notes.

11. Startup/Shutdown Solution

Deionized Water	1000 ml
Detergent TX-10	2 – 4 ml

Add 2 to 4 ml of TX-10 to 1000 ml of deionized water. Mix well.

12. Open Tubular Cadmium Reactor (OTCR)⁽³⁾

The Astoria analytical cartridge uses an Open Tubular Cadmium Reactor coil to reduce nitrate to nitrite. Nitrogen is used to segment the analytical stream to prevent a pH increase due to reaction between oxygen in ambient air and cadmium. Contact with oxygen will also deactivate the OTCR.

A. OTCR Activation

The OTCR (API p/n 303-0500-24) is a coiled cadmium tube (24") that has been cleaned of manufacturing oils inside and coated with plastic outside. The outside diameter is 0.090 inches, with an inside diameter of 0.050 inches, and a wall thickness of 0.020 inches. Short lengths of 0.034" ID polyethylene are sleeved to the reactor coil to allow installation of the reactor in the manifold. These sleeves are joined by a N-13 (N-2) nipple.

B. Reagents for OTCR Activation

1. Stock Imidazole Buffer
2. Copper Sulfate Solution
3. 1.0 N Hydrochloric Acid (100 ml)

Hydrochloric Acid, concentrated	8.3 ml
HCl (FW 36.46)	
Deionized Water	

Add 8.3 ml of concentrated hydrochloric acid to about 70 ml of deionized water contained in a 100 ml volumetric flask. Dilute to the mark with deionized water.

C. Procedure

NOTE: Do not introduce air into the cadmium tube during this process.

1. Detach one end of the polyethylene tubing from the N-13 (N-2) nipple.
2. Using a 10 cc plastic syringe fitted with 0.040" ID PVC tubing and a short 0.034" ID polyethylene extension, flush the OTCR with the described solutions using the following procedure:
 - a) Deionized Water

b) 1.0 N Hydrochloric Acid

CAUTION: The hydrochloric acid may cause pitting of the cadmium reactor interior surface if left in the OTCR for longer than a few seconds. After the HCl flush, proceed quickly to Step C.

c) Deionized Water

d) 2% Copper Sulfate

Slowly flush the OTCR with 10 cc of 2% copper sulfate. Repeat. Precipitated copper may be observed exiting the reactor (black particles).

e) Deionized Water

Flush with deionized water until no more precipitated copper is flushed from the reactor. This requires a forceful flush. Repeat 2-3 times.

f) Stock Imidazole Buffer

Fill the OTCR with Stock Buffer. The reactor should be stored with stock buffer when not in use.

D. Installation of the OTCR

The analytical cartridge is provided with a jumper of 0.034"ID polyethylene sleeved at both ends in the position where the OTCR is to be installed.

1. With the N-13 nipple in place, pump reagents segmented with nitrogen until a stable flow is established.

NOTE: The working buffer must be in the cartridge before the OTCR is installed.

2. Turn the pump off and disconnect the N-13 in the jumper connection.
3. Install the OTCR in the jumper, attaching each free end with one N-13 nipple.
4. Resume pumping and wait until a stable bubble pattern is established before proceeding with the determinations.

E. Removal of the OTCR

1. Before the reagent lines are removed from the reagents, stop the pump, remove the OTCR and reconnect the N-13 nipple in the jumper connection.
2. Resume pumping. Place the reagent lines in Startup/Shutdown solution and pump until the cartridge has been thoroughly rinsed.
3. Attach the syringe to the N-13 nipple on the OTCR. Draw 10 to 15 ml of Stock Buffer through the OTCR. Leaving buffer in the OTCR, remove the syringe and join the tubing ends with the N-13 nipple.

NOTE: Do not leave any air in the OTCR. It must be stored filled with Stock Buffer.

F. Reduction Efficiency and Stabilization of the OTCR

In the OTCR, nitrate is reduced to nitrite. However, under some conditions reduction may proceed further with nitrite being reduced to hydroxylamine and ammonia. These reactions are pH dependent.



At the buffered pH of the reactions, equation 1 predominates. However, if the cadmium surface is overly active, equation 2 will proceed sufficiently to give low results. If the cadmium surface is insufficiently active, there will be a low recovery of nitrate as nitrite.⁽³⁾ This latter is defined as poor reduction efficiency.

To determine the reduction efficiency, run a high level nitrite calibrant followed by a nitrate calibrant of the same nominal concentration. A reduction efficiency range of 90% - 110% is acceptable. The reduction efficiency is calculated as follows:

$$\frac{\text{Peak Height (NO}_3^-) \times 100}{\text{Peak Height (NO}_2^-)} = \% \text{ efficiency}$$

If the response of the nitrite is as expected but the reactor efficiency is poor, it may be necessary to repeat the activation procedure. However, if the nitrite response is much less than expected, it is an indication that the nitrite is being further reduced and stabilization of the OTCR is necessary.

G. Stabilization

When an OTCR is first activated, it may be necessary to stabilize the activity of the reactor. In order to stabilize the OTCR, pump a mid or high calibrant continuously and record the steady state signal. Continue the steady state until a drift is no longer observed. Return the sampler probe to wash and proceed with determinations when the baseline has stabilized. An alternate procedure for stabilizing the OTCR is to pump a more concentrated nitrate solution through the column for 5-10 minutes, but do not attempt to monitor the signal.

F. Calibrants

Specific Stock and Working Calibrant preparation instructions can be found on the back of the flow diagram. Be sure to use the flow diagram which covers the concentration range you wish to analyze.

Working calibrants may be prepared to cover alternate ranges by adding the appropriate volumes of stock or intermediate calibrant to 100 ml volumetric flasks that contain approximately 80 ml of sampler wash solution. Dilute the solution to 100 ml with sampler wash solution and mix well.

The following formula can be used to calculate the amount of stock (or intermediate) calibrant to be used.

$$C_1V_1 = C_2V_2$$

Where:

C_1 = desired concentration (in mg/L) of working calibrant to be prepared

V_1 = final volume (in ml) of working calibrant to be prepared (generally 100 ml)

C_2 = concentration (in mg/L) of stock (or intermediate) calibrant

V_2 = volume (in ml) of stock (or intermediate) calibrant to be used

Rearranging the equation to solve for V_2 yields:

$$V_2 = \frac{C_1 V_1}{C_2}$$

For example, to prepare a 1.0 mg/L working calibrant from a 1000 mg/L stock calibrant, use 0.1 ml (100 μ l) of the stock calibrant in 100 ml final volume.

$$V_2 = \frac{(1.0 \text{ mg/L})(100 \text{ ml})}{1000 \text{ mg/L}}$$

$$V_2 = 0.1 \text{ ml}$$

Add this amount of stock calibrant to the volumetric flask and then dilute to volume with the sampler wash solution.

G. Operation Procedure

1. Set up the cartridge as shown in the flow diagram. Check all tubing and connections. Replace if necessary.
2. Place reagent lines in startup solution.
3. Turn on power to all units and latch platens to begin liquid flow.
4. Verify that the bubble size and spacing is consistent throughout the cartridge. If bubbles are splitting up as they enter or exit a coil, check and replace fittings if necessary. The bubbles should flow smoothly without dragging. If dragging occurs, add more TX-10 to the startup solution.
5. Check all reagent containers on the instrument for particulate matter. Reagents should be filtered. Be sure all containers are properly labeled and filled before pumping reagents.
6. After a stable baseline has been verified on the startup solution, place reagent lines in reagent bottles.
7. If using data collection software, set up the appropriate sample table.
8. Allow reagents to run for 5 to 10 minutes and verify a stable baseline.
9. Once the reagent baseline is satisfactory, add the OTCR into the cartridge flow. Always connect the inlet first and the outlet second. It is important to avoid the introduction of air into the coil during this procedure.
10. Once the OTCR is on-line, run for 5-10 minutes and then re-verify the bubble pattern and baseline stability. Make any necessary adjustments.
11. Load the sampler tray with calibrants, blanks, samples, and QC or monitor samples.
12. Select the appropriate parameters for the detector and sampler. (See Flow Diagram at the end of

methodology.)

13. Begin analysis.
14. At the end of analysis remove the OTCR from the cartridge. Disconnect the outlet first, then the inlet. Flush the OTCR with buffer which contains no surfactant (TX-10).
15. Place all reagent lines in startup solution. Pump for 5 to 10 minutes to flush all of the reagents out of the cartridge.
16. Turn off the power to all units and release pump platens.

