

QUALITY ASSURANCE PROJECT PLAN

Ambient Water Quality Monitoring in the Far Western Long Island Sound

Prepared by
Interstate Environmental Commission

May 21st, 2018
Version 5.0

EPA Grant Nos.
LI00A00372

RFA Number 18077

Project Plan Title:

Ambient Water Quality Monitoring in the Far Western
Long Island Sound

Coordinating Organization:

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Effective Date of the Plan:

May 21st, 2018

Timeline:

It is intended that this plan be valid for the duration of EPA Grant No. LI00A00372, which currently extends through September 30, 2018. A request to amend the grant, including an extension of the grant and project period to December 31st, 2019 was submitted to EPA Region 1 on June 1st, 2018.

The QAPP will be reviewed annually and updated as needed. Any significant deviations will be reported.

Plan Description:

This project plan covers Ambient Water Quality Monitoring in the Far Western Long Island Sound.

Approvals:

The undersigned reviewers recommend this Quality Assurance Project Plan (QAPP) for the ambient water quality monitoring in the Far Western Long Island Sound for approval and implementation.

SIGNATURE: Heather Radcliffe DATE: 6/5/2018
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Project Manager
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SIGNATURE: Michael Jennings DATE: 6/5/18
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SIGNATURE: Evelyn Powers DATE: 6/5/18
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SIGNATURE: Leah O'Neill DATE: 6/11/18
Leah O'Neill
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SIGNATURE: Nora J. Conlon DATE: 6/5/2018
Nora Conlon
QA Manager
Office of Environmental Measurement & Evaluation
USEPA, Region 1- New England

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NEIWPCC (IEC District) Laboratory Analytical Standard Operating Procedures included:

Analytical Method	SOP Number	Revision Number	Effective Date
DISSOLVED OXYGEN (Optical Luminescence Based Probe)	Field III	8	March 2016
SALINITY (Electrical Conductivity Method)	FIELD II	3	March 2016
DISSOLVED OXYGEN (Modified Winkler with Full-Bottle Technique)	IX	7	January 2016
pH	XIX	14	January 2018
TEMPERATURE	XXIV	8	March 2016
SECCHI DISK DEPTH	FIELD IV	1	January 2015
TOTAL SUSPENDED SOLIDS (TSS)	XXIIIC	10	June 2017
BIOCHEMICAL OXYGEN DEMAND (BOD)	III	10	August 2017
CHLOROPHYLL <i>a</i>	XXXVII	6	January 2018
NUTRIENT SAMPLE FILTRATION	XXXVIII	3	January 2015
NITRATE, NITRITE, AND TOTAL NITROGEN	XXXXI	3	June 2017
PHOSPHORUS (TOTAL AND DISSOLVED) ORTHOPHOSPHATE	XXXX	3	December 2017 2017
AMMONIA	XXXIX	3	January 2018
SILICATE	XXXXII	2	January 2018

Note: The most current SOP will be used for each parameter during the length of this QAPP. SOP effective dates and revision numbers listed are current as of the date of the preparation of this QAPP. SOPs may be revised one or more times per year. *The laboratory Standard Operating Procedures Manual, Document 002, should always be referred to in order to ensure the use of the most current revision of any SOP.*

A3. Distribution List

Individuals, who will receive copies of the approved Quality Assurance Project Plan, and any subsequent revisions, are identified below.

<u>Individual</u>	<u>Title</u>	<u>Organization</u>
Evelyn Powers	Executive Director	IEC District
Jessica Haley	Environmental Analyst	NEIPWCC (IEC District)
Heather Radcliffe	Project Manager	NEIWPC
Michael Jennings	QA Manager	NEIWPC
Jerry Frank	Laboratory Manager	UMCES CBL NASL
Leah O'Neill	Project Officer	USEPA – Region 1
Nora Conlon	Quality Assurance Officer	USEPA – Region 1
Mark Tedesco	Data User	USEPA – LISO

A4. Project Organization

Table 1 lists the names and titles of all individuals participating in this project, along with their organization name and their responsibilities for this project.

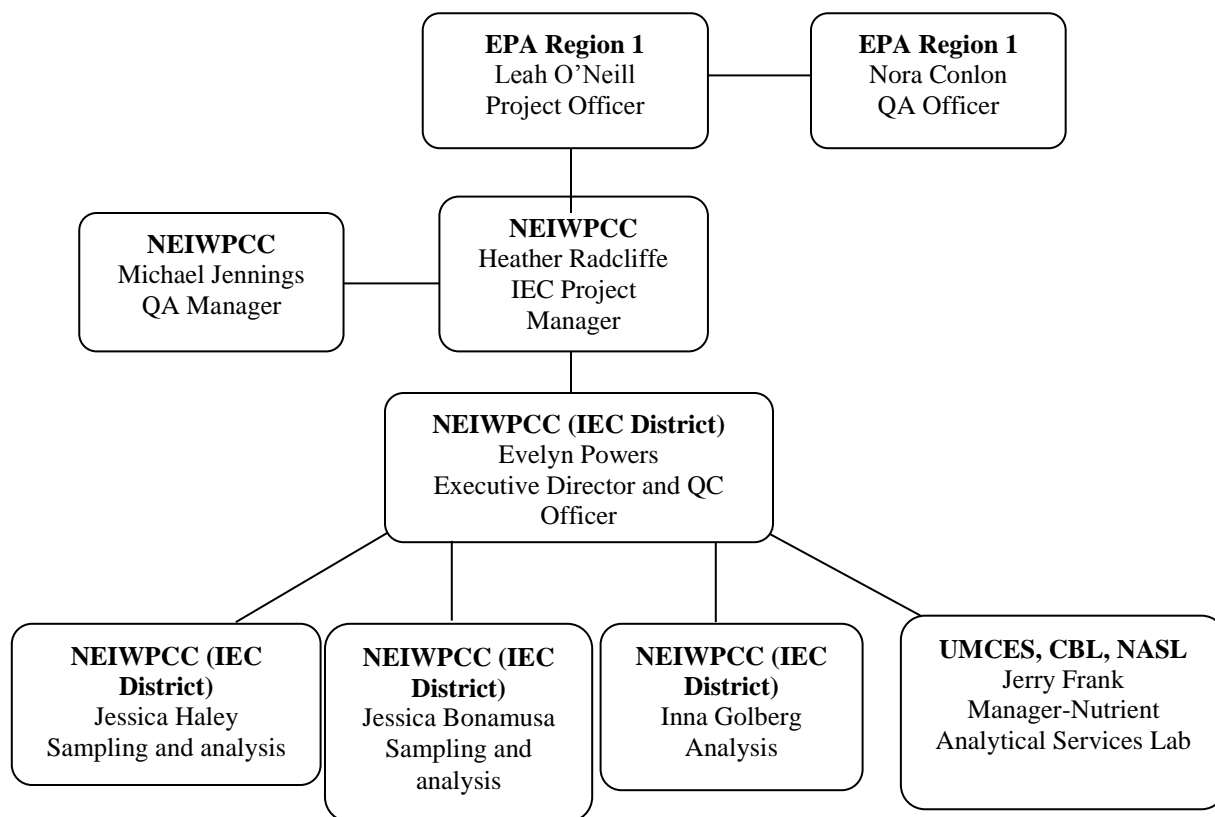
TABLE 1: Individuals Participating in the Project

<i>Person</i>	<i>Title</i>	<i>Organization</i>	<i>Role/Responsibilities</i>
Heather Radcliffe 978-349-2522 hradcliffe@neiwppcc.org	Program Manager	NEIWPCC	Project Manager.
Michael Jennings 978-323-7929 mjennings@neiwppcc.org	QA Program Manager	NEIWPCC	Review and approval of QAPP
Evelyn Powers 718-982-4059 epowers@iec-nynjct.org	Executive Director & QC Officer	IEC District	Oversees sample collection and NEIWPCC-IEC analyses & Quality Assurance, coordinates with contract laboratory, performs QA/QC review of contract lab data. Maintains and distributes the official approved QAPP.
Jessica Haley 718-982-3792 jhaley@iec-nynjct.org	Environmental Analyst I	NEIWPCC (IEC District)	Field sampling and analysis, data dissemination, interagency monitoring coordination, database management.
Jessica Bonamusa 718-982-3792 jbonamusa@iec-nynjct.org	Environmental Analyst I	NEIWPCC (IEC District)	Field sampling and analysis
Inna Golberg 718-982-3792 igolberg@iec-nynjct.org	Environmental Analyst III	NEIWPCC (IEC District)	Sample analyses
Jerry Frank 410-326-7252 frank@umces.edu	Manager-Nutrient Analytical Services Laboratory	UMCES, CBL, NASL	Director of contract laboratory, QA/QC

TABLE 1: Individuals Participating in the Project (*Continued*)

<i>Person</i>	<i>Title</i>	<i>Organization</i>	<i>Role/Responsibilities</i>
Leah O'Neill (617) 918-1633 oneill.leah@epa.gov	Project Officer	USEPA	EPA – Region 1 Coordination
Nora Conlon (617) 918-8335 conlon.nora@epa.gov	Quality Assurance Officer	USEPA	Review and approval of QAPP

Figure 1: Project Organization Chart

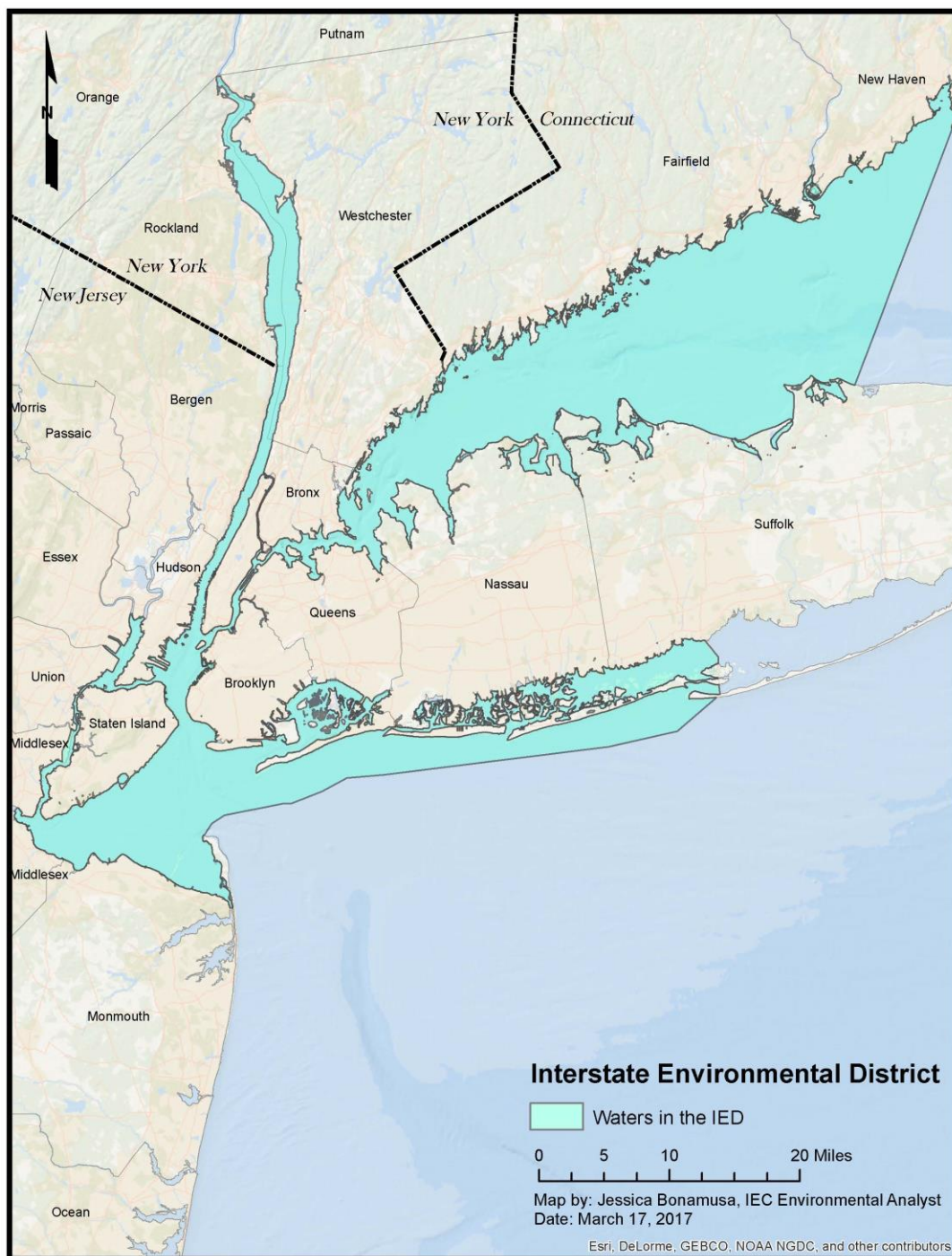


A5. Project Background

On May 15, 2012, the Interstate Environmental Commission (IEC) entered into a Memorandum of Understanding with the New England Interstate Water Pollution Control Commission, and the NEIWPCC (IEC District) was established. Through the formation of the NEIWPCC (IEC District), NEIWPCC has met diverse needs by providing fiduciary responsibility, staff and technical assistance, analysis and support for a broad array of water quality program areas for the terms outlined in the MOU. In 2018, IEC is transitioning from being a program of NEIWPCC to being an independent interstate agency.

In support of its member states' (Connecticut, New Jersey, and New York) NPDES programs and regional priorities, NEIWPCC (IEC District) conducts year-round effluent investigations on a regular basis, as well as periodic ambient monitoring surveys within NEIWPCC (IEC District) waterways. See Figure 2 on the next page for a map of the region in which this monitoring takes place.

Figure 2: NEIWPCC (IEC District)



Since 1991, NEIWPCC (IEC District) has participated in a cooperative sampling effort during the critical summer season on the Long Island Sound in support of the National Estuary Program's Long Island Sound Study. As part of a multi-agency collaborative effort, the IEC District continues to perform monitoring along with state and local entities such as the Connecticut Department of Energy and Environmental Protection (CTDEEP-<http://www.ct.gov/deep>), the University of Connecticut Department of Marine Sciences (UConn-<http://lisicos.uconn.edu/index.php>), New York City Department of Environmental Protection (NYCDEP-<http://nyc.gov/dep>), municipal health departments, and citizen science groups. With an ongoing need to document the hypoxic conditions in Long Island Sound and its embayments where the majority of primary recreational activities take place, US EPA - Region 1, in August 2017, awarded funding to the Interstate Environmental Commission to continue intensive ambient water quality survey in support of the Long Island Sound Study in summer 2018. In April 2018, the LISS Management Committee recommended the LISS fund, through its FY18 appropriations, an enhancement proposal to IEC's summer monitoring. The enhancement funds an expansion of IEC's LIS monitoring to include 8 additional monthly "winter" surveys. The monthly surveys will be performed at a frequency of one survey per month from October 2018 through May 2019, resulting in a year-round monitoring program in western Long Island Sound. The scope of work was outlined in a workplan that was submitted to and approved by USEPA Region 1. Documents in support of an amendment to grant LI00A00372 to increase the budget, revise the narrative and extend the project period to cover the enhancement are currently being developed for submission to LISS and EPA Region 1 based on guidance received by EPA Region 1 May 09, 2018. The information will be used to measure the effectiveness of management activities and programs implemented under the Comprehensive Conservation and Management Plan.

A6. Project Description

While some of the parameters and sampling stations have changed since 1991, the main focus of the monitoring program has remained the monitoring of dissolved oxygen and key water quality indicators during the summer when the Sound is most at risk for hypoxia. This is normally a 12-week period from late June through mid- September. If results indicate hypoxic conditions prevail at the end of a typical season, NEIWPCC (IEC District) may extend the duration of its LISS monitoring program to perform an additional week of sampling. Historically, NEIWPCC (IEC District) has performed one weekly sampling survey at 22 stations in the far western Long Island Sound (see Figure 3) during the sampling season described above. The final sampling schedule is determined annually in conjunction with CTDEEP in order align with its summer monitoring schedule. Sampling locations were originally chosen in discussions between NEIWPCC (IEC District) and NYSDEC's Division of Marine Resources to fill a monitoring gap in the Western Long Island Sound. See Figure 3 and Table 2 on the following pages. The scope of data collection has historically included weekly *in situ* measurements of water temperature, salinity, dissolved oxygen, pH, as well as biweekly collection of chlorophyll *a* grab samples at each station. Secchi disk depth is also measured as an indicator of water clarity at each station weekly.

Figure 3: Long Island Sound Sampling Locations

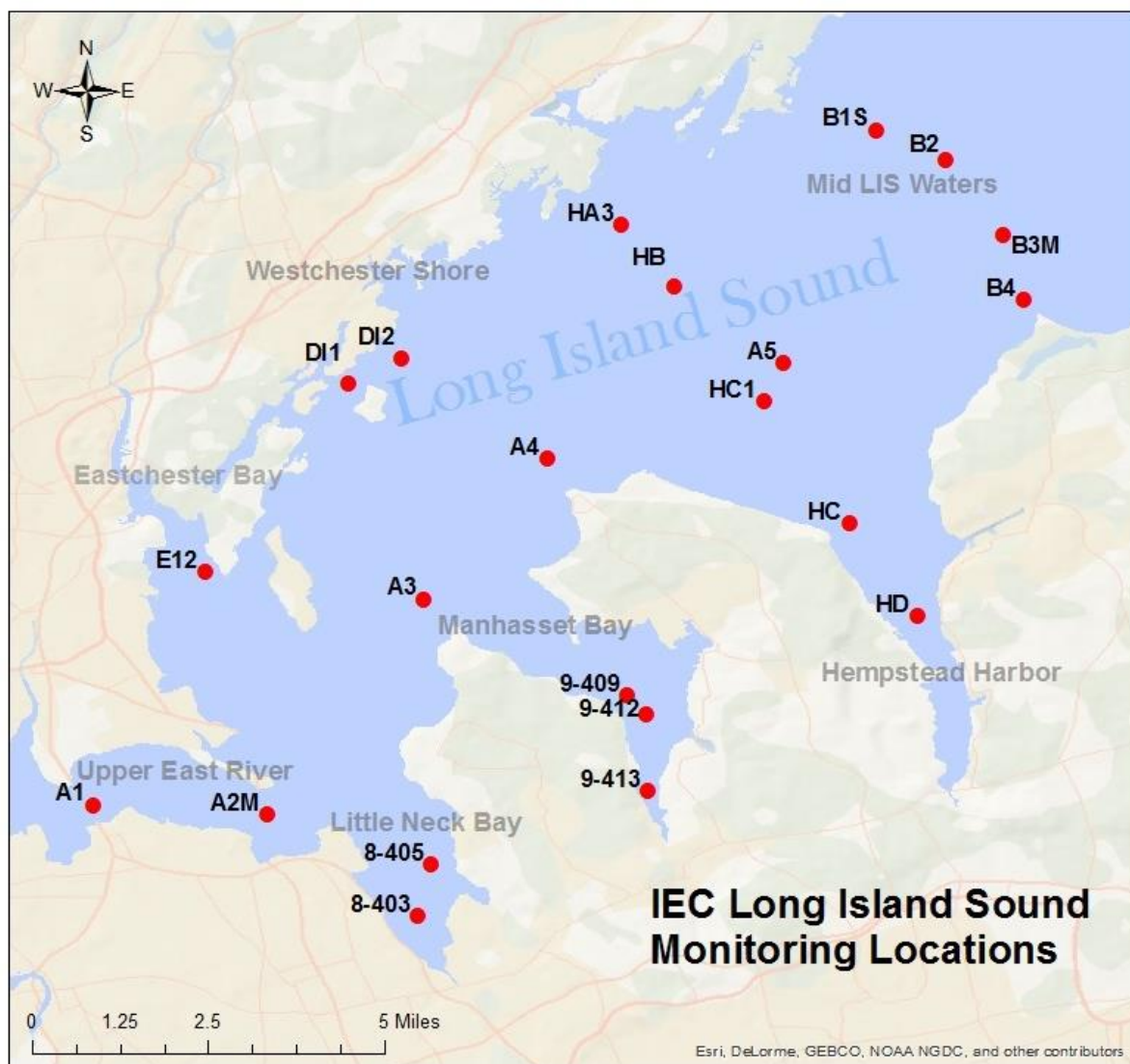


Table 2: IEC Long Island Sound Study Sampling Stations

STATION	WATER COLUMN DEPTH (meters)	LOCATION				DESCRIPTION
		LATITUDE D M	LONGITUDE D M	LATITUDE DD	LONGITUDE DD	
E-12	4	40°50.920N	73°48.270W	40.8487	-73.8045	Eastchester Bay mid-channel at N 6
A1	26	40°48.080N	73°49.610W	40.8013	-73.8268	East of Whitestone Bridge
A2M	35	40°47.950N	73°47.480W	40.7992	-73.7913	East of Throgs Neck Bridge
8-403	3	40°46.670N	73°45.650W	40.7778	-73.7608	Little Neck Bay - ~0.2 nm W of yellow nun “B”
8-405	3	40°47.330N	73°45.490W	40.7888	-73.7582	Little Neck Bay - ~0.15 nm North of LNB mid-channel buoy
A3	12	40°50.600N	73°45.540W	40.8433	-73.7590	Hewlett Point South of Fl G 4 Sec “29”
9-409	4	40°49.440N	73°43.050W	40.8240	-73.7175	Manhasset Bay
9-412	4	40°49.200N	73°42.810W	40.8200	-73.7135	Manhasset Bay
9-413	3	40°48.245N	73°42.796W	40.8041	-73.7133	Manhasset Bay
A4	35	40°52.350N	73°44.060W	40.8725	-73.7343	East of Sands Point, mid-channel
A5	13	40°53.540N	73°41.120W	40.8923	-73.6853	~2.6 nm East of Execution Lighthouse
B1S	15	40°56.420N	73°40.000W	40.9403	-73.6667	Porgy Shoal South of Fl G 4 Sec R “40”
B2	20	40°56.060N	73°39.120W	40.9343	-73.6520	Matinecock Point 1.6 nm North of Gong “21”
B3M	19	40°55.120N	73°38.420W	40.9187	-73.6403	Matinecock Point 0.7 nm North of Gong “21”
B4	15	40°54.321N	73°38.157W	40.9054	-73.6360	Matinecock Point South of Gong “21”
DI1	10	40°53.300N	73°46.490W	40.8883	-73.7748	Davids Island North of Nun “10A”
DI2	6	40°53.580N	73°45.850W	40.8930	-73.7642	Davids Island East of Nun “4”
H-A3	3	40°55.240N	73°43.120W	40.9207	-73.7187	Delancy Point South of Can “1”
H-B	12	40°54.480N	73°42.540W	40.9080	-73.7090	0.7 nm Southeast of Daymarker Fl R 4 Sec

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H-C	8	40°51.540N	73°40.300W	40.8590	-73.6717	Hempstead Harbor East of R Bell “6”
H-C1	11	40°53.120N	73°41.420W	40.8853	-73.6903	Hempstead Harbor~ 2.0 nm East of Sands Point
H-D	7	40°50.410N	73°39.430W	40.8402	-73.6572	Hempstead Harbor East of Can “9”

Nutrient and BOD samples will be collected from stations in **Bold**.

In 2018, NEIWPCC-IEC staff will perform 12 weekly surveys during the summer months (June- September) and 8 monthly surveys during the fall and winter (October 2018 through May 2019) of 22 stations in the far western Long Island Sound. The 12 weekly summer surveys will include weekly *in situ* water column profiles of temperature, salinity, dissolved oxygen, pH, the collection of chlorophyll a surface grab samples, and measurement of Secchi disk depth. Six of the 12 summer surveys will include collection of additional samples for parameters relevant to hypoxia at 11 of the 22 stations. These samples will be analyzed for nutrients, Biochemical Oxygen Demand (BOD), and Total Suspended Solids (TSS), in addition to the suite of *in situ* parameters listed above. The eight monthly surveys performed from October 2018 through May 2019 will include *in situ* water quality profiles of water temperature, salinity, dissolved oxygen, pH, chlorophyll a, and Secchi disk depth at all 22 stations. These surveys will also include collection of surface grab samples for parameters relevant to hypoxia at 11 of the 22 stations, as with the summer surveys. These samples will be analyzed for nutrients, Biochemical Oxygen Demand (BOD), and Total Suspended Solids (TSS). The specific nutrient parameters that will be analyzed include Ammonia, Nitrate+Nitrite, Particulate Nitrogen, Orthophosphate/Dissolved Inorganic Phosphorus, Total Dissolved Phosphorus, Particulate Phosphorus, Dissolved Organic Carbon, Particulate Carbon, Dissolved Silica, and Biogenic Silica. These stations and additional parameters were selected following a review of the scope of work of previous surveys and recommended modifications from the LISS Water Quality workgroup. They include both embayment and open water stations. The surveys that entail sample collection will occur bi-weekly (June-September) and once monthly (October-May), and will align, to the extent possible, with water quality sampling performed by CTDEEP farther east in Long Island Sound. The 22 sampling locations comprising the weekly surveys are identified in Figure 2 and Table 2. The subset of 11 stations selected for expanded parameter sampling highlighted in **bold** in Table 2. TSS, BOD, and chlorophyll *a* analyses will be performed in-house at NEIWPCC’s IEC District Laboratory located in Staten Island, NY. Additionally, the NEIWPCC IEC District Laboratory will perform sample analyses for Ammonia, Nitrate+Nitrite, Total Dissolved Nitrogen, Orthophosphate/DIP, Total Dissolved Phosphorus, Particulate Phosphorus, Dissolved Silica, and Biogenic Silica. Samples for Dissolved Organic Carbon, Particulate Carbon, and Particulate Nitrogen analysis will be subcontracted to the University of Maryland’s Center for Environmental Science, Chesapeake Biological Laboratory, Nutrient Analytical Services Laboratory (UMCES CBL NASL) in Solomons, Maryland.

If any of the sampling locations are modified (added or subtracted), all those on the distribution list (Section A3) will be notified of any changes. If any additional parameters (not listed in the QAPP) are requested to be sampled and analyzed, NEIWPCC (IEC District) would be required

to submit an amendment to the QAPP, stating the reason for the additional samples and describing the methods used to sample and analyze them. Any additional samples would be analyzed by the NEIWPCC's IEC District Laboratory only if the parameter is within the scope of the laboratory's capabilities. Parameters beyond the capabilities of the IEC District Laboratory's will be analyzed by a competent contract laboratory. All those on the distribution list will be notified of the modification and receive the approved addendum.

A7. Quantitative QA Objectives: Precision, Accuracy, Bias, Sensitivity, Completeness, Comparability, and Method Detection Limit

Data Quality Objectives (DQOs) are set to ensure that data generated in this project will be of sufficient quality and quantity to reduce the chance of making an error to a tolerable level. Qualitative and quantitative DQOs are characterized by the following terms: precision, accuracy, bias, sensitivity, completeness, comparability and representativeness.

A7.1 Precision

For this project, precision is defined as the degree of agreement among replicate measurements of the same property. Precision is measured by submitting two "identical" samples of unknown value from the same location (field duplicates) to the laboratory and seeing whether the results of the analyses reflect comparable values. In addition to the use of duplicates throughout the project's duration, an extended list of standard laboratory quality control measures further enhances the quality of results, from the perspective of precision. Please refer to Section B5 (Analytical Quality Control) and Tables 5-7 for further information on quality control measures to ensure and monitor precision.

A7.2 Accuracy

Accuracy is defined as the degree of conformity of the results obtained by the analysis of the test sample for a certain variable to the true value of that variable. Accuracy can be affected by sampling and analytical operations. It could be determined by analyzing a reference material of known pollutant concentrations or by reanalyzing a sample spiked with a known amount of pollutant.

Accuracy will be assured by employing, and strictly adhering to, sample collection procedures that minimize sampling variability (*e.g.*, through training of sampling crews and standardization of collection methods) and analytical methods capable of providing the best measurement performance (*e.g.*, low bias and acceptable precision). These procedures will not be modified throughout the duration of the project.

In addition to semi-annual proficiency samples and duplicate analyses by NEIWPCC (IEC District) throughout the project's duration, an extended list of standard laboratory quality control measures, further enhance the quality of results, from the perspective of accuracy (See B5 Analytical Quality Control and individual analytical SOPs).

A7.3 Bias

Bias is the systematic distortion of a measurement process that causes errors in one direction. To minimize meter bias, be sure the instrument is calibrated before taking measurements. These are discussed in Section B4 as well as individual analytical SOPs. Measurements for pH, salinity and temperature are typically quick and accurate. Care must be measured when handling the probe specifically for the determination of dissolved oxygen. The instrument must be turned on for 5-15 minutes before taking measurements. Be sure the sensor guard is attached to protect the sensor from debris that could cause damage. After placing the probe in the water to be measured, stir the probe in the sample to overcome the stirring dependence of the dissolved oxygen sensor. Once the values plateau and stabilize, you may record the measurement. The dissolved oxygen reading will drop over time if stirring is ceased. Sampling location information is determined using an on-board GPS (receiver) unit. GPS receivers calculate position by timing the signals sent by GPS satellites maintained by the US Government. Sources of bias can be classified as either satellite dependent, receiver dependent, or resulting from unpredictable delays or dispersal of signal propagation. Receiver bias can be monitored by annually verifying coordinates at each sampling location with a second, handheld GPS unit.

A7.4 Sensitivity

For this project, sensitivity is defined as the minimum concentration of an analyte above which a data user can be reasonably confident that the analyte was reliably detected and quantified. Method sensitivity is expressed by the use of method detection levels (MDLs) and/or reporting limits (provided in Table 4). NEIWPCC's IEC District Laboratory's reporting limits are 2-5X the laboratory-determined MDL and are verified annually (or more often if specified by the method) by analyzing a sample at or near the reporting limit. UMCES CBL NASL uses MDL's to analyze sensitivity. The quantitation limits (reporting limits) listed in Table 4 and analytical SOPs are mathematically determined. However, UMCES CBL NASL reports down to each method's analytically-determined MDL. UMCES CBL NASL MDL's are determined annually and are calculated as the standard deviation of multiple low-level replicate analysis times the student t-test value (students T-test value for 99% confidence that the value is greater than zero, for seven replicates the value is 3.14, seven replicates is the minimum number used). Any values below UMCES CBL NASL's MDL is reported as the MDL and given a code of "L." As with accuracy, sensitivity is inherent to the technique. All samples will be analyzed according to the methods listed in Table 4. Adherence to equipment calibration and maintenance procedures, as well as the standard laboratory quality control measures and standard operating procedures, described previously in more detail (see Section B5 Analytical Quality Control), will maintain sensitivity.

A7.5 Completeness

Overall completeness is defined as the total number of acceptable samples divided by the number of samples planned for collection. For all data collected, the objective is for 100% completeness. However, as a practical matter, this might not be possible. The project goal is 95% completeness.

A7.6 Comparability

For the purpose of this project, comparability expresses the confidence that two data sets can contribute to a common analysis. It is a reflection of the degree of difficulty one would have in comparing or merging the two data sets because of differences in collection and analytical methods used.

Data will be generated from samples collected by and analyzed by NEIWPCC (IEC District). It is anticipated that intra-organization sample collection and analyses comparability will approach 100%, since standard guidelines for sample collection and analytical procedures will not be modified throughout the duration of the project. The use of standard methods for sample analysis will allow for comparability of the results to other data sets collected/analyzed using the same methodology.

A7.7 Representativeness

For this project, representativeness is defined as the degree to which a subset of data, the one actually collected, reflects the characteristics of a larger set of data. Generally, the larger the subset, the greater the representativeness. The homogeneity of the larger set also has a major influence on representativeness. The typical approach used to maximize representativeness is to increase the size of the subset that is sampled. However, to maintain efficiency of the project, only a limited number of samples will be analyzed. Nevertheless, sample collection during this project has been designed to provide the representativeness required to achieve the project's goals. Sampling stations are distributed within an area covering the Western Long Island Sound. Sampling stations include open water stations as well as near-shore and embayment stations in order to encompass the full scope of Long Island Sound waters. This will also assist in evaluating any variation amongst the sampling locations. Sampling locations were selected between discussion between NEIWPCC (IEC District) and NYSDEC's Division of Marine Resources to fill a monitoring gap in the Western Long Island Sound. The sampling will occur during the summer months when the possibility of hypoxia in these waters is at its highest.

A7.8 Method Detection Limit

Table 4 lists the method detection limits and reporting limits for the parameters analyzed by NEIWPCC's IEC District Laboratory. Reporting limits are verified analytically at least annually and are typically in the range of 1-10 times the method detection limit. The laboratory reporting limits and the sensitivity of the field meters meet the sensitivity needed for the water quality monitoring portion of the project. Table 4 also lists the MDLs and reporting limits for UMCES CBL NASL.

A7.9 QA/QC Checks

The QC procedures shall follow the QA objectives stated earlier in this document. Quality control samples (also known as laboratory control samples) will be procured or prepared and

analyzed in conjunction with the above sampling events. Quality control samples will consist of a minimum of the following: blanks at a rate of 5%, duplicate analyses at a rate of 5%. For the field measurements of salinity, temperature, pH and dissolved oxygen, and Secchi disk depth, a duplicate reading will be measured at each depth measured for two sites per sampling run and recorded. Because of currents, ambient water is continuously in flux and duplicate measurements in the field may not demonstrate the precision yielded by laboratory analyses of discrete samples. Nonetheless, target acceptance criteria for replicate measurements of field measurements are within 15% of their average.

All field measurements and sample collection will be performed by the NEIWPCC (IEC District)'s laboratory staff in accordance with IEC's Laboratory Quality Control Manual, the Quality Assurance Project Plan, and NEIWPCC's Quality Management Plan. Sample analyses for chlorophyll *a*, TSS, BOD, and nutrients (with the exception of Dissolved Organic Carbon, Particulate Carbon, and Particulate Nitrogen) will be performed by NEIWPCC'S IEC District Laboratory. Sample analyses for Dissolved Organic Carbon, Particulate Carbon, and Particulate Nitrogen will be performed by UMCES CBL NASL. UMCES CBL NASL will serve as a backup laboratory for IEC's nutrient analyses. Tables 5 and 7 outline NEIWPCC IEC District Laboratory Quality Control checks required. More detailed quality control procedures for field measurements, chlorophyll *a*, TSS, BOD, and nutrients analyzed in this study can be found in IEC's SOPs in Appendix C and UMCES CBL NASL SOP's in Appendix D and the published, certified EPA or Standard Methods. In addition, the QC Officer participates in at least one survey per summer to perform a field audit (assessment). Typically, this is the first survey of the season, so that any issues with field measurements or sample collection or site selection (*e.g.*, navigational hazards making it difficult to reach a specific sample site) can be addressed, corrective actions implemented, and the QAPP amended if necessary and the appropriate parties notified.

Also, NEIWPCC (IEC District)'s QC Officer will review all of the data before the report is ready to be finalized. A final report is then generated. Prior to final report dissemination, the final report is again reviewed by NEIWPCC (IEC District)'s QC Officer.

NEIWPCC may implement, at their discretion, various audits or reviews of this project to assess conformance and compliance to the quality assurance project plan in accordance with the NEIWPCC Quality Management Plan.

A8. Certification and Training

Analyses for TSS, BOD, chlorophyll *a*, and nutrients (with the exception of Dissolved Organic Carbon, Particulate Carbon, and Particulate Nitrogen which will be subcontracted to UMCES CBL NASL) are performed in-house by the NEIWPCC (IEC District)'s laboratory, currently located on the campus of The College of Staten Island, Staten Island, NY. The NEIWPCC (IEC District)'s laboratory is a nationally certified environmental testing laboratory. The laboratory is accredited by the National Environmental Laboratory Accreditation Program, known as NELAP. The New York State Department of Health (NYS DOH) Environmental Laboratory Approval

Program (ELAP) is the primary accrediting authority for the majority of the laboratory's certified parameters. The laboratory also holds primary NELAP certification from the New Jersey Department of Environmental Protection (NJ DEP) for certain parameters for which New York State does not offer certification. Furthermore, the laboratory also holds secondary NELAP certification through the NJ DEP for those parameters that it already holds primary certification from the NYS DOH. The Laboratory is also a State of Connecticut Department of Public Health Approved Environmental Laboratory.

NEIWPCC (IEC District)'s laboratory participates annually in two rounds of proficiency testing for NYS DOH and NJ DEP. In addition, as part of its NELAP requirements, biennial on-site assessments are conducted by both agencies.

Prior to performing analyses on any samples, NEIWPCC (IEC District)'s laboratory staff must complete a thorough training program. All staff must satisfactorily complete an initial demonstration of capability study (IDC) for all field and laboratory analyses they perform, as required by NELAP. In subsequent years, staff must perform an annual continuing demonstration of capability study (CDC) for all analyses they perform. Additionally, all NEIWPCC (IEC District) staff members responsible for collecting samples, performing field measurements, collecting data, analyzing samples, or completing or reviewing final reports must complete initial and annual ethics and data integrity training. This training program is designed, coordinated, and documented by the NEIWPCC (IEC District) QC Officer. Documentation is maintained in the laboratory's Training Manual.

NEIWPCC (IEC District)'s sampling, field measurements, data collection and sample analyses are carried out by NEIWPCC (IEC District)'s staff. Initially, newly hired staff carry out investigations jointly with a QC Officer. Staff initially observe/assist in several investigations/sampling events after which they may take the lead in investigations/sampling events. Ambient sampling on Long Island Sound requires two staff members.

UMCES CBL NASL performs analyses for numerous local, state, and federal agencies as well as the private sector. NASL performs most of the nutrient analysis for the Chesapeake Bay Program and the Maryland Department of the Environment TMDL program. UMCES CBL NASL participates in the USGS Standard Reference Sample Project Inter-Laboratory Comparison and coordinates its own inter-laboratory comparison effort with fifteen individual laboratories that participate in the comparison.

A9. Documentation and Records

As NEIWPCC (IEC District)'s sampling data becomes available during the study, the NEIWPCC (IEC District) submits excel data sheets to the Technical Director of US EPA's Long Island Sound Study Office; the New York State Department of Environmental Conservation (NYS DEC), Division of Marine Resources; the New York City Department of Environmental Protection, Bureau of Wastewater Pollution Control, Marine Science Section; Connecticut Department of Environmental Protection, Bureau of Water Management; Nassau County Health Department; and other stakeholders. The data are distributed on a weekly basis. After the

sampling period is completed, all the data are presented in a final report in conjunction with CT DEEP. All electronic data files are backed up weekly. The approved QAPP will be distributed via email to all individuals listed in the distribution list in Section A3 by the QC Officer.

The STORET Data Warehouse is EPA's repository of the water quality monitoring data collected by water resource management groups across the country. Like many other organizations, including states, tribes, watershed groups, federal agencies, volunteer groups, and universities, NEIWPCC (IEC District) submits data to the STORET Warehouse to make it publically accessible. After the completion of each monitoring year, the NEIWPCC (IEC District) submits data to the STORET Data Warehouse through a framework called the Water Quality Exchange (WQX).