H. Operating Notes

1. The OTCR may be conditioned by running a mid scale standard through the manifold for 10-15 minutes.
2. Life expectancy of the OTCR varies and is difficult to predict. It is recommended that a nitrite standard of the same nominal concentration as the high scale standard be run as a check on column reduction efficiency.
3. There are special considerations when running seawater samples on any flow system.

A. Standards

Primary standards should be prepared from the best grade of chemicals available. Certificates of Analysis are available from the chemical manufacturer. These should be consulted to identify impurities.

Standard material should be oven dried for two hours at 110°C before weighing.

It is advisable to periodically verify the concentrations of the working standards. This can be done by running standards against standards from an outside source.

The matrix of the standards should be consistent with that of the samples. If deionized water standards are used it becomes important to determine the salt effects of each individual test. (See number 2 under Operating Notes.)

B. Matrix

Optimal system performance can be expected if the sample matrix is carried over into the sampler wash solution and the standards. Care should be taken when using deionized water wash solution with seawater standards. Many investigators recommend segregating the samples by salinity and running as a group to make corrections easier.

There are many options with respect to the matrix of the calibrant and sampler wash solutions. The relative merits of several types of material are presented here. ⁽¹⁾

Deionized Water

Advantages:

1. The quality of the water is usually well known.
2. The quality of the water is usually not highly variable.
3. The prepared standards are relatively stable with time.
4. Large volumes of water are easily available.

Disadvantages:

1. The chemical factors may be different than in salt solution (salt effect).

Artificial Seawater Solution (and/or Deionized Water - Sodium Chloride Solution)

Advantages:

1. Salt effects on the chemical factors are minimized.
2. Sodium chloride solution is easy to prepare and is not expensive.

Disadvantages:

1. Ammonium impurity is quite large in sodium chloride.
2. Large quantities of sodium chloride are sometimes required.

Low Nutrient Seawater

Advantages:

1. Salt effects are eliminated.
2. In certain regions of the ocean it is easily obtained.

Disadvantages:

1. It always contains some nutrients.
2. If not used immediately it must be filtered to remove any particulate matter.
3. Often it is difficult to obtain when working in eutrophic waters.
4. Storage is difficult, so large quantities are not easily obtained.

4. As stated above, the system is optimal when the sample matrix is carried over into the sampler wash solution and the standards. If this is not possible (i.e. if deionized water is used for the sampler wash and/or calibrants), it is advisable to check for refractive index disturbance effects. This can be accomplished by removing the sodium hypochlorite reagent. After the initial sample run is finished, place the data collection in pause, replace the hypochlorite reagent with deionized water, wait for a stable baseline, autozero the detector and run the samples again. The peaks seen are due to the refractive index disturbance.
5. As stated above, the system is optimal when the sample matrix is carried over into the sampler wash solution and the standards. If this is not possible (i.e. if deionized water is used for the sampler wash and/or calibrants), it is advisable to check for refractive index disturbance effects. This can be accomplished by running without the NED present. After the initial sample run is finished, place the data collection in pause, replace the NED reagent with deionized water, wait for a stable baseline, autozero the detector and run the sample again. The peaks that are seen are due to the refractive index disturbance.

6. If bubbles are sticking in a debubbler, cleaning the debubbler will allow bubbles to escape smoothly out the debubble line. Bubbles sticking in the debubbler can cause a loss in the overall precision of the peak height. To clean, soak the debubbler for 2-3 hours in a mixture of 20-30% Contrad[®]NF (API p/n 80-0007-04) and hot tap water. Rinse thoroughly.
7. If the flowrate of the sample pump tube is $\leq 226 \mu\text{l}/\text{minute}$ (a blk/blk pump tube) a helper line must be added when the cartridge is run alone. See Section 9 of the Astoria Analyzer Operation Manual for information on how to add a helper line.

NOTE: If the sample line is debubbled, a helper line is not necessary.

8. Cover all reagents and other solutions to avoid interference due to dust and other particulates. This will also help prevent contamination of the solutions from absorbance of analytes in the air.

I. References

1. Standard Methods for the Examination of Water and Wastewater: Centennial Edition, 21st Ed., 2005, American Public Health Association, Washington, D.C. (Method referenced: Automated Cadmium Reduction, 4500 – NO₃F, pp. 4-125 – 4-126).
2. F. Nydahl, Talanta, 23, Pages 349-357 (1976).
3. Patton, C.J., Doctoral Dissertation, Michigan State University, 1982, Page 87-121.
4. Fox, J.B., Anal. Chem., 51, 1493 (1979).
5. Automated Nutrient Analysis in Seawater, Technical Report, Brookhaven National Laboratory, Whitley, Veidt, et. al., May 1986.
6. Methods and Guidance for Analysis of Water, 1999, Office of Water, Environmental Protection Agency (USEPA), Cincinnati, OH (Method referenced: 353.3)

ACKNOWLEDGEMENTS

Astoria[®] and FASPac[™] are trademarks of Astoria-Pacific, Inc., Clackamas, Oregon

Parafilm is a registered trademark of American National Can, Norwalk, CT

Contrad[®]NF and Neutrad[®] are registered trademarks of Decon Labs., Inc., Bryn Mawr, Pennsylvania

Standard Operating Procedure 004 Ortho-Phosphate Analysis

Date: Nov 2007

Revision: 2

Primary Method: EPA 365.5

A. Scope and Application

This method is used for the determination of ortho-phosphate (dissolved reactive phosphate) in seawater. The applicable range of this method is 0.02 – 3.0 μM phosphate. However, this method is also applicable to other ranges.

B. Summary of Method

Ortho-phosphate reacts with molybdenum (VI) and antimony (III) in an acidic medium to form a phosphoantimonylmolybdenum complex. This complex is subsequently reduced by ascorbic acid to a heteropolyblue with an absorbance maximum at 880 nm.

C. Sample Handling and Preservation

All samples will be filtered using 0.4 μm Nucleopore filters. Samples must be kept frozen until analysis. Holding time should not exceed 28 days.

D. Raw Materials Required

NOTE: Chemicals should be of ACS grade or equivalent.

Ammonium Molybdate, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ (FW 1235.86)

Antimony Potassium Tartrate, $\text{K}_2\text{Sb}_2\text{C}_8\text{H}_4\text{O}_{12}\cdot 3\text{H}_2\text{O}$ (FW 667.87)

Ascorbic Acid, $\text{C}_6\text{H}_8\text{O}_6$ (FW 176.13)

Deionized Water (ASTM Type I or Type II)

Low Nutrient Seawater (LNSW)*

Magnesium Sulfate $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ (FW 246.48)*

Potassium Dihydrogen Phosphate, KH_2PO_4 (FW 136.09)

Sodium Bicarbonate NaHCO_3 (FW 84.01)*

Sodium Chloride NaCl (FW 58.44)*

Sodium Lauryl Sulfate, $\text{C}_{12}\text{H}_{25}\text{O}_4\text{SNa}$ (FW 288.38)

Sulfuric Acid Concentrated, H_2SO_4 (FW 98.08)

* See operating notes for information on matrix choices.

E. Reagent Preparation**1. Sodium Lauryl Sulfate (SLS), 15% w/w**

Deionized Water	85 ml
Sodium Lauryl Sulfate (SLS).....	15 g
$C_{12}H_{25}O_4SNa$ (FW 288.38)	

Dissolve 15 g SLS in 85 ml deionized water contained in a 250 ml Erlenmeyer flask. Gentle warming may be needed for complete dissolution.

2. Sulfuric Acid, 5 N (1000 ml)

CAUTION: Mixing sulfuric acid with water generates a great amount of heat.

Sulfuric Acid, concentrated.	140 ml
H_2SO_4 (FW 98.08)	
Deionized Water	

Cautiously add 140 ml concentrated sulfuric acid to 600 ml deionized water contained in a 1000 ml Erlenmeyer flask. Cool to room temperature and transfer to a 1000 ml volumetric flask. Dilute to the mark with deionized water.

3. Antimony Potassium Tartrate (50 ml)

Antimony Potassium Tartrate	0.15 g
$K_2Sb_2C_8H_4O_{12} \cdot 3H_2O$ (FW 667.87)	
Deionized Water	

Dissolve 0.15 g antimony potassium tartrate in 40 ml deionized water contained in a 50 ml volumetric flask. Dilute to the mark with deionized water. Store at 2-8° C in a dark bottle.