Each sampling event will be assigned a unique investigation number as per NEIWPCC (IEC District) procedures. Field data sheets, chain-of-custody forms, field equipment QA/QC, and calibration record forms will be completed in the field and, in the case of chain-of-custody forms, upon transfer of samples to the lab. All laboratory-generated data, including calculations where applicable, are recorded directly onto analyte-specific forms or the analysts' logbooks. These forms and photocopies of all records in analysts' logbooks, including calculations, QC results, and calibration records are included in the investigation folder. Data and all calculations are reviewed by NEIWPCC (IEC District)'s QC Officer. In addition to the review, the QC Officer will verify that the QAPP was followed, including but not limited to, verifying that samples were properly preserved in the field, QC samples were collected, chain of custody was maintained, no transcription errors occurred, holding times were met, laboratory QC samples were analyzed, and laboratory samples were properly stored.

B1. Sampling Process Design

The primary focus of the project is the monitoring of dissolved oxygen and other water quality parameters relevant to hypoxia in the Far Western Long Island Sound during the summer, when the Sound is most at risk for hypoxia. This is normally a 12-week period from late June through mid-September. If results indicate hypoxic conditions prevail at the end of a typical season, NEIWPCC (IEC District) may extend the duration of its LISS monitoring program to perform an additional week of sampling.

Historically, NEIWPCC (IEC District) has performed one weekly sampling survey at 22 stations in the Far Western Long Island Sound during the sampling season described above. The proposed sampling schedule is outlined in the Table 3 below. The sampling schedule is projected to align with CTDEEP's ambient water quality monitoring. As with all proposed sampling, minor modifications to this schedule may be made due to weather or other unforeseen circumstances (unsafe sea state, boat mechanical issues). Sampling locations were originally chosen in discussions between NEIWPCC (IEC District) and NYSDEC's Division of Marine Resources to fill a monitoring gap in the Western Long Island Sound. In coordination with the LISS Water Quality Workgroup, IEC District staff have been working on opportunities to improve the overall monitoring program for the Sound by collaborating with CTDEEP and

others involved in monitoring in the Sound. This has resulted in the implementation of recommendations regarding the scope of monitoring including the modifications of parameters included in the monitoring program, and the release of a joint season summary of the 2016 monitoring season.

Consistent with the approved workplan, IEC will continue to coordinate with LISS partners, especially the WQWG, to ensure an effective and efficient monitoring program for Long Island Sound. In particular, IEC will examine, in consultation with the WQWG, whether overlap or duplication exists between IEC's monitoring program and NYCDEP's Harbor Water Quality Survey to the west of IEC's monitoring area. IEC will consult with LISS WQWG and may eliminate or reduce monitoring at certain stations to reduce redundancies and improve efficiency of the overall monitoring program.

The scope of data collection includes weekly field measurements of water temperature, salinity, dissolved oxygen, pH, as well as biweekly collection of chlorophyll *a* and TSS grab samples at each station, and biweekly collection of BOD and nutrient grab samples at 11 of the 22 stations. Secchi disk depth is also measured as an indicator of water clarity at each station weekly. Observations of percent cloud cover and sea state are recorded at each station. Weather conditions during the duration of the survey and precipitation data for the 48 hours prior to survey start are also recorded. Monthly Sampling events executed from October through May will include field measurements of temperature, salinity, dissolved oxygen, pH, as well as chlorophyll *a* and TSS grab samples at each station, and a collection of BOD and nutrient grab samples at 11 of the 22 stations.

In summer 2018, NEIWPCC-IEC staff will perform 12 weekly surveys each summer at 22 stations in the far western Long Island Sound. Proposed 2017 survey dates are listed in Table 3. The 12 weekly surveys will include weekly *in situ* measurements of water temperature, salinity, dissolved oxygen, pH, and Secchi disk depth. Six of the 12 surveys will include collection of additional samples for parameters relevant to hypoxia at 11 of the 22 stations. These samples will be analyzed for nutrients, Biochemical Oxygen Demand (BOD), Total Suspended Solids (TSS), and chlorophyll *a*, in addition to the suite of *in situ* parameters listed above. Samples for TSS and chlorophyll *a* will be collected during six surveys at all 22 stations. Samples for BOD, and nutrients will be collected at 11 of the 22 stations. The specific nutrient parameters that will be analyzed include Ammonia, Nitrate+Nitrite, Total Dissolved Nitrogen, Particulate Nitrogen, Orthophosphate/DIP, Total Dissolved Phosphorus, Particulate Phosphorus, Dissolved Organic Carbon, Particulate Carbon, Dissolved Silica, and Biogenic Silica. These stations and additional parameters were selected following a review of the scope of work of previous surveys and recommended modifications from the LISS Water Quality workgroup. They include both embayment and open water stations. The six surveys that entail sample collection will occur approximately bi-weekly, and will align, to the extent possible, with water quality sampling performed by CTDEEP farther east in Long Island Sound. The 22 sampling locations comprising the weekly surveys are identified in Figure 2 and Table 2. The subset of 11 stations selected for expanded parameter sampling during 6 of the 12 surveys are highlighted in **bold** in Table 2. TSS, BOD, and chlorophyll *a* analyses will be performed in-house at NEIWPCC's IEC District Laboratory located in Staten Island, NY. Additionally, the NEIWPCC IEC District Laboratory

will perform sample analyses for Ammonia, Nitrate+Nitrite, Total Dissolved Nitrogen, Orthophosphate/DIP, Total Dissolved Phosphorus, Particulate Phosphorus, Dissolved Silica, and Biogenic Silica. Samples for Dissolved Organic Carbon, Particulate Carbon, and Particulate Nitrogen analysis will be subcontracted to the University of Maryland's Center for Environmental Science, Chesapeake Biological Laboratory, Nutrient Analytical Services Laboratory (UMCES CBL NASL) in Solomons, Maryland.

**Table 3: PROPOSED 2018 NEIWPCC-IEC DISTRICT LONG ISLAND SOUND
SURVEY DATES**

Survey Number	Proposed Date	Parameters	Proposed CTDEEP survey
1	6/26/2018	<i>in situ</i> parameters only	
2	7/3/2018	<i>in situ</i> , chlorophyll <i>a</i> , TSS, BOD, nutrients	7/2/2018-7/6/2018
3	7/10/2018	<i>in situ</i> parameters only	
4	7/17/2018	<i>in situ</i> , chlorophyll <i>a</i> , TSS, BOD, nutrients	7/16/2018-7/20/2018
5	7/24/2018	<i>in situ</i> parameters only	
6	7/31/2018	<i>in situ</i> , chlorophyll <i>a</i> , TSS, BOD, nutrients	7/30/2018-8/3/2018
7	8/7/2018	<i>in situ</i> parameters only	
8	8/14/2018	<i>in situ</i> , chlorophyll <i>a</i> , TSS, BOD, nutrients	8/13/2018-8/17/2018
9	8/21/2018	<i>in situ</i> parameters only	
10	8/28/2018	<i>in situ</i> , chlorophyll <i>a</i> , TSS, BOD, nutrients	8/27/2018-8/31/2018
11	9/4/2018	<i>in situ</i> parameters only	
12	9/11/2018	<i>in situ</i> , chlorophyll <i>a</i> , TSS, BOD, nutrients	9/10/2018-9/11/2018
M1	10/2018	<i>in situ</i> , chlorophyll <i>a</i> , TSS, BOD, nutrients	10/1/2018-10/5/2018
M2	11/2018	<i>in situ</i> , chlorophyll <i>a</i> , TSS, BOD, nutrients	11/5/2018-11/9/2018
M3	12/2018	<i>in situ</i> , chlorophyll <i>a</i> , TSS, BOD, nutrients	12/3/2018-12/7/2018
M4	1/2019	<i>in situ</i> , chlorophyll <i>a</i> , TSS, BOD, nutrients	TBD
M5	2/2019	<i>in situ</i> , chlorophyll <i>a</i> , TSS, BOD, nutrients	TBD
M6	3/2019	<i>in situ</i> , chlorophyll <i>a</i> , TSS, BOD, nutrients	TBD
M7	4/2019	<i>in situ</i> , chlorophyll <i>a</i> , TSS, BOD, nutrients	TBD
M8	5/2019	<i>in situ</i> , chlorophyll <i>a</i> , TSS, BOD, nutrients	TBD

If any of the sampling locations are modified (added or subtracted), all those on the distribution list (Section A3) will be notified of any changes. If any additional parameters (not listed in the QAPP) are requested to be sampled and analyzed, NEIWPCC (IEC District) would be required

to submit an amendment to the QAPP, stating the reason for the additional samples and describing the methods used to sample and analyze them, and the laboratory performing the analyses. All those on the distribution list will be notified of the modification and receive the approved addendum.

The coordinates for each station are programmed into the on-board GPS. The boat has two GPS systems onboard. The primary system is a Raymarine E120. The backup system is a Garmin GPS MAP 1040XS. Both systems use the North American Datum 1983 (NAD83) system for reference coordinates. Station locations are verified at least annually using a second GPS. Sampling is performed during dry or wet weather. Weather conditions in days prior to the sampling does not affect sampling. However, the weather conditions are noted on field data sheets. Given that sampling is performed in open waters, and safety of its personnel is NEIWPCC (IEC District)'s highest concern, summer sampling surveys may be cancelled, rescheduled, or shortened (*e.g.*, some stations not sampled) if conditions are unsafe or deteriorate during the run.

B2. Sampling Procedures

TSS, BOD, Chlorophyll *a*, and nutrients (with the exception of Dissolved Organic Carbon Particulate Carbon, and Particulate Nitrogen which will be subcontracted to UMCES CBL NASL) will be analyzed by NEIWPCC's IEC District Laboratory. Complete sample collection, preservation, and transport procedures specific to chlorophyll *a*, BOD, TSS, and nutrient analyses are addressed in detail in NEIWPCC IEC District Laboratory's Standard Operating Procedure and UMCES CBL NASL Standard Operating Procedures, attached in Appendices C and D, respectively.

Samples collected for chlorophyll *a* and TSS are collected as surface grabs (within one meter of the surface) into a clean, dry, 1000mL polypropylene sample bottle, which is rinsed with three successive portions of the ambient surface water at the station to be sampled as follows. At the station, the bottle is unsealed, the lid is held, inverted, by second staff or sealed in ziplock bag, and the bottle is clamped to a sampling stick. Sample aliquots for both chlorophyll *a* and TSS must be kept in the dark and cooled to $\leq 4^{\circ}\text{C}$ during the rest of the survey and transport. In addition to the collection of a field duplicate at one station per run, one grab sample per run should be collected with sufficient volume to perform a duplicate from the sample bottle (laboratory duplicate).

For BOD and nutrient sampling, a 2000 mL surface grab will be collected. One 2-liter polypropylene bottle will be filled using the sampling stick or Kemmerer using the method described above for TSS and chlorophyll *a* sampling. Subsamples for nutrient analyses will be aliquoted from Kemmerer, and filtered or preserved for each analyte, as appropriate, in the NEIWPCC-IEC District laboratory. Table 6A lists the volume required for each analyte. In addition to the collection of a field duplicate at one station per run, one grab sample per run should be collected with sufficient volume to perform a duplicate from the sample bottle (laboratory duplicate).

B2.1 Field Measurements

NEIWPCC (IEC District) staff, working in one team consisting of a minimum of two people, will complete each survey. All 22 stations will be monitored and sampled in one day. To avoid disruption of bottom sediments and as monitoring at each station can be completed quickly, ideally the boat does not anchor at sampling stations. The captain maintains the vessel in idle mode, monitoring the GPS and adjusts location in case of drift. In the event of strong currents or high seas, the boat may anchor, in which case sampling should be delayed for 5 minutes after anchoring. In the field, the sampling team will perform measurements of temperature (°C), salinity (ppt), dissolved oxygen (mg/l), and pH (S.U.). These measurements will be performed *in situ* using a YSI EXO 1 Multiparameter Sonde. General description of meter operation is included in this QAPP. For more specific meter set-up, testing, inspection, maintenance, and analytical information, refer to Sections B.6 and B.7. For calibration procedures, refer to analyte-specific standard operating procedures (attached) as well as the YSI EXO 1 Multiparameter Sonde user manual. The YSI EXO 1 Multiparameter Sonde is equipped with a 66 m cable attached to a sensor bulkhead capable of holding 4 sensors. Meters are transported on the boat in a manufacturer-supplied case. A fully-equipped back-up meter and two backup size “D” batteries should be on hand during all sample trips due to the difficulty of troubleshooting and replacing sensors on the boat. Open the meter case and turn the meter on approximately 15 minutes before arriving at the first sample site. To take measurements, unpack the meter from the case, unwind the cable, and place the probe in the water. The first reading taken is the bottom reading. Unwind the cable *slowly* until the probe just barely hits the bottom of the water. The onboard depth finder is used to estimate the depth of the water. The actual depth recorded is read from the YSI EXO handheld device. Gently pull the cable up 3 feet off the bottom, provide moderate stirring, wait for the readings to stabilize and record. The weighted cable minimizes drift to ensure bottom depth is as accurate as possible. Measurements are recorded on the Long Island Sound data sheet (Appendix B). Recorded data includes station, depth (meters), time, temperature (°C), salinity (ppt), D.O. (mg/L), % saturation, pH, percent cloud cover, sea state, and Secchi disk depth. The meter simultaneously displays the measured pH, temperature, salinity, and DO to be recorded. Repeat for mid-depth (one-half the bottom depth) measurements, if applicable, and surface measurements (1 meter below surface). Refer to Section B.6 for additional information on meter set-up, calibration, testing, inspection, and maintenance. Refer to Section B.4 for additional information on analytical methods.

In addition, water clarity (Secchi disk depth) will be measured *in situ*. Clarity will be determined using a Secchi disk. The Secchi disk will be lowered into the water on the shady side of the boat until it is no longer visible. The depth at which the disk disappears and at which it reappears is averaged (if different) and recorded. Secchi readings are performed on the shaded side of the boat to prevent interference from glare. Where there is no shaded side, staff should stand with their back to the sun.

B2.2 Field Data Transfer and Dissemination

Data recorded on the Long Island Sound data sheet is entered in a spreadsheet upon arrival back

at the laboratory. A second analyst checks the spreadsheet against the original field data sheet to ensure there are no transcriptional errors and to ensure the QA/QC checks highlighted in this QAPP, the IEC QC Manual, and analytical SOPs have been adhered to. This spreadsheet is disseminated weekly, along with a narrative highlighting and summarizing the data, to: EPA's LIS office, CT DEEP's Bureau of Water Management, Nassau County Health Department, NYSDEC Division of Marine Resources, EPA Region II, NYCDEP Marine Sciences Section, Westchester County Department of Health, and several other agencies and stakeholders on a Long Island Sound distribution list via email.

B2.3 Sampling Points

All surveys include *in-situ* monitoring of **all** 22 stations identified in Figure 3 and Table 2. Six of the twelve surveys conducted during the summer (June-September) and all surveys conducted in October through May will include surface grabs for chlorophyll *a* and TSS at all 22 stations. These surveys will also include sampling for nutrients and BOD analyses at the 11 stations listed in **bold type** in Table 2.

Surface (1 meter below surface), mid-depth, and bottom (approximately 1 meter above the bottom) measurements for temperature, salinity, pH, and dissolved oxygen are taken weekly at each stations with depths greater than 10 feet. For stations with depths less than 10 feet, surface, and bottom measurements are taken. For stations with a depth of 3 feet only one measurement is taken.

B2.4 Sampling Equipment

The following list describes the equipment that NEIWPCC's IEC District staff use during each sampling survey. Not all equipment will be used for every event.

- a. Coolers with ice and field thermometer
- b. Safety Equipment:
 - i. Boots, sneakers, or other close-toed shoe with rubber soles that provide adequate traction on wet boat deck
 - ii. Safety goggles
 - iii. Rain gear
 - iv. Life jacket (personal floatation device)
 - v. Sunscreen and hat (Boat offers little protection from the elements.)
 - vi. Potable water
 - vii. Gloves
- c. Sampling bottles:

- i. One 2 liter or two (2) one-liter polyethylene bottles for the collection of grab samples to be collected for TSS and Chlorophyll *a* analysis
 - ii. Nutrients: One 2-liter polypropylene bottle for collection of water sample to be subsampled and filtered, as necessary, for nutrients analyses
 - iii. Extra bottles in case of loss, leakage, or breakage.
- d. Labeling supplies:
 - i. Durable labels/water resistant labeling tape, “Sharpies”/indelible ink
- e. Field Meters:
 - i. YSI EXO 1 Multimeter Sonde equipped with 66-meter cable with conductivity/temperature, DO, pH and port plug installed in sensor hub
 - ii. Fully equipped back-up YSI EXO 1 Multimeter Sonde
- f. Watch or clock
- g. Appropriate paperwork, sampling plan, analytical SOPs, QAPP, field record book, sampling data sheets, chain of custody sheets, and black pens.
- h. Sampling equipment, including sampling stick and clamp
- i. Additional handheld GPS unit such as Trimble or Garmin Rino 130 to verify coordinates

B3. Sample Handling and Custody

Once filled with appropriate sample volume, bottles are tightly sealed and labeled. Each sample bottle is identified with a unique identification number. The sample bottle’s label will bear the NEIWPCC (IEC District) investigation #, initials of the collector, exact sampling location, time and date of collection, and analyses to be performed on that sample. The sealed, labeled bottle is then placed in a cooler containing ice and a cooler thermometer for transport to the NEIWPCC (IEC District) laboratory. A thermometer is kept inside the cooler at all times to ensure samples are kept at $\leq 4^{\circ}$ C. Upon arrival in the laboratory, the temperature of the temperature blank is read and recorded and the samples are logged into the sample log-in book and transferred to the laboratory refrigerators until filtration. Subsamples for nutrient analyses are partitioned and filtered in NEIWPCC’s IEC laboratory. Filtration and preservation should occur as soon as possible after sample collection.

A chain of custody sheet must accompany samples providing the full name of the collector, sample identification number, NEIWPCC (IEC District) investigation number, exact sampling location, time sample container was sealed, analyses to be performed on that sample, as well as

the time the sample arrived at the laboratory. Inadequately identified samples are not accepted for examination.

All staff involved in sampling are trained as to the exact location where NEIWPCC (IEC District) should take the sample and are required to review the sampling plan with the QC Officer prior to commencing sampling. Navigational coordinates for station locations and survey network are entered into the vessel's on-board GPS navigational system. Handheld GPS units are also used to verify the location of each station. Any deviation in the sample location (due to limited access or safety issues) will be detailed on the sampling plan. Staff are instructed to always have at least one extra of each required bottle in case of breakage or loss. Each sampling plan is stored and maintained within the investigation folder and file.

All chlorophyll *a*, BOD, and TSS sample bottles are placed in the refrigerator at $\leq 4^{\circ}\text{C}$ unless analysis is to be performed immediately. Samples for Chlorophyll *a* analysis must be filtered within 48 hours of sample collection. Samples must be kept $\leq 4^{\circ}\text{C}$ and in the dark until filtration and filtered in subdued light. BOD analysis must begin within 48 hours of samples collection. Once filtered, filters are frozen, and the rest of the analysis must be completed within 28 days. Samples for TSS analysis must be analyzed within 7 days.

Sample filtration and shipment processing for nutrients analyses will occur on the same day of collection, immediately upon arrival in the IEC District Laboratory. After the required sample filtration and processing, samples for DOC, PC, and PN analyses will be frozen overnight and shipped, frozen via next day carrier to UMCES CBL NASL. The person responsible for sample packaging will ensure that samples are properly packed to prevent spillage or breakage during transport. A temperature blank or temperature logger will be included in the cooler shipped to UMCES CBL NASL.

B4. Analytical Methods

Table 4 outlines the procedures used for those parameters analyzed in the field or in the IEC District Laboratory. Temperature, pH, dissolved oxygen, % saturation and salinity are field measurements. Samples collected for chlorophyll *a*, BOD, TSS, and nutrients (with the exception of Dissolved Organic Carbon, Particulate Carbon, and Particulate Nitrogen) are analyzed in the IEC District Laboratory. Samples for Particulate Carbon are filtered by the IEC District Laboratory and shipped, frozen or chilled as required, to UMCES CBL NASL for analysis along with samples for Dissolved Organic Carbon and Particulate Nitrogen analysis. A summary of filtration methods is outlined below. For more specific filtration procedures, please refer to the Nutrient Filtration SOP in Appendix C and Table 6A.

Subsamples for dissolved nutrient analyses will be taken from the 2-liter polypropylene bottle and filtered through **25 mm 0.7 μm GF/F filters (filters provided by UMCES CBL NASL)**. Filtrations will be performed on the same day of collection, immediately upon arrival in the laboratory. Time filtered will be recorded on chain of custody forms, along with sample collection information. A 200 ml aliquot of sample is filtered.

The filtrate from the filtration (undiluted; taken prior to deionized water rinse of filtration

apparatus) is transferred to two bottles. One 125 mL polypropylene bottle is filled to be used for Total Dissolved Nitrogen, Total Dissolved Phosphorus, Orthophosphate, Nitrate+Nitrite, and Ammonia analyses. Another 30 ml Teflon bottle is filled (allowing some headspace for expansion) to be used for DOC analysis. Bottles and COC must be clearly and completely labeled identify sample ID, analyses to be performed, time and date of collection, time and date of filtration, and sampler's initials.

For Particulate Carbon and Particulate Nitrogen, the filter from the above filtrations are sent to UMCES CBL NASL. Duplicate filters must have the same volume filtered through them. Particulate Carbon and Particulate Nitrogen will be analyzed from the same filter. Both (duplicate) filters will be placed in the same foil packet, not touching each other, and frozen immediately after filtration for preservation.

For Dissolved Silica, 200 mL sample will be filtered through 47mm 0.4µm polycarbonate filters, and the filter placed in a labeled centrifuge tube provided by UMCES CBL NASL and frozen for preservation. No glass of any type can be used in the sample measuring, filtering, or processing steps for silica determinations. Filtrate will be transferred to a 125 mL polypropylene bottle and chilled (not frozen) for use in dissolved silica (SiO₂) analysis. Sample collection information, including volume filtered and filter number for particulate phosphorus, will be recorded on the IEC chain of custody document (Appendix A).

All filters for PC/PN and filtrate DOC analyses will be shipped to UMCES CBL NASL via overnight carrier. Samples will be transported packed in ice to ensure filters and filtrates remain frozen.

Table 4 outlines the analytical methods used for those parameters (nutrients) subcontracted to UMCES CBL NASL for analysis.

Additional information on analytical methods is presented in the IEC Standard Operating Procedure for each parameter (Appendix C), UMCES CBL NASL Standard Operating Procedures (Appendix D) as well as the published, EPA-approved method reference, listed in Table 4. Although Appendix C and D include the current analytical SOPs, SOPs may be updated periodically due to method updates. The most current version of all SOPs is maintained on file in the laboratory and should always be referred to before beginning analyses.

B5. Analytical Quality Control

The QC guidelines apply those practices necessary to minimize systematic and random errors resulting from personnel, instrumentation, equipment, reagents, supplies, sampling and analytical methods, data handling, and data reporting. The quality control measures employed for the analyses of samples collected in this project are numerous, vary by method, and adherence to all, including those mentioned in standard operating procedures, are mandatory. A listing of key Field and IEC District Laboratory QC practices is given in Table 7 at the end of this document. The QC measures in Table 7 address accuracy, precision, bias, and sensitivity. IEC also plans to split a subset of nutrient samples and submit the split samples to UMCES NASL for method

validation and comparison purposes.

B5.1 Analytical Quality Control UMCES CBL NASL (subcontract laboratory)

For nutrient parameters analyzed by UMCES CBL NASL, a number of routine quality control (QC) checks are analyzed with each batch of samples, including continuing calibration verification, calibration blanks, laboratory duplicates, and spike sample analyses. Detailed method-specific Quality Control checks are also summarized in each of UMCES CBL NASL'S Standard Operating Procedures (Appendix D) and at <http://nasl.cbl.umces.edu/>.

TABLE 4: Analytical Methods

<i>Analytes</i>	<i>Sample Matrix</i>	<i>Project Action Level</i>	<i>Analytical Method</i>	<i>Method Detection Limit</i>	<i>Laboratory Reporting Limit</i>
Temperature	Non-Potable Water	N/A	SM2550 B-00	-5 to 65 Degrees C	-5 to 65 Degrees C
Dissolved Oxygen	Non-Potable Water	N/A	ASTM D888-09C	0-.1-50 mg/L	0.1-50 mg/L
Chlorophyll <i>a</i>	Non-Potable Water	N/A	EPA 445.0	0.7µg/L	02.1µg/L
pH	Non-Potable Water	6.0 to 9.0 SU	SM 4500-H B-11	0.01 to 14 SU	0.01 to 14 SU
Salinity	Non-Potable Water	N/A	SM 19-20 2520 B	0 to 70 ppt	0 to 70 ppt
Total Suspended Solids (TSS)	Non-Potable Water	N/A	SM 2540D 97,-11	2.5mg/filter*	2.5mg/filter*
Biochemical Oxygen Demand	Non-Potable Water	N/A	SM 5210B-01,-11	3mg/L	3 mg/L
Particulate Carbon*	Non-Potable Water	N/A	EPA 440	0.0633 mg/L	0.1899 mg/L
Particulate Nitrogen*	Non-Potable Water	N/A	EPA 440	0.0263 mg/L	0.0789 mg/L
Particulate Phosphorus	Non-Potable Water	N/A	EPA 365.1	10 µg/L	0.10mg/L
Biogenic Silica	Non-Potable Water	N/A	EPA 366.0	0.0009mg/L	0.01mg/L
Dissolved Silica	Non-Potable Water	N/A	EPA 366.0	0.0009 mg/L	0.01 mg/L

<i>Analytes</i>	<i>Sample Matrix</i>	<i>Project Action Level</i>	<i>Analytical Method</i>	<i>Method Detection Limit</i>	<i>Laboratory Reporting Limit</i>
Orthophosphate (DIP)	Non-Potable Water	N/A	EPA 365.1	0.001mg/L	0.005mg/L
Total Dissolved Phosphorus	Non-Potable Water	N/A	EPA 365.1	0.334mg/L	1.67mg/L
Dissolved Organic Carbon*	Non-Potable Water	N/A	EPA 415.1	0.16 mg/L	0.80mg/L
Total Dissolved Nitrogen	Non-Potable Water	N/A	EPA 353.2	0.05 mg/L	0.05 mg/L
Ammonia	Non-Potable Water	N/A	Lachat 10-107-6-1B	0.020 mg/L	0.1 mg/L
Nitrate+Nitrite (cadmium reduction method)	Non-Potable Water	N/A	EPA 353.2	0.004 mg/L	0.02mg/L
Nitrate	Non-Potable Water	N/A	EPA 353.2	0.006 mg/L	0.003mg/L
Nitrite	Non-Potable Water	N/A	EPA353.2	0.002 mg/L	0.01mg/L

*These analyses will be performed by UMCES CBL NASL in Solomons, MD.

**For TSS, a sample volume that yields a residue between 2.5 and 200 mg should be chosen. Accordingly, the MDL and reporting limit is 2.5 mg/filter (with volume filtered determining the final result). When 2.5 mg net weight of crucible is not achieved, samples results must be qualified as “estimated.”

B5.2 Quality Control Pre-Analysis

Table 5 partially describes the quality control activities that will be conducted in association with analyses in the field or in the NEIWPCC-IEC District Laboratory. See individual analytical SOPs (Appendix C and Appendix D) for parameter-specific quality control activities described in more detail.

B6. Equipment Testing, Inspection, and Maintenance

Initial instrument set-up (sensor installation, setting the date and time) and calibration for the YSI EXO Sondes is performed in the laboratory by an environmental analyst. Information on instrument calibration, frequency of calibration, inspection, and maintenance is summarized in Table 5. More detailed information, along with calibration and inspection criteria can be obtained from the respective standard operating procedures and manufacturer-supplied equipment manuals.

If a problem is indicated by any of the instrument performance checks or preventive maintenance measures, corrective action will be taken immediately. If laboratory personnel cannot satisfactorily remedy the malfunction, maintenance by manufacturer-approved service personnel will be immediately arranged. If the malfunctioning equipment makes it impossible for the analysis to proceed or to confidently yield results of high quality, analysis will **cease**, and no further sampling will be performed until the equipment is in satisfactory working order and passes all performance checks. In the case of service provided by an outside service provider, the receipt or work order form should indicate that the service provider has deemed the equipment in proper working order and be initialed by the service provider and the appropriate laboratory personnel. Samples that were being analyzed when the equipment was discovered to be malfunctioning will be properly disposed of and will not be used to generate any data. In this case, sampling rounds may need to be repeated to complete the project requirements.

TABLE 5: Key Analytical QC Activities for Field Parameters and NEIWPCC-IEC District Parameters

<i>Analyte</i>	<i>Data Quality Indicator</i>	<i>QC Check</i>	<i>Frequency</i>	<i>Acceptance Criteria</i>	<i>Corrective Action</i>
Salinity	Precision and Bias	Calibrate with NIST conductivity standards (calibrating the YSI for conductivity automatically calibrates for salinity)	Before each day's use	90-110%	Re-calibrate. Replace membrane/electrode if necessary.
Temp.	Precision and Bias	Calibrate with NIST thermometer in lab. Record correction factor.	Monthly, or more frequently if readings are suspected to be erroneous	temperature probe must read within 0.5°C of standard thermometer	Recalibrate. Replace probe if necessary.
Dissolved Oxygen	Precision and Bias	Perform DO % calibration according to manufacturer's instructions and record.	Before each day's use	90-110%	Recalibrate. Check moisture of calibration sleeve, replace membrane if necessary.
		Compare DO reading to winkler titration and record	Before each day's use	90-110%	Recalibrate. Repeat comparison. Check winkler against back-up meter.
		Compare meter reading to second meter and record	Before each day's use	Readings should vary by no more than 5% of their average	Recalibrate. Repeat comparison.
		Post calibration zero DO verification with	End of day	0-0.5 mg/L DO with addition of	Verify anhydrous sodium sulfite was used. Qualify data if

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<i>Analyte</i>	<i>Data Quality Indicator</i>	<i>QC Check</i>	<i>Frequency</i>	<i>Acceptance Criteria</i>	<i>Corrective Action</i>
		addition of sodium sulfite			DO reading does not zero after additional reading or with addition of additional sodium sulfite.
Chlorophyll a	Precision and Bias	A check standard and blank is analyzed at the beginning of each analytical batch	Each batch	90-110%	Re-analyze standard. Ensure fluorometer is properly warmed up and wavelengths/lamp correctly set.
pH	Precision and Bias	Calibrate meter with pH 4 and 10 buffer, read pH 4 buffer (buffers values used may vary depending on expected range of sample pH)	Before each day's use	pH 7 buffer must read 5.00±0.10 SU.	Re-calibrate, replace probe solution, buffers and/or probe as necessary.
		Duplicate samples with each batch (batch is defined as ≤ 20 samples).	Each batch	±20%	Recalibrate. Use back up meter if necessary. Inform Senior Manager (QC Officer)
		Perform CCV (analyze pH 7 buffer) at end of sample batch	Each batch	pH 7 buffer must read 7.00±0.10 SU.	Recalibrate and resample.
TSS	Precision and Bias	Read and record temperature of oven	Before and after each use	103°C to 105°C	Adjust temperature. Delay analysis until temperature is within limits. Avoid prolonged opening of oven door to minimize temperature change during analysis.
		Check accuracy of balances	Before each day's use	50g : 49.99700-50.0030 20g: 19.99500-20.00200 0.050g: 0.04900-0.05100	Make sure balance is level. Adjustment of internal calibration may be necessary (report to QC Officer). Re-check weights.
BOD	Precision and Bias	Glucose and Glutamic acid Check	Each Batch	198±30 mg/l	Flag data with appropriate qualifying notes. Consistent non-conformities indicate a problem with the quality system and analyses should be discontinued until root causes investigated
		seeded dilution blanks		0.6 to 1.0 mg/l	
		unseeded dilution blanks		0.2 mg/l	
Particulate Phosphorus, Orthophosphate, Total Dissolved Phosphorus	Precision and Bias	LCS	Each Batch	±10%	Flag data with appropriate qualifying notes. Consistent non-conformities indicate a problem with the quality system and analyses should be discontinued until root causes investigated
		Duplicates			
		CCV			

<i>Analyte</i>	<i>Data Quality Indicator</i>	<i>QC Check</i>	<i>Frequency</i>	<i>Acceptance Criteria</i>	<i>Corrective Action</i>
Particulate Nitrogen, Total Dissolved Nitrogen, Nitrate/Nitrite	Precision and Bias	LCS	Each Batch	±10%	Flag data with appropriate qualifying notes. Consistent non-conformities indicate a problem with the quality system and analyses should be discontinued until root causes investigated
		Duplicates			
		CCV			
Biogenic and Dissolved Silica	Precision	LCS	Each Batch	±10%	Flag data with appropriate qualifying notes. Consistent non-conformities indicate a problem with the quality system and analyses should be discontinued until root causes investigated
		Duplicates			
		CCV			

B7. Equipment Calibration and Frequency

Information on instrument calibration, frequency of calibration, inspection, and maintenance is provided in Table 5. More detailed information, along with calibration and inspection criteria can be obtained from the respective standard operating procedures as well as the manufacturer-supplied equipment manuals.

B8. Inspection/Acceptance of Supplies and Consumables

Samples for Chlorophyll *a* and TSS analysis are collected in 1000 mL (1 liter) opaque Nalgene polyethylene bottles that are inspected upon arrival and before each use to ensure they are free from leaks. Bottles are washed with laboratory detergent and deionized water and dried before each use. Spare parts for the YSI EXO 1 Multiparameter Sondes are ordered through YSI, the meter manufacturer. Persons receiving and unpacking a shipment of supplies and consumables will mark all contents with the date of receipt. All shipments are unpacked immediately upon receipt to ensure that proper storage conditions (*i.e.*, temperature) are provided. All chemicals, reagents, and standards must be logged in a Chemical Inventory Logbook upon unpacking. The name of the chemical, date received, manufacturer, catalog #, lot #, # of containers received, expiration date, and storage requirements are logged in this logbook. The catalog number of each chemical/reagent/standard is checked against the purchase order to make sure that the correct item was received. When the chemical/reagent/standard is first opened for use, it is marked with the date opened and initials of the person opening it. This information is also recorded in the chemical inventory logbook. If a staff member has any concern about the quality of a chemical/reagent/standard, the staff member should bring it to the attention of the QC Officer, who will determine if the consumable in question is appropriate for use. All supplies that have a manufacturer-designated expiration date will be used or discarded before that date.

B9. Non-Direct Measurements

Precipitation measured at LaGuardia Airport for the 24 hours and 48 hours prior to start of each

survey is obtained from the NOAA (NWS) website and recorded on the field data sheet. Last high and low tides at Kings Point, NY, and New Rochelle, NY, are obtained from NOAA's website and recorded on the field data sheet.

B10. Data Management

All original field and laboratory sheets will be scanned and stored electronically. Data will be made available upon request. All reports and the final report will be stored electronically for easier distribution. Computer files are backed up weekly.

C1. Assessments and Response Actions

NEIWPCC or IEC may, at their discretion, perform various audits or reviews of this project to assess conformance and compliance to the quality assurance project plan in accordance with the NEIWPCC Quality Management Plan. The QC Officer participates in at least one survey per summer to perform a field audit (assessment). Typically, this is the first survey of the season so that any issues with field measurements or sample collection or site selection (*e.g.*, navigational hazards making it difficult to reach a specific sample site) can be addressed, corrective actions implemented, the QAPP amended if necessary, and the appropriate parties notified.

Also, IEC District's QC Officer will review all data before the report is ready to be finalized. A final report is then generated in conjunction with CTDEEP. Prior to final report dissemination, the final report is reviewed by IEC's QC Officer.

C2. Reports to Management

Weekly updates of the sampling results will be disseminated weekly to management as well as LIS stakeholders discussed in Section A9. Quarterly progress is reported in the IEC's quarterly report. At the end of the sampling season, all data are summarized, after appropriate reviews in a final report that is disseminated to management, LIS stakeholders, and posted on the IEC website.

D1. Data Review, Verification, and Validation

Data review, verification, and validation will be the responsibility of IEC. Data sets produced during the project will be verified after each data set has been generated. The completeness, correctness, and compliance against the quality objectives and criteria set forth in this QAPP will be checked according to the following procedure:

- QC Officer will ensure that field and sample collection activities have been performed according to standard operating procedures and that the necessary documentation of calibration and post-survey QC activities has been generated by reviewing calibration and field data sheets.
- QC Officer will ensure that samples shipped to UMCES CBL NASL for analysis have been packaged properly to minimize the likelihood of leakage and ensure proper temperature preservation is maintained throughout shipment. QC Officer will verify that samples were received by UMCES CBL NASL within appropriate holding times and

within appropriate temperature (preservation ranges) and request final COC from UMCES CBL NASL.

- QC Officer will ensure that laboratory activities and sample analyses performed by both the NEIWPEC-IEC District Laboratory and UMCES CBL NASL have been performed according to standard operating procedures and that the necessary QC documentation and analyses (duplicates, matrix spikes) have been generated.
- QC Officer will review all field and laboratory documentation produced by both the NEIWPEC-IEC District Laboratory and UMCES CBL NASL and identify non-conformances to quality objectives and criteria.

Data validation will follow data verification after each data set has been generated and at the end of the data entry process. The Quality Control Officer will evaluate the impact of any technical or quality control non-conformities identified in the data verification process taking into consideration field and laboratory documentation.

Upon verifying and validating all data sets generated during the project, these sets will be disseminated to data users.

D2. Verification and Validation Methods

Analytical data will be validated by assembling planning documents (QAPP, standard operating procedures, field and laboratory logs), data to be validated; evaluating field documents for consistency and completeness; determining method and procedural quality control non-conformities (duplicate comparison, sample preservation, etc.); evaluating completeness objectives (% completeness), and evaluating the impact of those on the overall data quality of the complete data set.

D3. Reconciliation with User Requirement

In the event that non-conformities exist, they, as well as their overall impact on the project's goals, will be reported along with the final report. Any uncertainty in the validated data or any potential limitation on data use will be described in the final report.

E. REFERENCES

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5. ASTM November, 2009. D888-09(C) Standard Test Methods for Dissolved Oxygen in Water.
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11. State of Connecticut, Department of Environmental Protection, Bureau of Water Management, 2017. Quality Assurance Project Plan for Long Island Sound Ambient Water Quality Monitoring Program
12. State of New Jersey, Department of Environmental Protection, Office of Quality Assurance, September 2002. Regulations Governing Laboratory Certification and Standards of Performance, N.J.A.C. 7:18, as adopted June 6, 2001 and amended September 16, 2002, Trenton, N.J.
13. University of Maryland, Center for Environmental Science, Chesapeake Biological Laboratory, Nutrient Analytical Services Laboratory, 2009. Standard Operating Procedures.
14. US Environmental Protection Agency eCFR (online) Title 40, Parts 1-1700.