4. Ammonium Molybdate (150 ml)

Ammonium Molybdate	6 g
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (FW 1235.86)	
Deionized Water	

Dissolve 6 g ammonium molybdate in 75 ml deionized water. Add deionized water to final volume of 150 ml and mix well. Store at 2-8 °C in a polyethylene bottle.

5. Ascorbic Acid (300 ml)

Ascorbic Acid	5.4 g
---------------------	-------

C₆H₈O₆ (FW176.13)
Deionized Water

Dissolve 5.4 g ascorbic acid in 150 ml of deionized water. Add deionized water to a final volume of 300 ml and mix well. Stable for 10 days if stored at 2 – 8 °C.

6. Color Reagent (100 ml)

Sulfuric Acid, 5 N.....	50 ml
Antimony Potassium Tartrate	5 ml
Ammonium Molybdate.....	15 ml
Ascorbic Acid	30 ml

Add reagents in the order stated and mix after each addition. Add 3 – 5 ml SLS and filter to 0.45 µm. Prepare reagent daily.

7. Artificial Seawater (ASW) (4 L) (See Operating Notes)

Sodium Chloride	128.5 g
NaCl (FW 58.44)	
Magnesium Sulfate	28.5 g
MgSO ₄ ·7H ₂ O (FW 246.48)	
Sodium Bicarbonate.....	0.672 g
NaHCO ₃ (FW 84.01)	
Deionized Water	

Dissolve 128.5 g sodium chloride, 28.5 g magnesium sulfate and 0.672 g sodium bicarbonate in about 3 liters of deionized water. Dilute to 4 liters with deionized water. These reagents must be high quality, reagent grade to avoid excessive nutrient or trace metal contamination.

8. Startup/Shutdown Solution (100 ml)

Deionized Water	100 ml
SLS, 15%	2 ml

9. Sampler Wash Solution

See Operating Note #1.

F. Calibrants

See Nitrate + Nitrite Analysis SOP for information on calibrant preparation.

G. Operation Procedure

1. Set up the cartridge as shown in the flow diagram. Check all tubing and connections. Replace if necessary.
2. Place reagent lines in startup solution.
3. Turn on power to all units including heat bath and latch pump platens to begin liquid flow.
4. Verify that the bubble size and spacing is consistent throughout the cartridge. If bubbles are splitting up as they enter or exit a coil or heat bath, check and replace fittings if necessary. The bubbles should flow smoothly without dragging. If dragging occurs, add more SLS to the startup solution.
5. Check all reagent containers on the instrument for particulate matter. Reagents should be filtered weekly. Be sure all containers are properly labeled and filled before pumping reagents.
6. After the heat bath has reached the desired temperature and a stable baseline has been verified on the startup solution, place reagent lines in reagent bottles.
7. If using data collection software, set up the appropriate sample table.
8. Allow reagents to run for 5 to 10 minutes and verify a stable baseline.
9. Load the sampler tray with calibrants, blanks, samples, and QC or monitor samples.
10. Select the appropriate parameters for the detector and sampler. (See Flow Diagram.)
11. Begin analysis.
12. At the end of analysis place all reagent lines in startup/shutdown solution and turn off the heat bath. Pump startup/shutdown solution for 20 to 30 minutes to flush all of the reagents out of the cartridge and to allow the heat bath to cool.
13. Turn off the power to all units and release pump platens.

H. Operating Notes

See Nitrate + Nitrite Analysis SOP for information on sample matrices.

If the flowrate of the sample pump tube is ≤ 226 $\mu\text{l}/\text{minute}$ (a blk/blk pump tube) a helper line must be added when the cartridge is run alone. See Section 9 of the Astoria Analyzer Operation Manual for information on how to add a helper line.

NOTE: If the sample line is debubbled, a helper line is not necessary.

1. Low sensitivity and noise in the baseline can be caused by debris in the flowcell. Particulate matter from the reagents and samples can become lodged in the flowcell restricting the amount of light that is passed through the flowcell. Flushing the flowcell with approximately 10 ml of sampler wash solution with a syringe will dislodge any debris in the flowcell. Following proper filtration procedures for the reagents and samples will reduce the frequency of this occurring.
2. To prevent the accumulation of background contamination forming in the color reagent, keep the reagent bottle covered at all times. Baseline drift may also be reduced by placing the color reagent in an ice bath during analysis.
3. If increased carryover and drift are experienced, make sure the ascorbic acid and ammonium molybdate solutions are fresh.
4. If bubbles are sticking in a debubbler, cleaning the debubbler will allow bubbles to escape smoothly out the debubble line. Bubbles sticking in the debubbler can cause a loss in the overall precision of the peak height. To clean, soak the debubbler for 2-3 hours in a mixture of 20-30% Contrad[®]NF (API p/n 80-0007-04) and hot tap water. Rinse thoroughly.
5. For chronic carryover and drift problems, the following cleaning solution can be used to flush the analytical cartridge and flowcell.

Potassium Iodide Cleaning Solution (55 ml)

Potassium Iodide	1 g
KI (FW 166.00)	
5 N Sulfuric Acid (See Reagent Preparation)	25 ml
H ₂ SO ₄ (FW 98.08)	
Deionized Water	30 ml

Add 1 g KI to about 25 ml 5 N sulfuric acid. Stir vigorously until a strong yellow-orange color has formed. This may take at least one hour. Add about 30 ml deionized water. The solution will darken over time, and is usable for one month. Pump the cleaning solution through all lines in the cartridge for 10 to 15 minutes, followed by startup/shutdown solution.

6. Acid washed glassware should be used for all reagents and calibrants. Commercial detergents containing phosphorus should never be used to clean glassware used in phosphorus determination. Wash the glassware with 1:1 hydrochloric acid and rinse it thoroughly with deionized water. Store the glassware filled with deionized water. If the glassware is reserved for use only in phosphorus determination, treatment with hydrochloric acid is necessary only occasionally.

I. References

1. Standard Methods for the Examination of Water and Wastewater: Centennial Edition, 21st Ed., 2005, American Public Health Association, Washington, D.C. (Method referenced:

- Automated Ascorbic Acid Reduction, 4500-P-F, pp. 4-155 – 4-156).
2. Methods for Chemical Analysis of Water and Wastewater, March 1984, EPA-600/4-79-020, "Sample Preservation", p. xvii, Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency Cincinnati, OH 45286.
 3. Methods and Guidance for Analysis of Water, 1999, Office of Water, Environmental Protection Agency (USEPA), Cincinnati, OH (Method referenced: 365.5)
 4. Automated Nutrient Analysis in Seawater, Technical Report, Brookhaven National Laboratory, Whiteledge, Veidt, et. al., May 1986.

ACKNOWLEDGEMENTS

Astoria® and FASPac™ are trademarks of Astoria-Pacific, Inc., Clackamas, Oregon

Dowfax™ 2A1 is a trademark of Dow Chemical, USA

Contrad®NF and Neutrad® are registered trademarks of Decon Labs., Inc., Bryn Mawr, Pennsylvania

Standard Operating Procedure 005 Ammonia

Date: Nov 2007

Revision: 2

Primary Method: EPA 350.1

A. Scope and Application

This method is used for the determination of Ammonia in seawater and is applicable to many ranges.

B. Summary of Method

Ammonia reacts with alkaline phenol and hypochlorite to form indophenol blue. Sodium nitroferricyanide intensifies the blue color formed which is measured at 640 nm.

C. Sample Handling and Preservation

All samples will be filtered using 0.4 μm Nucleopore filters. Samples must be kept frozen until analysis. Holding time should not exceed 28 days.

D. Raw Materials Required

NOTE: Chemicals should be of ACS grade or equivalent.

Ammonium Sulfate $(\text{NH}_4)_2\text{SO}_4$ (FW 132.15)

Brij[®]-35, 30% w/v (API p/n 90-0710-04)

Chloroform, CHCl_3 (FW 119.38)

Deionized Water (ASTM Type I or II)

Low Nutrient Seawater (LNSW)*

Magnesium Sulfate $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (FW 246.48)*

Nitrogen gas source

Phenol, liquefied 88% $\text{C}_6\text{H}_5\text{OH}$ (FW 94.11)

Potassium Sodium Tartrate $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ (FW 282.23)

Sodium Bicarbonate NaHCO_3 (FW 84.01)*

Sodium Chloride NaCl (FW 58.44)*

Sodium Citrate $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ (FW 294.11)

Sodium Hydroxide NaOH (FW 40.00)

Sodium Hypochlorite NaOCl 5.25% (household bleach)

Sodium Nitroferricyanide $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$ (FW 297.97)

* See Operating Notes for information on matrix choices.