TABLE 6: Parameter Holding Times

Parameter	Analytical Method (Reference)	Sample Preservation	Holding Time
pH	SM 4500-H B-11	N/A	Immediately
Temperature	SM 2550 B-00	N/A	Immediately
Dissolved Oxygen	ASTM D888-09C	N/A	Immediately
Chlorophyll <i>a</i>	EPA 445.0	Cool to <4°C, dark	Filter within 48 hours, complete analysis within 28 days
Salinity	SM 20 2520 A&B	N/A	Immediately
Total Suspended Solids (TSS)	SM 2540D-11	Cool to <4°C	7 days
Biochemical Oxygen Demand	SM 5210B-11	Cool to <4°C	48 hours
Particulate Carbon*	EPA 440	Filter/Freeze to -10°C	1 year
Particulate Nitrogen*	EPA 440	Filter/Freeze to -10°C	1 year
Particulate Phosphorus	EPA 365.1	Filter/Freeze to -10°C	1 year
Biogenic Silica	EPA 366.0	Filter/Freeze to -10°C	1 year
Dissolved Silica	EPA 366.0	Filter/Filtrate Refrigerated to 4°C (do not freeze)	28 days
Orthophosphate (DIP)	EPA 365.1	Filter/Filtrate Frozen to -10°C	1 year
Total Dissolved Phosphorus	EPA 365.1	Filter/Filtrate Frozen to -10°C	1 year
Dissolved Organic Carbon*	EPA 415.1	Filter/ Filtrate Frozen to -10°C	1 year
Total Dissolved Nitrogen	EPA 353.2	Filter/Filtrate Frozen to -10°C	1 year
Ammonia	EPA 350.2	Filter/Filtrate frozen to -10°C	1 year
Nitrate+Nitrite	EPA 353.2	Filter/Filtrate frozen to -10°C	1 year
Nitrate	EPA 353.2	Filter/Filtrate frozen to -10°C	1 year
Nitrite	EPA 353.2	Filter/Filtrate frozen to -10°C	1 year

*These parameters will be analyzed by UMCES CBL NASL. Holding times, sample preservation and analytical methods are summarized here from UMCES CBL NASL Standing Operating Procedures (Appendix D).

**Frozen filters/filtrates are stable for 1 year, but typically analyzed within 28 days. Acidified and refrigerated (4°C) samples must be analyzed within 28 days.

Table 6A. Summary of sample type, containers, preservation, and holding times for Ambient Water Quality Monitoring in Western Long Island Sound for Nutrients

<u>VARIABLE</u>	<u>SAMPLE TYPE</u>	<u>SAMPLE CONTAINER</u>	<u>PRESERVATION METHOD</u>	<u>HOLDING TIME</u>
Ammonia (NH_3)	Filtrate, generally 200 mL filtered through 25 mm/0.7 μm GF/F	250-ml poly bottle with lid	Sample frozen	14 days
Nitrate + Nitrite (NO_3^-) + (NO_2^-)	Filtrate, generally 200 mL filtered through 25 mm/0.7 μm GF/F	250-ml poly bottle with lid	Sample frozen	1 year
Total Dissolved Nitrogen (TDN)	Filtrate, generally 200 mL filtered through 25 mm/0.7 μm GF/F	250-ml poly bottle with lid	Sample frozen	1 year
Particulate, Nitrogen (PN)	Filter, generally 200 mL filtered through 25mm 0.7 μm GF/F filter	aluminum foil packet; 2 duplicate filters per packet	Filter frozen	1 year
Orthophosphate (PO_4^{3-}) / Dissolved Inorganic Phosphorus (DIP)	Filtrate, generally 200 mL filtered through 25 mm/0.7 μm GF/F	250-ml poly bottle with lid	Sample frozen	1 year
Total Dissolved Phosphorus (TDP)	Filtrate, generally 200 mL filtered through 25 mm/0.7 μm GF/F	250-ml poly bottle with lid	Sample frozen	1 year
Particulate Phosphorus (PP)	Filter, generally 200 mL filtered through 47mm 0.7 μm GF/F filter	pre-labeled, filter-specific aluminum cup	Filter frozen	1 year
Dissolved Organic Carbon (DOC)	Filtrate, generally 200 mL filtered through 47 mm/0.7 μm GF/F	250-ml poly bottle with lid	Sample frozen	1 year
Particulate Carbon (PC)	Filter, generally 200 mL filtered through 25mm 0.7 μm GF/F filter	aluminum foil packet; 2 duplicate filters per packet	Filter frozen	1 year
Dissolved Silica (SiO_2)	Filtrate, generally 200 mL filtered through 47 mm/0.4 μm polycarbonate filter; filtrate chilled	125-ml poly bottle with lid	Sample chilled (not frozen)	28 days
Particulate (Biogenic) Silica (BioSi)	Filter, generally 200mL filtered through 47mm 0.4 μm polycarbonate filter	50-ml poly centrifuge tube with lid	Filter frozen	1 year

TABLE 7: Quality Control Measures NEIWPCC-IEC District Laboratory

Item	Laboratory/Field QC Check	Frequency	Acceptance Criteria	Corrective Action
YSI EXO 1 Multi-parameter Sonde	See Table 5 for Key Analytical QC measures	Before each use	See table 5	See table 5
	Meter is checked/calibrated in the laboratory	Weekly during summer, monthly	See table 5	See Table 5
Reagent grade water	Chemical Test: Conductivity pH Heavy metals, single (Cd, Cr, Cu, Ni, Pb, and Zn) Heavy metals, Total Total residual chlorine TOC Ammonia/Organic Nitrogen *Reagent grade water is used for rinse purposes only for chlorophyll analyses.	Monthly Each use Annually Annually Monthly Annually Annually	<2 mhos/cm at 25 ⁰ C 5.5 – 7.5 <0.05 mg/L <0.10 mg/L <0.01 mg/L <1.0 mg/L <0.10 mg/L	Stop utilizing the water. Repeat the test to confirm results. Identify and remedy the malfunction.
Thermometers	Professional Calibration of NIST Traceable	Annually	Calibration certificates and correction factors provided by the manufacturer	Stop using if certificates and correction factors are not provided.
	Calibration of other thermometers	Annually	<±0.5 ⁰ C (Tag with correction factor for less than ±0.5 ⁰ C)	Stop using if temperature differs by more than ±0.5 ⁰ C. Use back up.
Balances and weights	Check accuracy	Each day of use	±2% from certified weights	Stop using. Request professional maintenance. Use back up.
	Manufacturer's Inspection and Maintenance	Annually	Certificates provided by the manufacturer	Stop using if certificates are not provided

TABLE 7: Quality Control Measures (*Continued*)

Item	Laboratory QC Check	Frequency	Acceptance Criteria	Corrective Action
Oven	Check thermometer against NIST thermometer	Annually	Mark correction factor, not to exceed $\pm 1^{\circ}\text{C}$.	Procure new thermometer.
	Check oven thermometer	Before and after each oven use	103°C-105°C	Adjust oven temperature. Delay analysis until temperature is within specifications.
Refrigerator	Check temperature	Twice Daily	$<4^{\circ}\text{C}$	Discontinue use.
BOD incubator	Check temperature	Twice Daily	19-21°C	Discontinue use until temperature has been adjusted and remained in acceptable range for 4 hours.
Glassware	Inspect for cleanliness, chips, etching, and pH	Each Use	No signs of breakage.	Discard and use back up.
			Bromothymol blue test must be neutral (no color change) and the pH should fall within the range of 6.0-7.6.	Redo cleaning.
Samples	5% are run in duplicates	Each batch	Duplicates should not vary by more than 15% of each other.	Do not use the results.

Appendix A

CHAIN OF CUSTODY FORM

Interstate Environmental Commission Laboratory

2800 Victory Boulevard, Bldg. 6S Rm. 106

Staten Island, NY 10314 Ph: 718-982-3792

Page _____ of _____

SITE/INVESTIGATION #:					Date/Time Sampling Began:				
Address:					Date/Time Sampling Ended:				
City:					Sampled by:				
State:		Zip:			Date/Time Transferred to IEC Lab:				
SPDES #					Relinquished By:		Received By:		
Sample ID	Sealed		Matrix	Preservative	Sample Description		Unsealed	Destroyed	Analyses
	Time/Date	By			Grab/Comp	Location	D/T/I	D/T/I	

Matrix Code: WW= Wastewater SW= Surface (ambient) Water

D/T/I= Date/Time/Initials

COMMENTS: _____

Appendix B

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NEIWPCC IEC DISTRICT LABORATORY
LONG ISLAND SOUND SURVEY FIELD DATA SHEET

DATE OF SAMPLING: _____ IEC INVESTIGATION #: _____

CREW/SAMPLING TEAM: _____

WEATHER CONDITIONS: _____

LAST HIGH/LOW TIDE: _____ H/L SOURCE: _____

_____ H/L SOURCE: _____

RAIN FOR PREVIOUS 24hr. _____ SOURCE: _____

48hr. _____ SOURCE: _____

STATION (FEET)	DEPTH (METERS)	TIME (DST)	TEMP. (°C)	S ‰ (PPT)	D.O. (mg/l)	% saturation	pH	% CLOUD COVER	SEA STATE	SECCHI DEPTH (FEET)
9-413										
9-412										
9-409										
A3										
8-405										
8-403										

WERE SAMPLES TAKEN? YES _____ NO _____; If YES, attach chain of custody.

METER CALIBRATION:

Dissolved Oxygen: WINKLER _____ METER #1 _____ METER#2 _____
pH Calibration: pH 4 _____ pH 10 _____ pH 7 (read) _____
Salinity (Conductivity) Calibration: Certified value _____ Meter value _____

DOCKSIDE METER READINGS:

D.O. _____ TEMP _____ SALINITY _____ pH _____
 DATE _____ TIME _____ INITIALS _____

COMMENTS:

<u>Analyte</u>	<u>Method</u>
Temperature	SM2550 B-00
Salinity	SM19-20 2520 B
Dissolved Oxygen	SM4500-O G-11
pH	SM4500-H B-11

IEC EXO1 Meter Calibration Sheet: **Pre Sampling**

YSI Meter: _____

Serial Number: _____

PH Calibration Pre Sampling: Date: _____ Time: _____ Initials: _____

Manufacturer	Buffer Value	Catalog Number	Lot Number	Expiration Date	Pre Cal.	Post Cal.
FISHER	4.00	SB101-500				
FISHER	10.00	SB115-500				
FISHER	7.00	SB107-500			READ:	

Specific Conductance/Salinity Calibration Pre Sampling: Date: _____ Time: _____ Initials: _____

Manufacturer	Specific Value	Catalog Number	Lot Number	Expiration Date	Pre Cal.	Post Cal.
RICCA	50,000 μs/cm	2248-1				

Dissolved Oxygen Calibration Pre Sampling: Date: _____ Time: _____ Initials: _____

Temp.	Baro. Pressure	Pre Cal. %	Post Cal. %

Winkler mg/L	Meter 1 %	Meter 1 mg/L	Meter 2 %	Meter 2 mg/L

Depth Calibration Pre Sampling: Date: _____ Time: _____ Initials: _____

Location	Pre Cal.	Post Cal.
LAB		

Chlorophyll A Probe Calibration Pre Sampling: Date: _____ Time: _____ Initials: _____

Chl. A	Manufacturer	Type	Catalog Number	Lot Number	Expiration Date	Pre Cal.	Post Cal.
RFU	RICCA	ASTM TYPE II	9150-5				
μg/L	RICCA	ASTM TYPE II	9150-5				

YSI Meter Calibration Sheet **Post Sampling**

YSI Meter: _____

Serial Number: _____

Sampling Event: _____

PH Calibration Check Post Sampling: Date: _____ Time: _____ Initials: _____

Manufacturer	Buffer Value	Catalog Number	Lot Number	Expiration Date	Post Sampling
FISHER	7.00	SB107-500			

Did the meter read within ± 0.2 of the buffer value? _____

Specific Conductance/Salinity Check Post Sampling: Date: _____ Time: _____ Initials: _____

Manufacturer	Specific Value	Catalog Number	Lot Number	Expiration Date	Post Sampling
RICCA	50,000 $\mu\text{S}/\text{cm}$	2248-1			

Did the meter read within $\pm 500\mu\text{S}$ of the specific value? _____

Dissolved Oxygen Check Post Sampling: Date: _____ Time: _____ Initials: _____

Temperature	Baro. Pressure	Reading %, mg/L	Addition of Sodium Sulfite Anhydrous?	If Yes, Zero Reading mg/L

Sodium Sulfite Anhydrous Manufacturer: _____ Catalog Number: _____

Lot Number: _____

Depth Check Post Sampling: Date: _____ Time: _____ Initials: _____

Location	Reading
LAB	

APPENDIX C: **IEC SOPS**

DISSOLVED OXYGEN
(Optical Luminescent-Based Sensor Method)

Section FIELD III Revision No. 1, Effective Date March 2016

1) Test Method

Based on ASTM D888-09(C)

2) Applicable Matrix or Matrices

This method is applicable to all water and wastewater matrices that are free from interferences. Optical luminescence based sensors provide an excellent method for rapid DO analysis in the field.

3) Method Detection Limit

The reporting limits for the specific dissolved oxygen meter currently used (EXO1 sonde) is 0.1mg/L-50 mg/L.

4) Scope and Application

This method is used to measure dissolved oxygen in-situ of ambient waters in conjunction with ambient water quality monitoring surveys.

5) Summary of Method

The principle of operation of the optical luminescence based dissolved oxygen sensor is on the concept that dissolved oxygen quenches both the intensity and the lifetime of the luminescence associated with a carefully chosen chemical dye. The EXO DO sensor currently utilized in this method operates by shining a blue light of the proper wavelength on this luminescent dye which is immobilized in a matrix and formed into a disk. The blue light causes the immobilized dye to luminesce and the lifetime of this dye luminescence is measured via a photodiode in the probe. To increase the accuracy and stability of the technique, the dye is also irradiated with red light during part of the measurement cycle to act as a reference in the determination of the luminescence lifetime. When there is no oxygen present, the lifetime of the signal is maximal; as oxygen is introduced to the membrane surface of the sensor, the lifetime becomes shorter. Thus, the lifetime of the luminescence is inversely proportional to the amount of oxygen present and the relationship between the oxygen pressure outside the sensor and the lifetime can be quantified by the Stern-Volmer equation. For most lifetime-based optical DO sensors, this Stern-Volmer relationship $((T_{\text{zero}}/T)-1)$ versus O_2 pressure is not strictly linear (particularly at higher oxygen pressures) and the data must be processed by polynomial non-linear regression. Fortunately, the non-linearity does not change significantly with time so that, as long as each sensor is characterized with regard to its response to changing oxygen pressure, the curvature in the relationship does not affect the ability of the sensor to accurately measure oxygen for an extended period of time.

6) Definitions

Dissolved Oxygen- The term refers to oxygen which is dissolved in water. Oxygen can become dissolved in water through a variety of methods. Atmospheric oxygen can become dissolved through wave and wind action.

Dissolved Oxygen mg/L- A measurement of oxygen in mg/L.

Dissolved Oxygen %- A measurement of oxygen in percent of saturation.

7) Interferences

Variables that could affect dissolved oxygen measurements include temperature, salinity, and barometric pressure. Temperature and salinity are compensated for during instrument calibration and field use with the use of additional sensors and/or instrument software settings. Barometric pressure relates to the pressure of oxygen in the calibration environment, and barometric pressure changes due to a change in altitude or local weather. Generally, the effect of barometric pressure is overcome by proper sensor calibration to a standard pressure.

8) Safety

There are minimal safety concerns when utilizing this method. When performing Winkler titration, use appropriate personal protective equipment (PPE) including gloves, goggles, and a labcoat to minimize exposure to the reagents utilized in the azide-modification Winkler titration.

9) Equipment and Supplies

1. Sample container (only if necessary). Use narrow-mouth glass-stoppered BOD bottles of 300 mL with tapered and pointed ground-glass stoppers.
2. EXO 1 sonde multi-meter equipped with EXO handheld unit and 66 m (216 ft). A fully-equipped back-up meter should be available in case of meter malfunction.
3. Spare D batteries (4)

10) Reagents and Standards

1. See Dissolved Oxygen Winkler Titration (SOP ID IX) for required reagents for comparing EXO1 readings to Winkler titration.
2. Sodium Sulfite, anhydrous (to zero out DO as post run CCV)

11) Sample Collection, Preservation, Shipment and Storage

Because the EXO1 sonde offer the advantage of analysis in situ they eliminate errors caused by sample handling and storage. If sampling is required, collect samples very carefully. Do not let

sample remain in contact with air or be agitated, because either condition causes a change in its gaseous content. Samples from any depth in streams, lakes, or reservoirs need special precautions to eliminate changes in pressure and temperature. Collect surface water samples in narrow-mouth glass-stoppered BOD bottles of 300 mL capacity with tapered and pointed ground-glass stoppers and flared mouths. Avoid entraining or dissolving atmospheric oxygen. Let bottle overflow two or three times its volume and replace stopper so that no air bubbles are entrained. Use a Kemmerer-type sampler for samples collected from depths greater than 2 m. Record sample temperature to nearest degree celsius. Collected samples must be tested within 15 minutes of sample collection. Alternatively, samples may be fixed in the field (by adding the first two reagents (manganous sulfate and alkali-iodide-azide reagents) and iodide liberated within 8 hours of collection by addition of sulfuric acid. Sample must be tested within 15 minutes of liberating the iodide. See Dissolved Oxygen Modified Winkler (IEC SOP ID IX) for procedure for fixing and liberating iodide.

12) Quality Control

Calibrate meter in the laboratory by comparing to a Winkler titration of dissolved oxygen in aerated reagent-grade water in the lab at least once a month or more often in the case of regular surveys (within 24 hours of start of survey) or if meter malfunction is suspected. Calibrate meter on day of use by performing an air %DO calibration in the field and by performing a zero point check with a 0 mg/L DO sample (prepared by adding sodium sulfite to reagent grade water until meter reads the sample as 0mg/L). Inspect meter and probe during these checks for any issues that could affect performance such as fouled probes or dull sensor. Inspect meter case for spare parts and batteries. Compare the measured values against the readings obtained from the Winkler titration. Winkler readings must be within ± 0.3 mg/L of the meter value.

Refer to EXO1 Sonde user manual, sections 1.8, 2.1, 2.2, 2.3, 3.1, 3.3, 5.1, 5.3, 6.1, 6.2, 6.3, 6.8, 6.9, 6.16, 6.17 for information of battery replacement, calibration cup installation, sensor installation, basic sonde calibration, storage, maintenance, rehydration, sensor cap replacement and procedures to maintain the integrity of the sonde and DO sensor.

13) Calibration and Standardization

EXO sensors (except temperature) require periodic calibration to assure high performance. Calibration procedures follow the same basic steps with slight variations. Conduct calibrations in a temperature-controlled laboratory.

Review Basic Calibration in section 5.1 of EXO user manual.

ODO% sat and ODO % local- 1 point

Place the sonde with sensor into either saturated air or saturated water:

- (a) Saturated air: Ensure that there are no water droplets on the DO sensor or the thermistor. Place into a calibration cup containing about 1/8 inch of water that is vented by loosening the threads. (Do not seal the cup to the sonde.) Wait 10-15 minutes before proceeding to allow the temperature and oxygen pressure to equilibrate. Keep out of direct sunlight.

- (b) Saturated water: Place into a container of water which has been continuously sparged with an aquarium pump and air stone for one hour. Wait approximately 5 minutes before proceeding to allow the temperature and oxygen pressure to equilibrate.

In the Calibrate menu, select ODO, then select ODO% sat or ODO % local. Calibrating in ODO % sat automatically calibrates ODO mg/L and ODO% local and vice versa.

Enter the current barometric pressure in mm of Hg (Inches of Hg x 25.4 = mmHg).

NOTE: Laboratory barometer readings are usually “true” (uncorrected) values of air pressure and can be used “as is” for oxygen calibration. Weather service readings are usually not “true”, i.e., they are corrected to sea level, and therefore cannot be used until they are “uncorrected.” An approximate formula for this “uncorrection” (where BP readings MUST be in mmHg) is: True BP=[corrected BP]- [2.5*(Local Altitude in meters above sea level/100)]

Click 1 Point for the Calibration Points. Enter the standard value (air saturated.)

Click Start Calibration. Observe the readings under Current and Pending data points and when they are Stable (or data shows no significant change for approximately 40 seconds), click Apply to accept this calibration point.

Click Complete. View the Calibration Summary Screen and QC score. Click Exit to return to the sensor calibration menu, and then the back arrows to return to main Calibrate menu.

Mg/L- 1 point

Place the sonde with sensor in a container which contains a known concentration of dissolved oxygen in mg/L and that is within $\pm 10\%$ of air saturation as determined by one of the following methods:

- Winkler titration
- Aerating the solution and assuming it is saturated
- Measurement with another instrument

Carrying out DO mg/L calibrations at values outside the range of $\pm 10\%$ of air saturation is likely to compromise the accuracy specification of the EXO sensor. For highest accuracy, calibrate in % saturation.

In the Calibrate menu, select ODO, then select ODO mg/L. Calibrating in ODO mg/L automatically calibrates ODO% sat and vice versa.

Click 1 Point for Calibration Points. Enter the known mg/L concentration for the standard value. Click Start Calibration. Observe the readings under Current and pending data points and when they are stable (or data shows no sign of significant change for approximately 40 seconds), click Apply to accept this calibration point. Click Complete.

Rinse the sonde and sensor(s) in tap or purified water and dry.

14) Procedure

Turn the instrument on and wait 5-15 minutes. Remove the calibration cup from the sensor/cable

and install the weighted sensor guard to protect the sensor and membrane. Place the sonde in the sample (waterbody) and deploy to the desired depth. Allow the temperature readings to stabilize. Once the values plateau and stabilize, you may record the measurement and/or log the data set. The dissolved oxygen reading may drop over time if stirring is ceased. If placing the DO sensor into a stream or fast flowing water it is best to place it perpendicular to the flow and NOT facing into the flow. In deep water, the probe may drift. Weights are stored within the sonde kit and can be anchored onto EXO1 sensor guard to minimize drift.

15) Calculations

1. Instrument is designed to provide measurements of dissolved oxygen % and dissolved oxygen mg/L and displays these readings on the LCD screen.

16) Method Performance

See SOP for Winkler Titration. Compare dissolved oxygen results with Winkler Titration method within 24 hours of start of run and record. The readings must correspond to within ± 0.3 mg/L of each other.

Perform a %DO saturation calibration prior to start of sampling run.

Analyze dissolved oxygen in aerated reagent-grade water in the lab at least once a month or more often in the case of regular surveys or if meter malfunction is suspected. Inspect meter and probe during these checks for any issues that could affect performance such as fouled membrane or dull sensor. Inspect meter case for spare parts and batteries. Compare the measured values against the readings obtained from the dissolved oxygen Winkler titration. The readings must correspond to within ± 0.3 mg/L of each other.

17) Pollution Prevention

The MSDS for all reagents are stored in a file in the laboratory. All analysts should review the relevant MSDS before using any reagent. Clean up all spills immediately. All cleaning or treatment of probe should be done in the lab, not in the field, to prevent spillage of detergents or reagents into the environment.

18) Data Assessment and Acceptance Criteria for Quality Control Procedures

The quality control measures must include the check for the accuracy of the method within 24 hours of use against the Winkler titration method (SM 4500-O C., IEC SOP ID IX). The acceptance criteria for such QC check must fall within ± 0.3 mg/L of the value calculated from the titration method.

19) Corrective Actions for Out-of-Control Data

Any out-of-control data must be reported at once to the Executive Director who in turn has to promulgate a corrective action plan in consultation with IEC's QA Officer.

20) Contingencies for Handling Out of Control or Unacceptable Data

The Executive Director or designee, will investigate the problem(s) in depth and take all necessary corrective actions that may include but are not confined to cleaning or replacing membrane, sending instrument for repair to an authorized service center, and/or comparing the measured values against that obtained by the titration method. All analysis of samples will be stopped until the problem is rectified.

21) Waste Management

Order or prepare all reagents in limited volumes that will be used before their expiration dates to minimize producing waste. See Pollution Prevention, section 17, above.

22) References

1. EXO User Manual. August 2014. YSI Incorporated, Yellow Springs, Ohio.
2. Standard Test Methods for Dissolved Oxygen in Water ATSM D888-09

23) Tables

None.

SALINITY

(Electrical Conductivity Method) Section No. FIELD II Revision No. 3 March 2016

1) Test Method

Based on Standard Methods, 20th Edition, 2520 A and B and EPA Method 120.1

2) Applicable Matrix or Matrices

Salinity is an important property of industrial and natural waters. This field parameter can be useful for assessing the source or origin of effluents and of the mixing between fresh and marine waters in coastal regions.

3) Method Detection Limit

The salinity range for the YSI Professional Plus and EXO1 is 0-70 ppt.

4) Scope and Application

This method is used to measure salinity in-situ of ambient waters in conjunction with ambient water quality monitoring surveys such as the Long Island Sound Survey.

5) Summary of Method

Salinity by definition is the ratio of the mass of dissolved salts in a given mass of solution. Thus, salinity values are commonly expressed as “grams of salt / kilograms of water” and recorded as ‰ or ppt. The experimental determination of the salt content by drying and weighing may result in a loss of sample, leaving the only reliable way to determine the true or absolute salinity is to make a

complete chemical analysis. This method is time-consuming and cannot yield the precision necessary for accurate work, thus salinity is determined by using indirect methods involving the measurement of a related physical property such as conductivity, density, sound speed, or refractive index with known empirical relationships to salinity. Due to its high sensitivity and ease of measurement, the electrical conductivity method is used in the field to calculate salinity.

6) Definitions

Conductivity – The term refers to the measure of the ability of an aqueous solution to carry an electric current. The ability depends on the presence of ions; on their total concentration, mobility and valence; and on the temperature of measurement.

7) Interferences

1. The conductivity sensor will provide quick readings as long as the entire sensor is submerged and no air bubbles are trapped in the sensor area.
2. Drifting readings or an inability to calibrate the sensor may indicate that deposits have formed on the electrode. Occasional cleaning of the sensor and the openings which allow sample access to the conductivity electrodes may be required to maintain accuracy and increase responsiveness.
3. Avoid any environment that contains substances that may attack probe materials such as concentrated acids, caustics, and strong solvents.

8) Safety

This method requires the use of a sodium chloride conductivity calibration standard. To protect the analyst from contact with the standard, appropriate personal protective equipment, including goggles and gloves should be worn.

The following first-aid procedures should be used as a guideline in the event of contact with NaCl. For more information, refer to the reagent-specific MSDS, which is on file in the laboratory.

Eyes:

In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention.

Skin:

Wash with soap and water. Cover the irritated skin with an emollient. Get medical attention if irritation develops.

Ingestion:

Do NOT induce vomiting unless directed to do so by medical personnel. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention if symptoms appear.

Inhalation:

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention if symptoms appear.

9) Equipment and Supplies

1. YSI Professional Plus multi-meter with 10 foot and/or 100 foot Quattro cable (depending on anticipated depth of waterbody to be monitored) or EXO1 sonde equipped with 66 meter (216 ft) cable.
2. Spare C batteries (4) for the YSI ProPlus. Spare D batteries (4) for the EXO 1 sonde and handheld device
3. Spare Conductivity/Temperature sensor YSI item #005560 for YSI ProPlus
4. Traceable Conductivity Standards (see reagents)
5. Calibration container – Supplied by YSI
6. Ring stand
7. Deionized water

10) Reagents and Standards

1. Traceable Conductivity Standard 5 $\mu\text{S}/\text{cm}$ at 25 °C: Manufactured by Ricca Chemical and purchased from Fisher Scientific (Catalog # 2236.02-16).
2. Traceable Conductivity Standard 100 $\mu\text{S}/\text{cm}$ at 25 °C: Manufactured by Ricca Chemical and purchased from Fisher Scientific (Catalog # 5877-16).
3. Traceable Conductivity Standard 1413 $\mu\text{S}/\text{cm}$ at 25°C. Purchased through Fisher Scientific (catalog # 09-328-11)
4. Traceable Conductivity Standard 10,000 $\mu\text{S}/\text{cm}$ at 25°C. Purchased from Fisher Scientific (Catalog # 09-328-4)
5. Traceable Conductivity Standard 50,000 $\mu\text{S}/\text{cm}$ at 25°C. Purchased from Fisher Scientific (Catalog # 2248-1)

Standards used for salinity calibration should be in the expected range of sample values. For Long Island Sound (brackish water) sampling, the 50,000 $\mu\text{S}/\text{cm}$ standards are typically used. Alternate sources of calibration standards may be used if they have been evaluated and determined to meet the data quality needs of the laboratory. Manufacturer's certificates of analysis are kept on file in the laboratory.

11) Sample Collection, Preservation, Shipment and Storage

Because electrodes offer the advantage of analysis in situ they eliminate errors caused by sample handling and storage. If sampling is required, collect samples according to standard field methods.

12) Quality Control

1. Occasional cleaning of the sensor may be necessary to maintain accuracy and increase the responsiveness. Regularly check electrode for formed deposits, cracks or other issues that could affect performance. Use a mild detergent (laboratory grade soap or bathroom foaming tile cleaner) with the manufacturer's supplied cleaning brush if necessary. Rinse thoroughly with clean water,

then check the response and accuracy of the conductivity cell with a calibration standard.

2. No special storage is required. Sensors can be stored dry or wet as long as solutions in contact with conductivity electrodes are not corrosive (for example, chlorine bleach). Sensors should be cleaned prior-to and after long-term storage. Long-term storage temperature: -5 to 70 °C.

3. Commercially purchased reagents must be used by the manufacturer's expiration date and certificates of analysis kept on file in the laboratory.

4. In the event that the electrode does not calibrate or read correctly, bring it to the attention of the Associate Laboratory Director and see the YSI ProPlus user manual for instructions on electrode replacement.

5. The YSI ProPlus and EXO1 meters are checked weekly in the laboratory when in routine use (typically during the Long Island Sound Surveys from June-September), typically within 24 hours of use, by an Environmental Analyst. Lab checks include general inspection of equipment condition, presence of spare batteries, any required electrode solution, spare probes as well as a calibration with traceable conductivity standards and comparison to the laboratory Conductivity meter (Accumet model 30). Checks and calibrations are documented on the YSI Meter Calibration Sheet.

13) Calibration and Standardization

On each day of use, calibrate the conductivity (salinity) meter (YSI ProPlus multimeter or EXO1) before taking sample measurements. All calibrations should be completed at a temperature which is as close as possible to the sample temperature. The following instructions for calibration are adapted from the YSI ProPlus Instruction Manual:

1. Place the sensor into a fresh, traceable conductivity calibration solution in the range of the expected sample values (10,000 $\mu\text{S}/\text{cm}$ for Long Island Sound) using the specialized calibration container connected to a ring stand for support. The solution must cover the holes of the conductivity sensor that are closest to the cable. Ensure the entire conductivity sensor is submerged in the solution
2. Press **Cal**. Highlight probe ID. Probe ID must be enabled in the system GLP menu to appear in the Calibrate menu. After selecting the Probe ID, highlight **Conductivity** and press enter. Highlight the desired calibration method: **Specific Conductance**, **Conductivity**, or **Salinity**. YSI recommends calibrating in specific conductance for greatest ease. Calibrating by any of these methods will calibrate the meter for the other two units of measurement as well. Place the sensor into a fresh, traceable conductivity calibration solution. The solution must cover the holes of the conductivity sensor that are closest to the cable. Ensure that the entire conductivity sensor is submerged in the solution or the instrument will read approximately half of the expected value!
3. Choose the calibration unit "C-uS/cm" and press enter.
4. Highlight "Calibration value" and press enter to input the value of the calibration standard. Then, once the temperature and conductivity readings stabilize, highlight "Accept Calibration" and press enter. Or, press "Esc" to cancel the calibration.
5. After completing the calibration, the message line at the bottom of the screen will display "Calibrating Channel..." and then "Saving Configuration..."

Calibration for EXO1 Sonde:

1. Place the correct amount of conductivity standard into a clean, dry, or pre-rinsed calibration cup. Select the 50,000 $\mu\text{S}/\text{cm}$ standard.
2. Carefully immerse the probe end of the sonde into the solution, making sure the standard is above the vent holes on the conductivity sensor. Gently rotate and/or move the sonde up and down to remove any bubbles from the conductivity cell.
3. Allow at least one minute for temperature equilibration before proceeding.
4. In the calibration menu, select Conductivity, and then a second menu will offer the options of calibrating conductivity, nLF conductivity, specific conductance, or salinity. Calibrating any one option automatically calibrates the other parameters. After selecting the option of choice (specific conductance is normally recommended), enter the value of the standard used during calibration. Be certain that the units are correct and match the units displayed in the second window at the top of the menu.
5. Click Start Calibration. Observe the readings under Current and Pending data points and when they are Stable (or data show no significant change for approximately 40 seconds), click Apply to accept this calibration point. NOTE: If the data do not stabilize after 40 seconds, gently rotate the sonde or remove/reinstall the cal cup to make sure there are no air bubbles in the conductivity cell.
6. Click Complete. View the Calibration Summary screen and read and record the QC score. Click Exit to return to the sensor calibration menu, and then the back arrows to return to the main Calibration menu.

At the end of calibration, check electrode against an additional known conductivity standard in the range of expected measurement, making sure to rinse the sensor thoroughly with deionized water before taking a reading. The electrode should read $\pm 15\%$ of the known standard value.

14) Procedure

Turn the instrument on and wait 5-15 minutes. Remove the plastic cup from the sensor/cable and install the sensor guard to protect the sensor and membrane. Completely submerge the probe in the sample to be measured and give the probe a quick shake to release any air bubbles. Allow the temperature readings to stabilize. Once the values plateau and stabilize, record the measurement and/or log the data set.

15) Calculations

The instrument is designed to provide measurements of salinity in ‰ or ppt and displays these readings on the LCD screen.

16) Method Performance

Analyze conductivity standard in the lab at least once a month or more often during routine surveys or if meter malfunction is suspected. The electrode should read $\pm 15\%$ of the known standard value.

17) Pollution Prevention

All cleaning or treatment of probe should be done in the lab, not in the field, to prevent spillage of detergents or reagents into the environment. If possible, use low-phosphorus detergents to prevent pollution of local water bodies caused by nutrient loading.

18) Data Assessment and Acceptance Criteria for Quality Control Procedures

Certificates of Analysis for conductivity calibration standards will remain on file in the laboratory in QC Log 27: Chemistry Certificates of Analysis.

19) Corrective Actions for Out-of-Control Data

Any deviation from the protocol or a questionable result will be brought to the attention to IEC's Executive Director/QC Officer who will promulgate a corrective action plan.

20) Contingencies for Handling Out of Control or Unacceptable Data

The QC Officer will investigate the problem(s) in depth and take all necessary corrective actions. This may include but is not confined to cleaning or replacing the electrode or sending instrument for repair to an authorized service center. All analysis of samples will be stopped until the problem is rectified.

21) Waste Management

Order and prepare all standards in limited volumes that will be used before their expiration dates to minimize producing waste.

22) References

1. Standard Methods for the Examination of Water and Wastewater, 20th Edition, Method 9020-A and B. pp (2-48) to (2-49). APHA, AWWA, WEF, 1998.
2. Standard Methods for the Examination of Water and Wastewater, 20th Edition, Method 2510-A. p (2-44). APHA, AWWA, WEF, 1998.
3. EPA Method 120.1
4. YSI Professional Plus User Manual. 2009. YSI Incorporated, Yellow Springs, Ohio.
5. EXO Sonde user manual, August 2014. YSI Incorporated, Yellow Springs, Ohio.

23) Tables

Not Applicable.

DISSOLVED OXYGEN

(Modified Winkler with Full-Bottle Technique) Section IX, Revision No. 8, Effective Date January 2016

1) Test Method

Based on Standard Methods, 4500-O C-11.

2) Applicable Matrix or Matrices

This method is applicable for use with most wastewaters and stream samples especially if samples contain more than 50 µg/l nitrite nitrogen and not more than 1 mg/l of ferrous iron. Other reducing or oxidizing materials should be absent.

3) Reporting Limit

The reporting limit to measure dissolved oxygen by this method is 0.2 mg/l. This is based on the visual detection of the endpoint. Although experienced analysts may be able to detect as low as 0.05 mg/l, 0.2 mg/l takes into account any variability with which analysts may read the buret or detect the end-point.

4) Scope and Application

This method is used to determine dissolved oxygen concentrations of wastewater and ambient water samples. Most often, this method is incorporated to facilitate measurement of initial and final dissolved oxygen concentrations in the biochemical oxygen demand. The method may also be used in the field to determine dissolved oxygen concentrations in ambient waters. The method may be subject to interferences, discussed in section 7 of this method, which may limit the application of the Winkler titration to certain samples.

5) Summary of Method

The sample is treated with manganous sulfate, alkaline-iodide-azide and finally sulfuric acid. The initial precipitate of manganous hydroxide, $\text{Mn}(\text{OH})_2$, combines with the dissolved oxygen in the sample to form a brown precipitate, manganic hydroxide, $\text{MnO}(\text{OH})_2$. Upon acidification, the manganic hydroxide forms manganic sulfate which acts as an oxidizing agent to release free iodine from the sodium iodide. The iodine, which is stoichiometrically equivalent to the dissolved oxygen in the sample, is then titrated with standard sodium thiosulfate. The volume of sample to be titrated is adjusted based on the normality of the titrant. The dissolved oxygen concentration in mg/l is equal to the volume in ml of sodium thiosulfate used in the sample titration.

6) Definitions

Dissolved Oxygen- The term refers to oxygen which is dissolved in water. Oxygen can become dissolved in water through a variety of methods. Atmospheric oxygen can become dissolved through wave and wind action.

Azide Modification- The addition of sodium azide (the alkaline-iodide-azide reagent) prior to the acidification in order to suppress any interference from NO_2^- in the sample.

7) Interferences

The azide modification is not applicable for the following types of samples:

- samples containing sulfite, thiosulfate, polythionate, appreciable quantities of free chlorine or hypochlorite;
- samples high in suspended solids;
- samples containing organic substances which are readily oxidized in a highly alkaline solution or which are oxidized by free iodine in an acid solution;
- untreated domestic sewage
- biological flocs;
- where sample color interferes with end point detection; and
- samples containing ≥ 5 mg/l ferric iron salts

Most organic matter is oxidized partially when the oxidized manganese precipitate is acidified, thus causing negative errors. Significant error may occur in testing waters having organic suspended solids or heavy pollution.

8) Safety

This method requires the use of concentrated sulfuric acid, which is extremely corrosive. Use extreme care when adding sulfuric acid and shaking bottles to avoid drips and spills. Activities involving the addition or transfer of sulfuric acid should be performed in a hood. To protect the analyst from contact with acid as well as all the other reagents, appropriate personal protective equipment, including goggles, labcoat, and gloves must be worn.

The following first-aid procedures are from the MSDS for sulfuric acid, but can be used as a guideline in the event of contact with other similar reagents as well. For more information, refer to the reagent-specific MSDS, which is on file in the laboratory.

Eyes:

Flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower lids. Get medical aid immediately.