E. Reagent Preparation

All reagents and calibrants are prepared with ammonia-free deionized or distilled water. See Operating Notes for preparation of ammonia-free deionized water.

1. Stock Complexing Reagent (1 L)

Sodium Citrate	140.0 g
Na ₃ C ₆ H ₅ O ₇ •2H ₂ O (FW 294.11)	
Potassium Sodium Tartrate	24.0 g
KNaC ₄ H ₄ O ₆ •4H ₂ O (FW 282.23)	
Sodium Hydroxide	5.0 g
NaOH (FW 40.00)	
Deionized Water	

Dissolve 140.0 g of sodium citrate, 24.0 g of potassium sodium tartrate and 5.0 g of sodium hydroxide in approximately 800 ml of deionized water contained in a 1 L volumetric flask. Cool and dilute to the mark with deionized water. Filter to 0.45 µm. Prepare at least one day in advance to help reduce background noise.

2. Working Complex Reagent (200 ml)

Stock Complexing Reagent	200 ml
Brij-35 (30% w/v)	0.5 ml (20 drops)

Add 20 drops of Brij-35 for each 200 ml of complexing reagent.

3. Stock 0.125 N Sodium Hydroxide (1 L)

Sodium Hydroxide	5.0 g
NaOH (FW 40.00)	
Deionized Water	

Add 5.0 g of sodium hydroxide to approximately 700 ml of deionized water contained in a 1 L volumetric flask. When the solution is cool, dilute it to the mark with deionized water and filter to 0.45 µm. Store in a tightly sealed plastic container.

4. Sodium Nitroferricyanide (1 L)

Sodium Nitroferricyanide	0.5 g
Na ₂ Fe(CN) ₅ NO•2H ₂ O (FW 297.97)	
Deionized Water	

Add 0.5 g of sodium nitroferricyanide to approximately 800 ml of deionized water contained in a 1 L volumetric flask. Dilute the solution to the mark with deionized water and filter to 0.45 µm. Store this solution in an amber bottle at room temperature where it is stable for at least 1 month.

5. Phenol/Nitroferricyanide

Sodium Nitroferricyanide solution	100 ml
Phenol, liquefied	2.0 ml
Phenol C ₆ H ₅ OH (FW 94.11) liquefied 88%	

Add 2.0 ml of liquefied phenol to 100 ml of the sodium nitroferricyanide solution. Swirl gently until dissolved. The resulting solution should be a light straw color. Store the reagent in a brown glass bottle and refrigerate at 2-8°C. Discard the reagent if it becomes dark amber in color. If necessary, filter the reagent to 0.45 µm with a syringe filter.

6. Sodium Hypochlorite (50 ml)

0.125 N Sodium Hydroxide	50 ml
Sodium Hypochlorite Solution	1.0 ml
NaOCl, 5.25% solution (household bleach)	

Add 1.0 ml of sodium hypochlorite solution to 50 ml of 0.125 N sodium hydroxide. This reagent is not stable; prepare it daily.

7. Sampler Wash Solution for Ammonia Analysis

See Operating Notes

8. Artificial Seawater (ASW) (4L) (See Operating Notes)

Sodium Chloride	128.5 g
NaCl (FW 58.44)	
Magnesium Sulfate	28.5 g
MgSO ₄ •7H ₂ O (FW 246.48)	
Sodium Bicarbonate	0.672 g
NaHCO ₃ (FW 84.01)	
Deionized Water	

Dissolve 128.5 g of sodium chloride, 28.5 g of magnesium sulfate and 0.672 g of sodium bicarbonate in about 3 liters of deionized water. Dilute to 4 liters with deionized water. These reagents must be high quality, reagent grade to avoid excessive nutrient or trace metal contamination.

9. Startup/Shutdown Solution (1L)

Deionized Water	1 L
Brij 35, 30%	1 – 2 ml

Add 1 to 2 ml of Brij-35 to 1000 ml of deionized water. Mix well.

F. Calibrants

See Nitrate + Nitrite Analysis SOP for information on calibrant preparation.

G. Operation Procedure

1. Set up the cartridge as shown in the flow diagram. Check all tubing and connections. Replace if necessary.
2. Place reagent lines in startup solution.
3. Turn on power to all units including heat bath and latch platens to begin liquid flow.
4. Verify that the bubble size and spacing is consistent throughout the cartridge. If bubbles are splitting up as they enter or exit a coil or heat bath, check and replace fittings if necessary. The bubbles should flow smoothly without dragging. If dragging occurs, add more Brij-35 to the startup solution.
5. Check all reagent containers on the instrument for particulate matter. Reagents should be filtered. Be sure all containers are properly labeled and filled before pumping reagents.
6. After the heat bath has reached the desired temperature and a stable baseline has been verified on the startup solution, place reagent lines in reagent bottles. Allow the complex reagent to reach the cartridge first, before the other reagents or the seawater matrix.
7. If using data collection software, set up the appropriate sample table.
8. Allow reagents to run for 5 to 10 minutes and verify a stable baseline.
9. Load the sampler tray with calibrants, blanks, samples, and QC or monitor samples.
10. Select the appropriate parameters for the detector and sampler. (See Flow Diagram.)
11. Begin analysis.
12. At the end of analysis place all reagent lines in startup solution and turn off the heat bath. Pump startup solution for 20 to 30 minutes to flush all of the reagents out of the cartridge and to allow the heat bath to cool.
13. Turn off the power to all units and release pump platens.

H. Operating Notes

A. Standards

Primary standards should be prepared from the best grade of chemicals available. Certificates of Analysis are available from the chemical manufacturer. These should be consulted to identify impurities.

Standard material should be oven dried for two hours at 110°C before weighing.

It is advisable to periodically verify the concentrations of the working standards. This can be done by running standards against standards from an outside source.

The matrix of the standards should be consistent with that of the samples. If deionized water standards are used it becomes important to determine the salt effects of each individual test.

B. Matrix

Optimal system performance can be expected if the sample matrix is carried over into the sampler wash solution and the standards. Care should be taken when using deionized water wash solution with seawater standards. See Nitrate + Nitrite Analysis SOP for information on sample matrices.

1. As stated above, the system is optimal when the sample matrix is carried over into the sampler wash solution and the standards. If this is not possible (i.e. if deionized water is used for the sampler wash and/or calibrants), it is advisable to check for refractive index disturbance effects. This can be accomplished by removing the sodium hypochlorite reagent. After the initial sample run is finished, place the data collection in pause, replace the hypochlorite reagent with deionized water, wait for a stable baseline, autozero the detector and run the samples again. The peaks seen are due to the refractive index disturbance.
2. Prepare ammonia free water by passing distilled water through a mixture of strongly acidic cation and strongly basic anion exchange resins.⁽²⁾
3. To prevent ammonia contamination from the air, segment the analytical stream with nitrogen or draw air through a 5 N sulfuric acid solution.
4. Precipitation following the addition of the phenol/ferricyanide solution may indicate poor reagent quality. Change the source of potassium sodium tartrate and sodium citrate.
3. Precipitation may also occur if the samples being determined contain calcium and/or magnesium in amounts that exceed the capacity of the complexing reagent. In such cases, increasing the amount of sodium citrate in the complexing reagent should alleviate the problem.
4. Clean precipitates from the system by pumping 10% HCl through the sample line and all reagent lines. Wash the system thoroughly by pumping startup solution through the reagent lines before proceeding with analyses.
5. If necessary, approximately 1.6 g to 1.8 g of solid phenol may be substituted for the 2 ml of liquefied phenol.
6. If bubbles are sticking in a debubbler, cleaning the debubbler will allow bubbles to escape smoothly out the debubble line. Bubbles sticking in the debubbler can cause a loss in the overall precision of the peak height. To clean, soak the debubbler for 2-3 hours in a mixture

of 20-30% Contrad[®]NF (API p/n 80-0007-04) and hot tap water. Rinse thoroughly.

7. If the flowrate of the sample pump tube is $\leq 226 \mu\text{l}/\text{minute}$ (a blk/blk pump tube) a helper line must be added when the cartridge is run alone. See Section 9 of the Astoria Analyzer Operation Manual for information on how to add a helper line.

NOTE: If the sample line is debubbled, a helper line is not necessary.

8. Cover all reagents and other solutions to avoid interference due to dust and other particulates. This will also help prevent contamination of the solutions from absorbance of analytes in the air.

I. References

1. Automated Nutrient Analysis in Seawater, Technical Report, Brookhaven National Laboratory, Whiteledge, Veidt, et. al., May 1986.
2. Methods and Guidance for Analysis of Water, 1999, Office of Water, Environmental Protection Agency (USEPA), Cincinnati, OH (Method referenced: 350.1)
3. Standard Methods for the Examination of Water and Wastewater: Centennial Edition, 21st Ed., 2005, American Public Health Association, Washington, D.C. (Method referenced: Automated Phenate Method, 4500-NH₃ G, pp. 4-115 – 4-116).

ACKNOWLEDGMENTS

Astoria[®] and FASPac[™] are trademarks of Astoria-Pacific, Inc., Clackamas, Oregon
Brij[®]-35 is a registered trademark of ICI Americas, Wilmington, Delaware
Contrad[®]NF and Neutrad[®] are registered trademarks of Decon Labs., Inc., Bryn Mawr, Pennsylvania

Standard Operating Procedure 006 TN and TP Analysis

Date: Nov 2007

Revision: 2

Primary Method: USGS Report: 03-4174

A. Scope and Application

This method is used for the determination of Total Nitrogen and Total Phosphorous in seawater and is applicable to many ranges.

B. Summary of Method

Alkaline persulfate digestion oxidizes all forms of inorganic and organic nitrogen to nitrate and hydrolyzes all forms of inorganic and organic phosphorous to ortho-phosphate. After digestion, samples are analyzed as described in SOPs for nitrate/nitrite and for ortho-phosphate.

C. Sample Handling and Preservation

Samples must be kept frozen until analysis. Holding time should not exceed 28 days.

D. Raw Materials Required

NOTE: Chemicals should be of ACS grade or equivalent.

Sodium hydroxide (NaOH, FW=40.0)

Potassium persulfate ($K_2S_2O_8$, FW=270.33)

Sodium bisulfate ($NaHSO_4 \cdot H_2O$, FW=138.08)

Glycine ($C_2H_5NO_2 \cdot HCl$, FW=111.5)

Glycerophosphate ($C_3H_7O_6PNa_2 \cdot 5H_2O$, FW=306.1)

Glucose ($C_6H_{12}O_6$, FW=180.2)

E. Reagent Preparation

1. Sodium Hydroxide, 1.5 M

Sodium Hydroxide	60.0 g
NaOH (FW 40.00)	
Deionized Water	

Dissolve 60.0 g of sodium hydroxide in about 800 mL of DI water in a 1-L volumetric flask [**Caution:** When NaOH dissolves in water, heat is released.] After dissolution is complete, allow the resulting solution to cool and dilute it to the mark with DI water. Transfer this reagent to a plastic bottle in which it is stable at room temperature for 6 months.

2. Alkaline Persulfate Digestion Reagent

Potassium Persulfate18.0 g
 $K_2S_2O_8$ (FW=270.33)
 Sodium Hydroxide, 1.5 M
 Deionized Water

Add 18.0 g of potassium persulfate and 45 mL of 1.5 M sodium hydroxide solution to about 350 mL of DI water in a graduated 500-mL Pyrex media bottle. Cap the bottle, swirl its contents, and place it in an ultrasonic bath until potassium persulfate dissolution is complete (about 10 minutes). Remove the bottle from the ultrasonic bath, dry its outer surfaces, and then add enough DI water to bring the volume to 450 mL. (Make a line on the side of the bottle that indicates this volume to within +/- 5mL.) Swirl the bottle to mix its contents and then divide the resulting solution among four, 125 mL bottles. Prepare this reagent daily.

3. Sampler Wash Reservoir Solution (0.05 M sodium bisulfate)

Sodium Bisulfate.....6.9 g
 $NaHSO_4 \cdot H_2O$ (FW=138.08)
 Deionized Water

Dissolve 6.9 g of sodium bisulfate in about 800 mL of DI water in a graduated 1-L Pyrex media bottle. Dilute this solution to the mark with DI water, mix it well, and store it tightly capped at room temperature

NOTE: This solution matches the matrix of sample digests. Use it as the matrix for continuing calibration verification (CCV) solutions and any other undigested check samples.

4. Glycine Digest-Check Stock Solution

Glycine3.98 g
 $C_2H_5NO_2 \cdot HCl$ (FW=111.5)
 Deionized Water

Dissolve 3.98 g glycine in about 400 mL of DI water in a 500 mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer the stock digest-check solution to a 500 mL Pyrex media bottle in which it is stable for 6 months at 4°C.

5. Glycerophosphate Digest-Check Stock Solution

Glycerophosphate1.976 g
 $C_3H_7O_6PNa_2 \cdot 5H_2O$ (FW=306.1)
 Deionized Water

Dissolve 1.976 g glycerophosphate in about 400 mL of DI water in a 500 mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and

shaking. Transfer the stock digest-check solution to a 500 mL Pyrex media bottle in which it is stable for 6 months at 4°C.

6. Glucose Digest-Check Stock Solution

Glucose1.564 g
 $C_6H_{12}O_6$ (FW=180.2)
 Deionized Water

Dissolve 1.564 g glucose in about 400 mL of DI water in a 500 mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer the stock digest-check solution to a 500 mL Pyrex media bottle in which it is stable for 6 months at 4°C.

7. Mixed Digest-Check Solution

Glycerine digest-check stock solution 1 mL
 Glycerophosphate digest-check stock solution..... 1 mL
 Glucose digest-check stock solution..... 10 mL
 Deionized Water

Dispense 1 mL each of glycine and glycerophosphate stock digest-check solutions, 10 mL of the glucose digest-check stock solution into a 250 mL volumetric flask that contains about 200 mL of DI water. Dilute the contents of the flask to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer the mixed digest-check solution to a 250 mL Pyrex media bottle in which it is stable for 1 month at 4°C.

F. Sample Preparation

Alkaline persulfate digests are prepared by dispensing samples and digestion reagent into 30-mL, screw-cap, Pyrex culture tubes in the volume ratio of 2 + 1 (i.e. 10.000 mL sample, 5.000 mL digestion reagent). All tubes are capped tightly and mixed thoroughly either by manual inversion (three times) or with a vortex mixer (3, 5 second cycles). The capped tubes are placed in an autoclave where they are digested at 121°C and 117 kPa for 1 hour.

When the digestion cycle is complete and pressure and temperature gages on the autoclave indicate 0 kPa and less than 80°C, remove the alkaline persulfate digests from the autoclave and allow them to cool sufficiently to be handled comfortably. Then mix the contents of each capped digestion tube by manual inversion (three times) or with a vortex mixer (3, 5 second cycles). Wait about 1 hour after mixing for any suspended solids to settle. If it is not possible to decant or pipet a clear supernatant solution from digest tubes into analyzer cups, then suspended solids must be removed by 0.45 µm filtration prior to colorimetric analysis. Note that the tightly capped digests can be stored at room temperature for several days before their nitrogen and phosphorous concentrations are determined as described in SOPs for nitrate/nitrite and ortho-phosphate.

G. Operation Procedure

Follow procedure described in SOPs for nitrate/nitrite and ortho-phosphate.

H. References

1. Patton, C.J. and J.R. Kryskalla. (2003). Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory – Evaluation of Alkaline Persulfate Digestion as an Alternative to Kjeldahl Digestion for Determination of Total and Dissolved Nitrogen and Phosphorous in Water. Water-Resources Investigations Report 03-4174. Denver, Colorado.

Standard Operating Procedure 007 Chlorophyll a Analysis

Date: Nov 2007
Revision: 2
EPA Method: 445.0

Field Sample Filtration: Generally two replicate samples are filtered from each water sample within 2 hours of sample collection. If filtration cannot take place immediately after sample collection, water samples must be refrigerated. In subdued light water samples are filtered through 0.7 um glass fiber filters (Whatman GF/F). Individual filters are folded in half, wrapped first in blotting filters (for example a piece of white paper towel or white coffee filter), and then in squares of aluminum foil. These foil packs are then placed in a Whirlpak labeled with the following information: location, date, amount of water filtered. The filters are kept frozen until just prior to extraction and analysis.

Lights must be kept off during analysis and preparations. Lighting should be the minimum that is necessary to read instructions and operate fluorometer.

Extraction Procedure: A glass fiber filter, through which has been filtered a known aliquot of water, is placed in a glass 16 x 150 mm test tube containing 10 ml of 90% acetone. The vial is capped, shaken vigorously and allowed to steep 18 - 24 hours at 4 deg. C. Prior to analysis the rack of vials is brought to room temperature.