Skin:

Flush skin with plenty of soap and water for at least 15 minutes while removing contaminated clothing and shoes. **SPEEDY ACTION IS CRITICAL!** Get medical aid immediately.

Ingestion:

Do NOT induce vomiting. If the victim is conscious and alert, give 2-4 cupfuls of milk or water. Get Medical aid immediately.

Inhalation:

Move from exposure to fresh air immediately. If not breathing, give artificial respiration and call 911. If breathing is difficult, give oxygen (if available) and call 911.

9) Equipment and Supplies

1. Sample bottles: 300 ml capacity BOD incubation bottles with tapered ground glass pointed stoppers and flared mouths.
2. Pipets with elongated tips capable of delivering 2.0 ml
3. Class A Auto-fill Buret with a minimum of 25 ml capacity for titration, suspended securely on a ringstand and a reservoir for sodium thiosulfate solution.
4. 300 ml wide-mouth Erlenmeyer flasks.
5. A graduated cylinder of 1 ml increments.

10) Reagents and Standards

1. Manganous sulfate solution: Manufactured by LabChem, Inc., purchased from Fisher Scientific

(Catalog #: LC16570-4), or Manganese (II) Sulfate Solution (Fisher Scientific Catalog # SM20-500)

2. Alkaline-iodide-azide solution: Manufactured by LabChem Inc., purchased from Fisher Scientific (Catalog #: LC10670-2) or Ricca Chemical Company (Catalog # 540-16)

3. Sulfuric acid, concentrated: Purchased from Fisher Scientific (Catalog #: A300-212).

4. Starch Indicator 1.0%: Manufactured by Lab Chem Inc., purchased from Fisher Scientific (Catalog #: LC25310-2), or Ricca Chemical Company (Catalog # 8050-32).

5. Sodium Thiosulfate Titrant, 0.025N: Manufactured by and purchased from Fisher Scientific (Catalog # SS370-1). The certificate of analysis must be retained on file in the laboratory. In addition, the normality of the titrant must be verified quarterly or when a new bottle of the same lot number is opened. This is done by standardizing the titrant in the lab according to the procedure outlined in Section 13.

6. Potassium Dichromate, 0.025N: Manufactured by Ricca company, purchased through Fisher Scientific (Catalog # 6050-16). For quarterly standardization of titrant.

7. Potassium Iodide, Granular: Manufactured by and purchased from Fisher Scientific (Catalog # P410-500). For quarterly standardization of titrant.

8. 10% Sulfuric acid solution: Prepared from concentrated sulfuric acid, above. Place approximately 800 ml deionized water in 1000 ml volumetric flask. Add 100 ml concentrated sulfuric acid **slowly**. Fill flask to 1000 ml mark with deionized water. Allow to cool to room temperature, adjust the volume if necessary. For quarterly standardization of titrant.

Additional buffers are required to be added to dilution water for BOD analysis. Please refer to BOD SOP (III) for description of these buffers.

11) Sample Collection, Preservation, Shipment and Storage

Where possible, collect the sample in a 300 ml BOD incubation bottle. Special precautions are required to avoid entrainment or dissolution of atmospheric oxygen or loss of dissolved oxygen. Do not let samples remain in contact with air or be agitated, because either condition causes a change in its gaseous content. Let the bottle overflow two to three times its volume and then replace the stopper so that no air bubbles are entrained. At the time of sampling, the sample temperature should be recorded as precisely as possible.

Determine DO immediately on all samples. As an analyze immediately parameter, the analysis (titration) must occur within 15 minutes of adding the concentrated sulfuric acid (see step 1 of Procedure). Samples can be held for up to eight hours if the manganous sulfate solution and alkaline-iodide-azide solution is added but **not the sulfuric acid**, and the samples kept in the dark. It is IEC's current practice to analyze all samples immediately.

Add 2 ml of manganous sulfate solution and then 2 ml of alkaline-iodide-azide solution the sample contained in the BOD bottle. Both reagents must be added well below the surface of the liquid.

Stopper the bottle immediately and mix the contents thoroughly. Let the sediment settle for approximately 10 minutes. Shake again and allow to settle. Complete the procedure by adding 2 ml H_2SO_4 at the time of analysis (titration). If sample is to be held, DO NOT add the acid, and keep the sample(s) in the dark for up to eight hours. Titrate the solution within 15 minutes of adding H_2SO_4 .

12) Quality Control

1. All reagents prepared must be used within six months. Commercially purchased reagents must be used by the manufacturer's expiration date.
2. See the SOP for BOD (Section No. III) for more quality control measures that are necessary when the Winkler Titration is used for measurement of BOD.
3. Perform a zero check daily. Remove all oxygen from a check sample (deionized water) by adding sodium sulfite (and cobalt chloride if needed) until 0 mg/L reading for DO is obtained.

13) Calibration and Standardization

Standardized sodium thiosulfate solution is purchased through Fisher Scientific and is certified. The certificate of analysis is retained in the laboratory. This eliminates the need for daily standardization. However, the normality of the titrant must be verified quarterly or when a new bottle of the same lot number is opened according to the following procedure:

1. Put 150 ml of deionized water into an Erlenmeyer flask
2. Add 2 grams of potassium iodide.
3. Add 10 ml of 10% sulfuric acid.
4. Add 25 ml of Potassium Dichromate, 0.025N.

Titrate with sodium thiosulfate to a straw yellow endpoint, add about 1 ml of starch indicator, and continue titrating to clear endpoint. If the normality is not 0.0248-0.0252N, the titration is repeated. If the normality continues to be out of acceptance ranges, a new lot or laboratory-prepared sodium thiosulfate is used.

14) Procedure

1. To the sample collected in the BOD incubation bottle, add 2 ml of the manganous sulfate solution followed by 2 ml of alkaline-iodide-azide solution, well below the surface of the liquid; stopper with care to exclude air bubbles, and mix well by inverting the bottle several times. When the precipitate settles, leaving a clear supernatant above the manganese hydroxide floc, shake again. Samples can be held for up to eight hours if the manganous sulfate solution and alkaline-iodide-azide solution is added but **not the sulfuric acid** (the next step), and the samples kept in the dark. It is IEC's current practice to analyze all samples immediately. When settling has produced at least 200 ml of clear supernatant, carefully remove the stopper and immediately add 2 ml of concentrated H_2SO_4 , by allowing the acid to run down the neck of the bottle, re-stopper, and mix by gentle inversion until the iodine is uniformly distributed throughout the bottle. Complete the analysis within 15 minutes.

2. Transfer the appropriate volume from the BOD bottle (determined by calculation 1, below) by inversion into a 300 ml wide mouth flask and titrate with 0.025N sodium thiosulfate solution to a pale straw yellow color. Add 1-2 ml of starch solution and continue to titrate to the first disappearance of the blue color.

4. The DO is calculated from a direct reading from the buret and recorded. For example, if 2 ml of the sodium thiosulfate is used in the titration, then the corresponding DO value of the sample is 2 mg/l.

15) Calculations

1. Volume to be titrated = $\frac{203 \text{ ml} \times \text{normality of sodium thiosulfate}}{0.025}$
(to the nearest ml)

2. 1 ml sodium thiosulfate = 1 mg/l dissolved oxygen

16) Method Performance

Analyze dissolved oxygen in aerated reagent-grade water once a week or as needed and compare the measured values against the readings obtained from dissolved oxygen electrode probe.

17) Pollution Prevention

After titration, samples are neutralized with 1N sodium hydroxide and disposed of. Sulfuric acid is stored in the acid cabinet, below the fume hood, which has a protective liner. The MSDS for all reagents are stored in a file in the laboratory. All analysts should review the relevant MSDS before using any reagent.

18) Data Assessment and Acceptance Criteria for Quality Control Procedures

The quality control measures must include the check for the accuracy of the method once a month or as needed against the electrode method using a dissolved oxygen selective probe. There are no QC samples available for dissolved oxygen. QC, as well as IDC and CDC studies are performed using a sample prepared by the laboratory director or assistant laboratory director. A volume of water is aerated using an airstone, and the DO is measured using the Winkler titration and an electrode (See IEC SOP # FIELD-1). The results of the two methods should vary by no more than 10% for each analyst. Perform a zero check daily. Remove all oxygen from a check sample (deionized water) by adding sodium sulfite (and cobalt chloride if needed) until 0 mg/L reading for DO is obtained.

19) Corrective Actions for Out-of -Control Data

Any out-of-control data must be reported at once to the IEC's Laboratory Director/QC Officer who in turn has to promulgate a corrective action plan in consultation with IEC's QA Officer.

20) Contingencies for Handling Out of Control or Unacceptable Data

The QC and QA Officers will jointly investigate the problem(s) in depth and take all

necessary corrective actions that may include but are not confined to checking the accuracy of measurements by running dissolved oxygen solutions of theoretically known strength, comparing the measured values against that obtained by electrode method and determining the standard deviation of replicate analyses or other contingency plans as needed. All analyses of samples will be stopped until the problem is rectified.

21) Waste Management

Order or prepare all reagents in limited volumes that will be used before their expiration dates to minimize producing waste.

22) References

1. Standard Methods for the Examination of Water and Wastewater, Method 4500-O C-11. APHA, AWWA, WEF, 2011.

23) Tables

Not Applicable.

pH

Section No. XIX, Revision No. 14, Effective Date January 2018

1) Test Method

Based on SM 4500-H B-11

2) Applicable Matrix or Matrices

Non-Potable and Potable Water.

3) Reporting Limit

pH of 0.01 S.U.

4) Scope and Application

The Interstate Environmental Commission regularly analyzes environmental samples for pH in the field at municipal and industrial facilities, as well as in conjunction with ambient water quality surveys aboard the Commission's boat. This method is applicable to surface, and saline waters, domestic and industrial wastes. This method is used by IEC most frequently to monitor the quality of the deionized water, as well as to measure the pH of prepared microbiological media and reagents. In the field, the pH of samples is taken using the Accumet AP 61 portable pH meter, the YSI ProPlus multimeter or the EXO1 sonde. Separate calibration and sample measurement procedures are outlined in the appropriate sections for each specific meter.

5) Summary of Method

The pH of a sample is measured electrometrically, using a combination electrode attached to a pH meter.

6) Definitions

The pH of a solution is defined as:

$$\text{pH} = -\log a_{\text{H}}$$

where a_{H} is the activity of the hydrogen ion in the solution. For hydrogen ions, the distinction between activity and concentration can usually be ignored.

Substituting the definitions of pH and slope into the Nernst equation, yields a working equation for computing sample pH:

$$E = E_0 + S_T \text{ pH}$$

In practice E_0 and S_T may be determined through standardization with buffers of known pH. The pH value of a sample then may be calculated from this equation and the measured output potential E of the electrode when placed in the sample.

7) Interferences

1. Oil and Grease or particulate matter, by coating the surface of the pH electrode, may interfere by causing sluggish response. These can usually be removed by gentle wiping or detergent washing, followed by deionized water rinsing. An additional treatment with hydrochloric acid (10%) may be necessary to remove any remaining film.
2. The pH meter automatically compensates for the temperature dependence of the electrode's response when measuring pH with an automatic temperature compensation (ATC) probe.

8) Safety

The reagents used in this analysis are not particularly hazardous. However, personal protective equipment including goggles, gloves and a labcoat should be worn at all times. Clean up any drips or spills of buffers or other reagents immediately using paper towels and a general cleanser.

9) Equipment and Supplies

1. Thermo Scientific Orion Star A215 pH/conductivity meter. In field, the Accumet Model AP61 pH meter, YSI Professional Plus multi-meter or EXO1 sonde is used.
2. pH electrode: The laboratory pH meter is equipped with an Orion 8157BNUMD Ross Ultra pH/ATC triode electrode. The field probe used is Fisher/Accumet Catalog #13-620 AP50. Use

flat-tipped pH electrode, Accumet Catalog # 13-620-289 with lab pH meter for checking pH of microbiological agar. The EXO1 sonde measures pH with two electrodes combined in the same probe: one for hydrogen ions and one as a reference. The sensor is a glass bulb filled with a solution of stable pH (usually 7) and the inside of the glass surface experiences constant binding of H⁺ ions. The outside of the bulb is exposed to the sample, where the concentration of hydrogen ions varies. The resulting differential creates a potential read by the meter versus the stable potential of the reference.

3. Beakers- 50 mL capacity.
4. Stir Plate and magnetic stirrers.

10) Reagents and Standards

1. Potassium Chloride Solution 4M- Saturated with Silver Chloride. Fisher Catalog # SP135.
2. pH Buffer 4.0 (Fisher Catalog # SB101-500)
3. pH Buffer 7.0 (Fisher Catalog # SB107-500)
4. pH Buffer 10.0 (Fisher Catalog # SB115-500)
5. Deionized water in squeeze bottle for rinsing electrode.

11) Sample Collection, Preservation, Shipment and Storage

Samples should be analyzed as soon as possible after sampling. High-purity waters and waters not at equilibrium with the atmosphere are subject to changes when exposed to the atmosphere, therefore the sample containers should be filled completely and kept sealed prior to analysis. Samples may be taken in either glass or plastic containers. They must be analyzed within 15 minutes of collection. Minimum sample volume required for analysis is 100 mL.

12) Quality Control

The meter must be calibrated on each day of use. Make sure that the level of electrode filling solution in the electrode is sufficient. The filling solution should be filled at least one inch above the level of the sample in the beaker. Ensure that the electrode filling hole is in the open position and free from crystallization. Field meters are calibrated in the field on the day of use prior to the measurement of any environmental samples. pH meters are calibrated with pH 4.00 and pH 10.0 buffers and the reading of the pH 7.0 buffer is read. The pH 7.0 buffer must read 7.0 ± 0.05 S.U. Daily or with each batch of twenty (or fewer samples) one sample must be analyzed in duplicate.

The Field meters are brought into the lab and inspected by the Senior Manager or designee monthly. During this monthly check, the pH buffers and deionized rinse water are replaced. The pH meter and kit is checked for cleanliness and the presence of a spare battery, backup pH paper, a

digital thermometer (to check pH probe temperature readings if they seem erroneous). The pH meter is calibrated with the pH 4.00 and 10.0 buffer and the pH of the pH 7.00 buffer is read. The pH 7.0 buffer must read 7.0 ± 0.05 S.U. The probe is inspected for breakage or crystallization. The ATC probe is calibrated against an NIST thermometer at least annually. A record of these monthly checks is logged into the Field Equipment log manual kept in the lab.

At the end of each analysis day, the meter will be checked with the mid-range buffer (pH 7.0) at the end of the run in addition to the required meter checks.

When in regular use, the pH sensor for the EXO1 should remain installed on the sonde in an environment of water-saturated air. Place approximately 0.5 in (1 cm) of deionized water in the bottom of the calibration cup. Insert the sonde and sensor into the cup and screw it on tightly to prevent evaporation. For long-term storage, remove the sensor from the sonde and insert its sensing end into the bottle that the sensor was shipped in. Install the bottle's o-ring and cap then tighten. This bottle contains a 2 molar solution of pH 4 buffer (tap water may be used as a replacement. DO not store the pH sensor in DI water.) If the pH sensor has been allowed to dry, soak the sensor for several hours (preferably overnight) in a 2 molar solution of KCl solution. If KCl is unavailable, tap water or pH 4 buffer soak may restore function. If the sensor is irreparably damaged, users must replace the sensor module.

13) Calibration and Standardization

Lab Benchtop Thermo Scientific Orion Star A215 pH/conductivity meter-*Clearing Existing Buffers*- Over time, both an electrode's slope and its zero potential will change. As a general rule of thumb, pH electrodes require a complete re-standardization at least once daily.

1. Make sure that the pH electrode (standard electrode is cat# 13-620-287) is attached to the BNC connector on the back of the meter. If using the meter to measure the pH of an agar, the flat-tipped electrode (cat#13-620-289) is used. **This may require replacing the installed electrode.**

2. In the measurement mode, press f1(cal) Press the arrow up button or arrow down button to highlight pH-Channel and press f2(select).

3. Calibrate according to the manufacturer's instruction manual, p. 4, utilizing pH 4 and 10 buffers to calibrate and pH 7 to check the calibration (read only).

- Rinse the electrode(s) in use with deionized water and blot dry with a kimwipe.
- Place in pH buffer 4.00. When the electrode and buffer are ready, press f3(start).
- Wait for the pH value on the meter to stabilize and stop flashing. Press f2(accept) to accept the displayed value, OR:
- Press f3(edit) to access the numeric entry screen and edit the value. **NOTE: Record temperature of buffer and adjust temperature on meter so that the meter indicates the pH value for buffers CORRECTED for buffer temperature.** Use the arrow buttons to

highlight a number, a decimal point, or negative sign; press f3(enter) to select the highlighted item and repeat until the value at the measured temperature is shown above the numeric entry screen

- Press f2(done) to exit the numeric entry screen.
- Press f2(accept) to accept the entered value.
- Press Record calibration in the pH logbook.

4. Press f2(next) to proceed to the next buffer (pH 10) and repeat the bulleted steps above. Press f3(cal done) to save and end the calibration. The meter will display the calibration summary including the average slope. Record calibration data in pH calibration logbook.

Measure the pH 7.00 buffer in the measure mode.

For both the pH 4.00 and pH 7.00 buffers, measure the mV reading by pressing the **mV** button. Record. Calculate the absolute value of the difference between the pH 4.00 and pH 7.00 readings and record in the pH meter log book.

Field Accumet Model 61 Portable pH Meter-

1. Turn on the meter by pressing the **on/off** button.
2. Press **setup** twice, followed by **enter** to clear existing standards.
3. Open the pH probe, by holding the middle of the probe in one end and twisting the blue band at the top so the hole in the blue band is open. Place the pH probe in the pH 4.00 buffer and press **std**. Let the probe sit for a few seconds and press **std** again. Rinse the probe with deionized water and repeat with pH 10.00 buffer, by pressing **std** twice. Record the reading of the pH 4.00 and 10.00 buffers after calibration. Rinse the probe with deionized water, place in the pH 7.00 buffer, allow to stabilize and record the reading of the pH 7.00 buffer. The pH 7.00 buffer must read 7.00 ± 0.10 SU. Perform a check with the pH 7.00 buffer at end of use for the day. The pH 7.00 buffer must read 7.00 ± 0.10 SU for acceptability

YSI Professional Plus Multi-meter-

Turn on meter. Press “**Cal.**” Highlight probe ID (ISE1 pH) by scrolling down with the up/down arrows until “ISE1pH” is highlighted. Press enter. The message line will show the instrument is “ready for point 1.” Place the sensor in pH 4 buffer solution. The instrument should automatically recognize the buffer value and display it at the top of the calibration screen. If the calibration value is incorrect, the auto buffer recognition setting in the Sensor Setup menu may be incorrect. If necessary, highlight the **Calibration Value** and press enter to input the correct buffer value.

Once the pH and temperature readings stabilize, highlight **Accept Calibration** and press enter to accept the first calibration point. The message line will then display “Ready for point 2.” To continue with the 2nd point, place the sensor in pH 10 buffer solution. The instrument should automatically recognize the 2nd buffer value and display it at the top of the calibration screen. If necessary, highlight the **Calibration Value** and press enter to input the correct buffer value. Once the pH and temperature readings stabilize, highlight **Accept Calibration** and press enter to confirm the second calibration point. Press **Cal** to complete the calibration. Read pH 7 buffer and record.