Instrumentation: Turner Trilogy Laboratory Fluorometer

Calibration: The fluorometer is calibrated at the beginning of each monitoring season with 2 liquid pure chlorophyll a standards and reagent blanks following the manufacturer's instructions. At the time of calibration a solid secondary standard is also analyzed and the formula for calculating chlorophyll a in samples is determined. That solid standard is analyzed with each batch of samples. When sample concentration is calculated an allowance for instrument drift is made using the daily batch readings of the solid standard. Blanks of 90% acetone, and an unused filter extracted with 90% acetone are set up with each rack of samples.

Cuvettes: 12x35 mm glass test tubes

Fluorometer Operation:

1. Make sure Trilogy is off.
2. Insert the Chlorophyll (acidification) Optical Application Module
3. Close lid and turn power on. Use the touch screen to identify the type of Optical Application Module installed.

Procedure:

1. The fluorometer should be on with Home screen displayed.
2. Select Chl A (chlorophyll, acidification)
3. In raw fluorescence mode, measure a blank (90% acetone) and the secondary standard. Record on data sheet.

4. On the screen, select Mode to switch to the calibration mode.
5. Use stored calibration – select the most recent stored calibration.
6. Samples: After the 24 hr extraction is complete, be sure cap is tightly sealed, and invert test tube several times to mix contents. Work in dim light; wear surgical gloves to keep acetone from your hands.
7. Remove cap, pour 5 ml into sample cuvette.
8. Check cuvette to be sure there are no finger prints on glass; polish with a Kimwipe if necessary.
9. Open the lid and insert the test tube. Close the lid.
10. Touch Measure Fluorescence to commence measurement. The sample will be measured for 6 seconds and report the average reading for the sample.
11. Remove sample, add 0.15 mL of 0.1 N HCl, cover and mix well. Wipe cuvette again with Kimwipe. Wait 90 sec and read a second time.
12. Record chlorophyll *a* and pheophytin *a* values on data sheet.
13. When finished measuring samples, switch to raw fluorescence mode and measure blank and secondary standard again. Record on data sheet.
14. Dispose of all acetone in Acetone Waste container. Rinse with tap water
15. Soak all test tubes and stoppers in soapy water (Liqui Nox) for 24 hours.
16. Rinse well with tap water and soak in DI water for 24 hours.
17. Rinse 3 times with DI water and dry in test tube rack.

Calculations: When in direct concentration mode the following calculations will be use to calculate corrected chlorophyll *a* and pheophytin values.

Variables stored during calibration phase of fluorometer

$C_{\text{stand}[1]}$ = Concentration of standard 1

F_{blank} = Fluorescence of Blank value

$F_{\text{stand}[1],B}$ = Fluorescence of standard 1 before acidification

$F_{\text{stand}[1],A}$ = Fluorescence of standard 1 after acidification

F_m = Acidification Ratio = $(F_{\text{stand}[1],B} - F_{\text{blank}}) / (F_{\text{stand}[1],A} - F_{\text{blank}})$

Variables required from the sample analysis phase

$F_{\text{samp},B}$ = Fluorescence of sample before acidification

$F_{\text{samp},A}$ = Fluorescence of sample after acidification

V_{solvent} = Volume of solvent used to extract sample

V_{water} = Volume of water filtered

Interpolation equation used in end calculation of chlorophyll *a* and pheophytin concentrations

$$\text{Interp}_{,B} = C_{\text{stand}[1]} * (F_{\text{samp},B} - F_{\text{blank}}) / (F_{\text{stand}[1],B} - F_{\text{blank}})$$

$$\text{Interp}_{,A} = C_{\text{stand}[1]} * (F_{\text{samp},A} - F_{\text{blank}}) / (F_{\text{stand}[1],B} - F_{\text{blank}})$$

End calculation for corrected chlorophyll and pheophytin *a*

$$\text{Chlorophyll a concentration} = [F_m / (F_m - 1)] * (\text{Interp}_{,B} - \text{Interp}_{,A}) * (V_{\text{solvent}} / V_{\text{water}})$$

$$\text{Pheophytin a concentration} = [F_m / (F_m - 1)] * [(F_m * \text{Interp}_{,A}) - \text{Interp}_{,B}] * (V_{\text{solvent}} / V_{\text{water}})$$

References:

EPA Method 445.0

Standard Methods for the Examination of Water and Wastewater: Centennial Edition, 21st Ed.,
Method 10200H, pp. 10-19 – 10-26 (2005).

Turner Designs Trilogy Laboratory Fluorometer User's Manual

[www.turnerdesigns.com/t2/doc/manuals/TrilogyUsersManual.pdf]

Standard Operating Procedure 008 Phytoplankton Collection and Analysis

The following methods for the collection, identification, and enumeration of phytoplankton species are similar to those described in Libby et al. (2002, 2005, 2006, and 2010) and have been used in Massachusetts Water Resources Authority Harbor Outfall Monitoring Projects HOM3 through HOM6. Thus data from current efforts will be comparable to data sets of previous years.

Collection

A Niskin bottle will be used to collect surface water at a depth of 1-2 meters at each station. A portion of the water from the Niskin sampling bottle (800 mls) will be measured with into a clean graduated cylinder that has been thrice-rinsed with the sampling bottle water. The sample will be mixed with Utermöhl's solution (1 ml Utermöhl's per 100 ml water sampled) in a 1-L HDPE bottle and stored in a cooler until it can be further processed in the lab. Utermöhl's solution is prepared as described in Guillard (1973): 100 g potassium iodide, 50 g iodine, and 50 g sodium acetate each are dissolved incrementally in distilled water to a final volume of 1 L.

Sample Preparation

At the laboratory, the Utermöhl's-preserved whole seawater samples will be prepared for analysis by concentrating the sample by gravitational settling as described by Borkman (1994), Borkman *et al.* (1993), and Turner *et al.* (1995). Samples will be settled in glass graduated cylinders with no more than a 5-to-1 height-to-width ratio. The preserved samples are stored at ambient temperature and in the dark until analysis. Prior to analysis, the 800 ml of seawater will be settled to 50 ml and decanted by low vacuum aspiration.

Counting and Abundance Estimates

One ml of the concentrate will be examined and phytoplankton counted in a gridded Sedgwick-Rafter chamber using a Olympus BH-2 research microscope with phase contrast optics.

Phytoplankton abundance is calculated by dividing the number of cells counted by the volume examined in a gridded Sedgwick-Rafter chamber. The theoretical maximum possible volume that would be examined would be an entire Sedgwick-Rafter chamber (1 ml). The grid subdivides the chamber into μl divisions so that if an entire chamber is not counted, an exact volume can still be determined. Typical volumes counted are one row of the chamber (50 1- μl cells or 1/20 of 1 ml). The volume of sample examined is dependent on number of cells encountered and how long it takes to reach cut-offs of 75 entities (unicellular forms, colonies, or chains) of each of the top 3 taxa, and 400 entities total. Calculation of abundance also accounts for the concentration factor used in the settling process. Normally, the volume processed is 800 ml of whole-water sample, settled to 50 ml of concentrate, for a 16:1 ratio.

The following equation results in the abundance estimate for cells counted:

$$C * [V_s / V_c] [1000 / V_{TOT}] = \text{cells/ L.}$$

where C = cells counted
 V_S = Volume of concentrated sample
 V_C = Volume of sample examined
 V_{TOT} = Original volume

Final abundance estimates will be reported in units of 10^6 cells per liter.

References

- Borkman, D. 1994. Phytoplankton and Nutrients in Buzzards Bay, Massachusetts 1987-1988. M.S. Thesis. University of Massachusetts Dartmouth, Dartmouth, MA. 203 pp.
- Borkman D, RW Pierce, JT Turner. 1993. Dinoflagellate blooms in Buzzards Bay, Massachusetts. Pp. 211-216 in Smayda, T.J., and Y. Shimizu (Eds.), *Proceedings of the Fifth International Conference on Toxic Marine Phytoplankton*, Elsevier.
- Guillard RRL. 1973. Division rates. Pages 289-311 In: J.R. Stein, (Ed.) *Phycological Methods*. Cambridge Univ. Press.
- Libby PS, Gagnon C, Albro C, Mickelson M, Keller AA, Borkman D, Turner JT, Oviatt CA. 2002. Combined work/quality assurance plan for baseline water quality monitoring: 2002-2005. Boston: Massachusetts Water Resources Authority. Report ENQUAD ms-074. 79 p.
- Libby PS, Gagnon C, Albro C, Mickelson M, Keller A, Borkman D, Turner J, Oviatt CA. 2005. Combined work/quality assurance plan for baseline water quality monitoring: 2004-2005. Boston: Massachusetts Water Resources Authority. Report ENQUAD ms-074 Version 1. 76 pp + apps.
- Libby PS, Mansfield A, Buhl R, Lescarbeau G, Leo W, Keller AA, Borkman DG, Turner JT and Oviatt CA. 2006. Combined work/quality assurance project plan (QAPP) for water column monitoring 2006 - 2007, tasks 4, 5, 6, 7, 8, 11. Boston: Massachusetts Water Resources Authority. Report 2006-03. 119 p.
- Libby S, Fitzpatrick M, Buhl, R, Lescarbeau G, Leo W, Borkman D, Turner J, Oviatt CA. 2009b. Quality assurance project plan (QAPP) for water quality monitoring: 2008-2009, Revision 1. Boston: Massachusetts Water Resources Authority. Report 2008-02. 92 p.
- Libby PS, Fitzpatrick MR, Buhl RL, Lescarbeau GR, Leo WS, Borkman DG, Turner JT, Oviatt CA. 2010. Quality Assurance Project Plan (QAPP) for water column monitoring 2010: Tasks 4-9 and 13. Boston: Massachusetts Water Resources Authority. Report 2010-2. 105 p.
- Turner JT, DG Borkman, RW Pierce. 1995. Should Red Tide Dinoflagellates be Sampled Using Techniques for Microzooplankton Rather than Phytoplankton? Pp. 737-742 in P. Lassus *et al.* (Eds.), *Harmful Marine Algal Blooms*, Lavoisier, Paris, France.

Standard Operating Procedure 009 Zooplankton Collection and Analysis

Collection

At each station, zooplankton is collected from the upper 19 meters of the water column. Samples are collected using standard 333-micrometer (μm) mesh conical nets fitted with General Oceanics helical flow meters. The flow meters are calibrated at the beginning of each year to attain the most accurate correction constant possible. The 333 μm mesh has been experimentally determined to represent the filtering ability of right whale baleen; however, any mesh size can be used to sample with these protocols.

Water column collections are initiated by vertically dropping a 60 cm-diameter net on-station. When the net has dropped the full 19 meters, the net is pulled obliquely through the water column by the boat until a mark on the rope reaches the surface, indicating that the net is now horizontal at the surface of the water column. At this point, the net is retrieved.

Once on board, the samples are washed from the nets carefully with a sea-water hose. The sample is concentrated into the bottom of the net to the collection bucket. From there it is concentrated further into a 333 μm mesh fluorette (PVC piping with 333 micron mesh at one end, 11.5 cm inside diameter, 9.5 cm length). This is rinsed into a sample jar and preserved with 10% buffered formalin. Samples are then placed into a cooler for the remainder of the cruise.

Subsampling the zooplankton net samples

If the sample is sparse enough, the entire sample is enumerated. Usually, the sample will have to be subsampled. To enumerate and identify the zooplankton in a net sample that is to be subsampled, all zooplankton are poured into a small fluorette (PVC piping with 333 micron mesh at one end, 5 cm inside diameter, 12cm length) and rinsed with fresh water to remove excess formalin. Once well rinsed, the entire sample is rinsed with fresh water in a squirt bottle into a glass beaker. The goal is to subsample 200 organisms in 5 ml of sample, so an appropriately sized beaker is approximated depending on the density and size of the organisms in the sample. Water is added to increase the volume of the entire sample to a known volume, read off the beaker. Again, this volume is approximated to get at least 200 organisms in 5 ml of sample. The lowest total volume used is 125 ml, to prevent poor subsampling. A Hensen-Stempel® Pipette is used to first stir the contents of the beaker, suspending and equally distributing the sample throughout the beaker, and then to subsample 5 ml of the sample from the middle of the beaker. The 5 ml is transferred directly into a glass watch-glass and the pipette's sample chamber is rinsed with fresh water from a squirt bottle, into the watch-glass, to ensure that no organisms from the subsample are lost. The sample is now ready to be enumerated and identified.

Sample Splitting

If the zooplankton net sample is too large to be sub-sampled in such a way that there will be approximately 200 organisms per 5 ml of sample, the sample must be split; for this a Folsom plankton splitter is used. First, the splitter is leveled by adjusting its legs. The sample is poured from a glass beaker (see steps above for transferring the sample into the glass beaker) into the opening in the main part of the splitter, oriented so that when it is rotated downward, the sample is split and exits the holes in the splitter, into the holding chambers. As the splitter is rotated, a squirt bottle is used to be sure that all organisms are included. Half of the split sample can now be put back into a beaker. If the sample is still too dense, the process is repeated.

Sample Enumeration and Identification

Once the desired subsample is in the watch-glass, it is placed under a Leica L2® dissecting microscope. Light source and zoom adjustments are dependent on the personal preferences of the individual counting and identifying the organisms. An Interface Systems® 20 channel digital counter is used to enumerate the organisms as they are identified. A number of keys and guides to Gulf of Maine zooplankton are used (see “zooplankton identification references”). 35 types of organisms are identified to a particular taxonomic level and some are staged (see zooplankton lab datasheet for details). As the individual identifying and enumerating counts an organism, he or she moves it aside so that no organism is counted more than once. If organisms are floating, they can be removed and then counted after the rest of the sample has been finished, for ease of accurate enumeration and identification. Once all organisms in the subsample have been identified, enumerated, and recorded with the digital counter (and paper should there be more than 20 taxa identified), the data on the digital counter are recorded onto the zooplankton lab datasheet. If less than 200 organisms have been found to make up the subsample, another 5 ml subsample is taken, processed, and added to the previous subsample counts; this may be done as many times as necessary, unless the volume of the sample being subsampled is no longer enough in which to completely submerge the pipette chamber. If this occurs, the individual enumerating must begin again. When taking additional subsamples, no water or organisms can be added to the total sample being subsampled. Upon counting 200 organisms or more, the recorded subsample volume counted is adjusted to reflect the number of ml counted. After the count has been completed and recorded, the entire sample is transferred back into a fluorette and then back into the sample jar. Formalin is added in a volume dependent on the sample jar size (Table 1), and water is added to fill the sample jar to the top. The point of contact between the sample jar and sample lid is wrapped in Parafilm® and the sample is archived.

Zooplankton Density Calculations

The following formula is used for calculating the org/m³ from the oblique net tow count data:

$$\text{Org/m}^3 = O \times (V_T/V_C) \times [1/(m_E - m_S) \times C_N]$$

Where O is the counted organisms, V_T is the total sample volume, V_C is the counted sample volume, m_E is the flow meter end reading, m_S is the flow meter start reading, and C_N is the net constant.

Table 1. Sample jar volume and volume of formalin added for sample archiving

Jar size (ml)	Formalin added (ml)
40	3
75-80	6
175	12
310-325	22

Zooplankton Identification References

Gerber RP. *An Identification Manual to the Coastal and Estuarine Zooplankton of the Gulf of Maine Region*. Freeport: Freeport Village Press, 2000.

Johnson WS and Allen DM. *Zooplankton of the Atlantic and Gulf Coasts*. Baltimore: Johns Hopkins University Press, 2005.

Smith DL and Johnson KB. *A Guide to Marine Coastal Plankton and Marine Invertebrate Larvae*. Dubuque: Kendall/Hunt Publishing Company, 1996.

Todd CD, Laverack MS and Boxshall GA. *Coastal Marine Zooplankton*. Cambridge: Cambridge University Press, 1996.

APPENDIX B
Data Forms

Field Data Sheet: Survey Data

Field Data Sheet: Station Data

Lab Data Sheet: Chlorophyll

Lab Data Sheet: Phytoplankton

Lab Data Sheet: Zooplankton

Survey Data

VESSEL

Alert

Seaway

Shearwater

Date

High Tide

Low Tide

Weather

Sea State

Wind

Time

Begin

End

Comments

Lab Data Sheet: Phytoplankton

							C	Cf
D	c		Bacteriastrium spp.					
D	c		Centric diatom sp. group 1 diam <10 microns					
D	c		Cerataulina pelagica					
D	c		Chaetoceros atlanticus					
D	c		Chaetoceros borealis					
D	c		Chaetoceros compressus					
D	c		Chaetoceros debilis					
D	c		Chaetoceros decipiens					
D	c		Chaetoceros didymus					
D	c		Chaetoceros laciniosus					
D	c		Chaetoceros laciniosus					
D	c		Chaetoceros lauderi					
D	c		Chaetoceros lorentianus					
D	c		Chaetoceros socialis					
D	c		Chaetoceros sp. group 1 diam <10 microns					
D	c		Chaetoceros sp. group 2 diam 10-30 microns					

D	c		Leptocylindrus minimus					
D	c		Lithodesmium undulatum					
D	c		Melosira nummuloides					
D	c		Paralia sulcata					
D	c		Porosira glacialis					
D	c		Pseudosolenia calcar-avis					
D	c		Proboscia alata					
D	c							

D	p	Pennate diatom sp. group 3 31-60 microns length					
D	p	Pennate diatom sp. group 4 61-100 microns length					
D	p						

Dino	Gymno	Gyrodinium sp. group 2 21-40 microns width, 21-50 microns length					
Dino	Gymno	Gyrodinium spirale					
Dino	Per	Heterocapsa rotundata					
Dino	Per	Heterocapsa triquetra					
Dino	Per	Protoperidinium bipes					
Dino	Per	Protoperidinium claudicans					
Dino	Per	Protoperidinium depressum					
Dino	Per	Protoperidinium pallidum					
Dino	Per	Protoperidinium pentagonum					
Dino	Per	Protoperidinium quinquecorne					
Dino	Per	Protoperidinium sp. group 1 10-30 microns width, 10-40 microns length					
Dino	Per	Protoperidinium sp. group 2 31-75 microns width, 41-80 microns length					
Dino	Per	Protoperidinium sp. group 3 76-150 microns width, 54.36-108.72 microns length					

C		Ciliatea (aloricate)					
C		Mesodinium rubrum					
C		Tintinnidae (hyaline)					
C		Tintinnids (agglomerate)					
Chl		Pediastrum spp.					
Chl		Scenedesmus spp.					

Zooplankton Count Sheet

Σ Late						
<i>Pseudocalanus</i> spp.						
<i>Para/Clausocalanus</i>						
<i>Pseudo</i> Early (C1 to C4)						
<i>Pseudo</i> Late (C5 & C6)						
COPEPODS						
<i>Temora longicornis</i>						
<i>Tortanus discuadatus</i>						
<i>Acartia</i> spp.						
<i>Eurytemora</i> spp.						
<i>Oithona</i> spp.						
<i>Metridia</i> spp.						
<i>Paraeuchaeta</i> spp.						
harpacticoids						
other/unidentified copepods						
OTHER ZOOPLANKTON						
Cyprids						
Nauplii						
Chaetognaths						
Cladocera						
Polychaetes						
Larvaceans						

Late larval crustaceans
Mysids
Urchin larvae
Veligers
Medusae
Salps
Ctenophores
Pteropods
Cliones
Ostracods
Other

COMMENTS

APPENDIX C

**Guidance Protocol on the Interaction with Whales Specifically Northern Right Whales for Vessels
Operated/Contracted by the Commonwealth of Massachusetts**

Guidance Protocol on the Interaction with Whales Specifically Northern Right Whales for Vessels Operated/Contracted by the Commonwealth of Massachusetts

Introduction

The northern right whale is the most endangered large whale in the world. In the western north Atlantic the population is estimated to be about 300 animals. Massachusetts coastal waters are part of the range of the northern right whale and Cape Cod Bay has been designated a critical habitat for the whale under the federal Endangered Species Act because of its high use by the species in the late winter and early spring for feeding. The Great South Channel, east of Cape Cod, has also been designated critical habitat because of its importance to the right whale as a feeding area. It has been determined that the most significant human induced causes of mortality are ship strike and entanglements in fishing gear.

Purpose

The purpose of this protocol is to give guidance to the vessels owned by the Commonwealth and those operating under contract to the Commonwealth as to proper operational procedures if the vessels should encounter whales - *i.e.*, sighting and reporting procedures, and entanglement and carcass reporting protocol.

Applicability

This protocol will apply to all vessels owned by the Commonwealth of Massachusetts and/or contracted out by the Commonwealth of Massachusetts.

Geographic Scope/Operational Scope

This protocol applies to all applicable vessels operating in or adjacent to Commonwealth waters. When vessels are operating in the designated critical habitat areas (Cape Cod Bay or the Great South Channel) heightened operation is applicable, especially during the late winter and spring when the right whales are expected to be located in these areas.

Sightings of Right Whales

The Executive Office of Environmental Affairs and the National Marine Fisheries Service is interested in receiving reports from individuals who observe right whales during vessel operations. Reports should be made to the National Marine Fisheries Service Clearinghouse. Patricia Gerrior, NMFS Right Whale Early Warning System Coordinator, who manages the Clearinghouse and her numbers are 508-495-2264 (work), 508-495-2393 (fax) and pager 508-585-8473. Please report your name, agency and phone numbers at which you can be contacted. The vessel's name, the date, time and location of the sighting, the numbers of whales sighted and any other comments that may be of importance. If a camera or video camera is available please take some photographs. These photographs should be provided to Pat Gerrior or Dan McKiernan, Massachusetts Division of Marine Fisheries. They will in turn send copies to the New England Aquarium for comparison to the Right Whale Photo Identification Catalog. **Please remember that Massachusetts has Right Whale Conservation Regulations (322 CMR 12:00) which establishes a 500 yard buffer zone surrounding a right whale. Vessels shall depart immediately from any buffer zone created by the surfacing of a right whale.**

Physical Contact with a Whale

If a vessel owned by the Commonwealth of Massachusetts or under contract with the Commonwealth of

Massachusetts comes into physical contact with any whale it should be noted in the vessel's logbook. The vessel's logbook should include the time and location of the incident; weather and sea conditions; vessel speed; the species of whale struck if known; the nature of any injuries to crew, and/or the whale, and/or damage to the vessel. Also record the name of any other vessels in the area that may have witnessed the incident or can provide information about circumstances. A copy of the vessel's log for the entire trip should be submitted to the Director of the Division of Marine Fisheries, the Director of the Division of Law Enforcement, the Secretary of Environmental Affairs and the National Marine Fisheries Service, Northeast Region in Gloucester.

If after hitting the whale, the animal is incapacitated or appears to have life threatening injuries and the vessel is safe and secure, immediately call the Center for Coastal Studies, entanglement hotline at 800-900-3622 or via their pager at 508-803-0204 and the Massachusetts Environmental Police Communications Center at 800-632-8075 or 617-727-6398. Stay with the whale until the Coast Guard or Center for Coastal Studies arrives on scene.

Entanglements

If the vessel come upon or entangles a right whale immediately notify the Center for Coastal Studies' entanglement hotline at 800-900-3622 or via their pager at 508-803-0204 and the Massachusetts Environmental Police Communications Center at 800-632-8075 or 617-727-6398. Do not attempt to remove any debris from the whale, stay on station with the whale or follow at a safe distance. As relocating an entangled whale can be extremely difficult, staying on station or following the animal is very important. However, if following the whale is not possible contact, the Coast Guard and/or the Center for Coastal Studies and note the last direction the animal was heading and any other pertinent information that would assist in relocating the whale.

Stranded Whales

For a stranded right whale please notify the Stranding Network immediately call Connie Merigo or Howard Krum, New England Aquarium, Central Wharf, Boston, MA 02110. The standing Network's hotline is 617-973-5247 (pager) or as a second resort call 617-973-5246/6551.

QUICK REFERENCE

Sightings & Photographs

Patricia Gerrior, NMFS Right Whale Early Warning System Coordinator, manages the Clearinghouse and her numbers are 508-495-2264 (work), 508-495-2393 (fax) and pager 508-585-8473

Photographs

Dan McKiernan, Massachusetts Division of Marine Fisheries, 19th Floor, 100 Cambridge Street, Boston, MA 02202. 617-727-3193 ext. 369.

Entanglements or Injured whales

Center for Coastal Studies, entanglement hotline at 800-900-3622 or pager at 508-803-0204
Massachusetts Environmental Police Communications Center at 800-632-8075 or 617-727-6398.

Stranded Animals

The standing Network's hotline is 617-973-5247 (pager) or as a second resort call 617-973-5246/6551.



Massachusetts Water Resources Authority
Charlestown Navy Yard
100 First Avenue
Boston, MA 02129
(617) 242-6000
<http://www.mwra.state.ma.us>