YSI EXO1 SONDE pH Calibration:

1. Begin with a clean, dry probe installed on the EXO sonde. Install the clean calibration guard over the probe(s). Go to the Calibrate menu in KOR software. Select the sensor you are going to calibrate from the list. Next select the parameter (pH) you are going to calibrate from the list. In the next menu, select a 2-point calibration. Pour of pH 4 buffer up to the first line in a pre-rinsed calibration cup. Carefully immerse the probe end of the sonde into the solution, making sure the sensor’s glass bulb is in solution by at least 1 cm. Allow at least 1 minute for temperature equilibration before proceeding.
2. In the Calibrate menu, select pH (may need to select twice). Select the number of points desired for calibration (2 points). Enter the values (4 and 10) of the pH buffers that will be used for the calibration. Observe the temperature reading above the standard value. The actual pH value of all buffers varies with temperature. Enter the correct value from the bottle label for your calibration temperature for maximum accuracy.
3. Click start Calibration. Observe readings under Current and Pending data points and when they are Stable (or data shows no significant change for approximately 40 seconds), click Apply to accept this calibration point. Confirm that the Pending data value is close to the Setpoint value. Click Proceed and wait for the software to prompt you to move the sensor to the next buffer solution.
4. Rinse the sensor and calibration cup with deionized water. Pour the next buffer (10) up to the first line and carefully immerse the probe end of the sonde into the solution. Allow at least 1 minute for temperature equilibration before proceeding. Repeat the calibration procedure and click Apply when the data are stable.
5. Click Complete. View the Calibration Summary screen and read and record the QC score. Click Exit to return to the sensor calibration menu, and then the back arrows to return to main Calibrate menu if additional sensors are to be calibrated. Rinse the sensor in tap or purified water.
6. Read a pH 7.0 buffer as a sample. pH 7.0 buffer must read 7.0 ± 0.1 SU.

14) Procedure

Thermo Scientific Orion Star A215 pH/conductivity meter.

Measuring pH procedures-See manufacturer's instruction manual, page 5.

Make sure the pH-Channel is selected.

1. Rinse the pH electrode, conductivity cell, and any other electrodes in use with deionized water, blot dry with a lint-free tissue and place into the sample.
2. Start the measurement and wait for it to stabilize.
 - a. If the meter is in AUTO-READ mode (default setting), press MEASURE (ESC) button to start the measurement. When the AR icon stops flashing, record the applicable measurement parameters and temperature of the sample. Press MEASURE (ESC) again to start a new measurement. AUTO-READ is the mode used for most applications.
 - b. If the meter is in continuous mode, the meter will immediately start taking a measurement and update the display whenever the measurement changes. Wait for the display to show **ready** and record the applicable measurement parameters and temperature of the sample.
 - c. If the meter is in timed mode, the meter will log measurements at the preselected time interval, regardless of the measurement stability. The meter will update the display whenever the measurement changes, so the applicable measurement parameters and temperature of the sample can be recorded when the display shows **ready**.
3. Remove the electrodes from the sample, rinse with deionized water, blot dry and place into the next sample. Repeat steps 2 through 3 for all samples.

Additional information on the use of the Orion Star A215 meter can be found in the manufacturer's instruction manual

Field Portable Accumet model 61 pH Meter/YSI Professional Plus Multi-meter and EXO1 Sonde

1. After calibrating with the pH 4.00 and pH 10.00 buffers, and recording the reading of the pH 7.00 buffer, place the field pH probe either directly in the water being measured, where practical and safe to do so. Provide moderate stirring, and when the reading is stable, record. If it is not possible to place the pH probe in the water, or the water being measured is a rapid flow such as from a spout or turbulent effluent stream, collect the sample in a clean, dry plastic or glass jar attached to a sampling stick. Rinse the jar 3 times with the water being measured, then collect a sample for pH analysis.

15) Calculations

None.

16) Method Performance

Each analyst must perform an initial demonstration of capability (IDC) prior to performing analysis on environmental samples. The IDC consists of analyzing a quality control sample, procured by the Quality Executive Director (QC Officer), or designee, and analyzed four times by the analyst. Thereafter, each analyst must perform a CDC, consisting of a sample analyzed in duplicate. The results of the IDC and CDC must be within the manufacturer's determined acceptance limits. In addition to in-house quality control measures, the laboratory performs two proficiency test samples procured by providers approved by NJDEP OQA. PT samples must be rotated through all field meters in use. Typically, the Interstate Environmental Commission has between two and four field meters in use, so each meter should be used to analyze a PT at least once every two years. Record in the analysis log book the model and serial number of the meter used.

17) Pollution Prevention

Clean up any spills immediately with paper towels and a general cleanser.

18) Data Assessment and Acceptance Criteria for Quality Control Measures

Quality Control samples must yield results within the manufacturer's acceptance limits. The pH of the 7.00 buffer must read 7.00 ± 0.1 S.U. As part of the calibration procedure the mV reading of the pH 4.00 buffer and pH 7.00 buffer are measured. The absolute value of the difference of these two measurements should be between >160 mV. This check of the electrode slope verifies acceptable electrode response. Duplicate readings, when measured, should be within 20% of each other.

19) Corrective Actions for Out-of-Control Data

Out-of-control data must be reported at once to the Senior Manager, who in turn will promulgate a corrective action plan.

20) Contingencies for Handling Out-of-Control or Unacceptable Data

The Executive Director will investigate the problem(s) at depth and take all necessary corrective actions that may include, but not be limited to: obtaining fresh QC standards from one or more suppliers and determining the accuracy of measurements, measuring standard deviations, consulting a service engineer and arranging on-site maintenance or repairing service. All analyses of samples will be stopped until the problem is rectified.

21) Waste Management

Dispose of measured samples and buffers down the drain, flushing with cold water. Samples that have been measured to be acidic ($\text{pH} < 5.00$) should be neutralized with 1 N NaOH prior to disposal.

22) References

Standard Methods for the Examination of Water and Wastewater, section 4500 H-B. APHA, AWWA, WEF, 2011.

Fisher Scientific. Accumet AP Series Handheld pH/mV/Ion Meter Instruction Manual. Rev. 1. 12/03

Fisher Scientific. Accumet pH/Temperature, Combination, Refillable Electrodes (Cat # 13-620-AP50) manual. October 2004.

Thermo Scientific Orion Star A215 Benchtop pH/Conductivity Meter Instruction Sheet, 2011.

YSI Professional Plus Multi-meter user manual. Revision D, March 2009. YSI Incorporated.

EXO1 Sonde user manual, August 2014. YSI Incorporated.

23) Tables, Diagrams, Flowcharts and Validation Data

None.

TEMPERATURE

Section No. XXIV, Revision No. 8, Effective Date March 2016

1) Test Method

Based on SM (21) 2550B

2) Applicable Matrix or Matrices

Non-Potable and Potable Water.

3) Reporting Limit

The reporting limit for temperature is 0.1°C. However, the lowest detection limit depends on the graduation of the thermometer used. The only thermometers that reach this reporting limit are those that are graduated in 0.1°C increments. The reporting limit for any given thermometer will be whatever the lowest graduation increment is on that thermometer. The maximum reporting limit also depends on the range of the thermometers used. Currently, the thermometers used in IEC TDS ovens is the thermometer with the highest range, from 0° to 200°C. For field work, temperature is measured by a thermistor-type thermometer attached to the pH meter. The temperature range of the field pH meters (Fisher Model AP61) is 0 to 100°C. The range for the EXO1 is -5 to 50°C. The reporting limit for these meters is 0.1°C.

4) Scope and Application

The Interstate Environmental Commission regularly analyzes environmental samples taken at municipal and industrial facilities, as well as samples collected in conjunction with ambient water quality surveys aboard the Commission's boat for temperature using this method. This method is

applicable to surface, and saline waters, domestic and industrial wastes. The IEC uses this method to measure the temperature of ambient water and wastewater in the field. In the lab, the method is used to monitor the temperature of refrigerators, waterbaths, incubators, coolers as well as to monitor the temperature of samples and standards during analytical processes (digestions, distillations, etc.) as required in specific analytical SOPs .

5) Summary of Method

Temperature is read using a Celsius thermometer or thermistor-type thermometer that displays a temperature on an LED screen. Thermometers must be checked at least annually against an NIST traceable thermometer

6) Definitions

Temperature is defined as the degree of hotness or coldness of a substance measured on a definite scale.

7) Interferences

Most interferences can be avoided by using good quality thermometers, annually checked against a NIST certified thermometer that is used with its certificate and correction chart. Thermometers must be regularly checked for imperfections such as chipped glass or gaps in the mercury column that could interfere with accurate readings. Thermometers calibrated for total immersion must be completely immersed to the depth of the etched circle around the stem just below the scale level.

8) Safety

Handle thermometers with extreme care to avoid breakage. Broken thermometers containing mercury must be cleaned up using a mercury spill kit. Notify the Associate Laboratory Director who will supervise the cleanup of the mercury spill. All technicians present in the laboratory must be notified of the location of the spill.

9) Equipment and Supplies

Temperature can be read using a thermometer or thermistor-type thermometer that displays a temperature on an LED screen. In the field, temperature measurement is made using one of two meters. The Fisher Scientific Accumet® AP 61 Handheld pH/mV/Ion meter is used in routine municipal and industrial treatment plant sampling events. The electrode used with these meters is the Accumet® pH/Temperature, Combination Refillable Electrode, Fisher Catalog number 13-620-AP50. The YSI ProPlus Multimeter Oxygen, Conductivity, Salinity, and Temperature System or EXO1 Sonde is used to measure temperature for ambient sampling events. Specifications are contained in the manufacturer's instruction manuals for the AP61, AP61 electrode, and the ProPlus.

10) Reagents and Standards

There are no reagents for temperature. All laboratory and field thermometers are checked annually against the NIST-traceable thermometer. The NIST-traceable thermometer is sent out for re-certification annually.

11) Sample Collection, Preservation, Shipment and Storage

In the field temperature measurements should ideally be taken directly, by submerging the pH probe or YSI probe into the water, if it is practical and safe to do so. For ambient water quality surveys, temperature reading should be taken 1 foot below the surface, unless the Quality Assurance Project Plan specifies particular depths. Samples can be collected in plastic or glass bottles, if the water cannot be reached directly by the probe. The method of collection depends on the location of the sampling site. The sample must be collected in a clean dry glass or plastic container. The sample may be taken by directly dipping the sampling container by hand into the water (if the field technician can do so safely) or using a bottle attached to a sampling stick. The sampling vessel should be rinsed out three times with portions of the water to be sampled. Temperature must be analyzed immediately (15 minutes or less from the time of sample collection). The sample aliquot should then be discarded in the field. Under no circumstances should a sample aliquot, having had a thermometer or temperature measuring probe inserted into the bottle for the purpose of measuring temperature, be used for any other analysis.

12) Quality Control

All thermometers- field and lab, must be checked against a precision thermometer or thermistors certified by the National Institute of Standards and Technology (NIST) annually. To do this, place the NIST thermometer alongside the device to be checked (the “working” thermometer), in the location where that device is normally used (e.g. refrigerator, 44.5°C water bath). Leave both thermometers side by side until they reach constant temperatures. Read the measurement on the NIST thermometer, apply any correction factor that has been determined from its annual recertification at that temperature, then read the measurement on the thermometer to be calibrated. The difference between the corrected NIST thermometer reading and the reading of the working thermometer is the correction factor for that working thermometer. As an example, if the NIST thermometer reads 44.7°C at a device set at 44.5°C, and the NIST thermometer is determined at its annual re-certification to have a correction factor of -0.1°C at 44.5°C, the analyst would subtract 0.1°C from the reading of the NIST thermometer to arrive at a reading of 44.6°C. The analyst would then use this as the NIST reading, and compare it to the reading of the working thermometer, to arrive at a correction factor for the working thermometer. Using the example of the 44.6°C corrected NIST reading above, if a working thermometer next to it stabilizes at a reading of 44.4°C, the analyst would add 0.2°C to reach the NIST-traceable reading of 44.6°C. The thermometer would therefore have a correction factor of +0.2°C. Each thermometer must be tagged with its unique identification number, correction factor, temperature at which the annual NIST check was made, date of the check, and the initials of the analyst performing the check.

In the case of field meters, a monthly check is done in the lab, by the associate laboratory director, comparing the pH probe temperature reading to the NIST traceable thermometer.

The Certificates from the annual certification of the NIST thermometer are retained on file in the lab in the black 3-ring binder labeled “Service records, Service contracts and Calibration Certificates.”

13) Calibration and Standardization

See Quality Control, Section 12, above, for requirements for calibrating thermometers.

14) Procedure

In the laboratory, the temperature of instruments (waterbaths, incubators, ovens, etc.) is monitored with thermometers that are permanently situated within the instrument. In the case of the waterbaths, the thermometers are inserted into a special thermometer opening in the hood of the waterbaths. The thermometer is immersed directly into the waterbath up to the immersion level etched on the thermometer. For refrigerators and dry incubators, the thermometer is immersed in deionized water in an ehrlenmeyer flask up to the immersion level etched on the thermometer. Care must be taken, especially in the case of dry incubators, to make sure that the level of water in the flask does not fall below the immersion point due to evaporation. In the case of the ovens, the thermometers are placed in beakers containing sand.

As part of daily routine laboratory quality control practices, the temperatures of the waterbaths, dry incubators, and refrigerators are checked twice a day, at least four hours apart. The temperatures are read directly off of the thermometer, the applicable correction factor applied, and the corrected temperature reading recorded in the temperature logbook.

Temperatures of environmental samples are measured in the field immediately upon collection. The sample is collected as described above in Section 11, Sampling, Preservation, Shipment, and Storage. If using the Accumet AP 61 pH/mV/Ion meter, immerse the probe directly into the sample and provide moderate stirring. Turn the meter on by pressing the on/off button. Wait until the temperature readout, displayed on the screen as °C, stabilizes. This should take less than 10 seconds. The temperature is always displayed on the AP61 screen while the meter is on, and changes as the temperature of the sample changes. Therefore it is critical to read and RECORD the temperature as soon as a stable reading is displayed.

If using the YSI ProPlus or EXO Sonde, immerse the probe in the solution (or water body at desired depth), provide moderate stirring, and turn on the meter. Like the AP61, the temperature of the sample will always be displayed on the screen as long as the meter remains on, and will change as the temperature of the sample changes. Read and record the first stable reading that the meter displays.

15) Calculations

None Required.

16) Method Performance

There are no proficiency samples available or required for temperature. Method performance is monitored by recording and reviewing data collected from daily checks of laboratory thermometers and annual checks of field and lab thermometers against the NIST thermometer.

17) Pollution Prevention

Under no circumstances are mercury filled thermometers to be disposed of in the regular waste. Broken thermometers must be cleaned up using a mercury spill kit under the direct supervision of the Executive Director or Senior Environmental Analyst. The Executive Director or designee will then arrange for a pickup of this waste by a licensed chemical waste company. The IEC currently uses Triumverate Environmental, Inc. for the removal of chemical wastes.

18) Data Assessment and Acceptance Criteria for Quality Control Measures

The correction factor for all laboratory and field thermometers must not be more than $\pm 0.7^{\circ}\text{C}$. In the event that this criterion is not met, the thermometer should be rechecked, and if the criteria is still not met, a new thermometer shall be procured, calibrated against an NIST thermometer and put into service. The NIST traceable thermometer correction factors must not exceed $\pm 0.3^{\circ}\text{C}$ at any temperature. In the event this criteria is not met, another NIST thermometer shall be procured.

19) Corrective Actions for Out-of-Control Data

Thermometers that are malfunctioning or appear to be malfunctioning must be reported at once to the IEC's Executive Director/QC Officer or Senior Environmental Analyst, who in turn has to promulgate a corrective action plan.

20) Contingencies for Handling Out-of-Control or Unacceptable Data

The QC Officer will investigate the problem(s) at depth and take all necessary corrective actions that may include, but not be limited to: comparing the thermometer against the NIST traceable thermometer, determining the accuracy of measurements, or replacing the thermometer, probe, or meter. All analyses of samples will be stopped until the problem is rectified. If the temperature device in question is monitoring a laboratory apparatus (incubator, waterbath, etc.), another thermometer, checked against the NIST thermometer at the temperature of use and assigned a correction factor, must be put into use in the apparatus.

21) Waste Management

See Pollution Prevention, Section 17, for information on managing mercury waste (broken thermometers). Environmental samples collected and solely used for temperature analysis in the field can be discarded in the field.

22) References

Standard Methods for the Examination of Water and Wastewater, Section 2550B 21th Edition.
APHA, AWWA, WEF, 2005.

EXO1 user manual, August 2014. YSI Incorporated.

23) Tables, Diagrams, Flowcharts and Validation Data

None.

SECCHI DISK DEPTH

Section No. FIELD IV, Revision No. 1, Effective Date January 2015

1) Test Method

Secchi Disk Depth, SOP ID FIELD IV, Revision 1, January 2015.

2) Applicable Matrix or Matrices

This method is applicable to open water, lakes, ponds, reservoirs and other slow-moving waterways. Monitoring water clarity in river waters is more difficult with a secchi disk as the current can interfere with a stable reading.

3) Method Detection Limit

0.5 feet

4) Scope and Application

The IEC uses this method as an in situ measurement to assess water clarity in the far western Long Island Sound as well as other ambient water quality projects, as needed.

5) Summary of Method

Secchi disk depth is measured by lowering a round weighted circular disk 20 cm in diameter with four alternating blank and white quadrants painted on the surface. The disk is lowered into the water from a boat until the point at which it disappears. It is then raised until it reappears. The depths, in feet, at which the Secchi disk disappears and reappears is averaged and the average recorded as the Secchi disk depth (also called Secchi depth).

6) Definitions

Secchi disk- a round weighted circular disk 20cm in diameter (approximately 8 inches) with four alternating black and white quadrants, which is suspended on a rope graduated at 0.5 foot intervals.

7) Interferences

This procedure may disrupt fish and microscopic organisms, such as phytoplankton and zooplankton, which can interfere with other sample collections (such as chlorophyll a). Secchi disk depth should be taken after these samples have been collected, but before any activity that may disturb bottom sediments to avoid increasing turbidity at the location. Ideally, secchi disk measurements should be taken between 10am and 2 pm to minimize glare. When this is not possible, the time the measurement is taken and, in the case of a series of surveys (such as the Long Island Sound surveys), take each station's secchi disk depth measurement at approximately the same time. Ideally the same analyst should take the Secchi disk depth at the same station throughout the season. As this is not always possible, at one station per survey, two analysts should measure Secchi disk depth to ensure consistent readings. The analyst should remove sunglasses but continue to wear any clear prescription glasses. The measurement should be taken on the shaded side of the boat to reduce glare. Where there is no shade, the analyst should keep their back to the sun to block glare and reflection.

8) Safety

All boat surveys consist of a minimum of two analysts for safety. Life vests and stable, flat, rubber soled shoes should be worn at all times on the boat.

9) Equipment and Supplies

Secchi disk secured to a weight and appropriately graduated rope (0.5ft increments) (The Science Source or other suitable supplier)

Spare Secchi disk in case of loss or damage

Long Island Sound Field Data Sheet or other project-specific data sheet.

Clipboard

Charter Boat with depth finder, GPS

Life jackets

Pen

Clock

Gloves

10) Reagents and Standards

No reagents or standards are required for Secchi disk depth measurement.

11) Sample Collection, Preservation, Shipment and Storage

Secchi disk depth is an *in situ* measurement. No samples are collected, preserved, shipped or stored for this method

12) Quality Control

While the procedure of this test is very simple, certain basic quality control requirements are still applicable. Procedures listed in section 7) Interferences as well as section 14 should be reviewed and followed to ensure accurate, consistent, and quality results. At two stations per run, duplicate Secchi disk measurements are taken. At one station per run, a second analyst performs the Secchi disk depth measurement to ensure consistent readings.

13) Calibration and Standardization

The rope used to suspend the Secchi disk is graduated in 0.5 foot increments.

14) Procedure

The field analyst should remove sunglasses (prescription or non-prescription) but continue to wear any clear prescription glasses. Determine which side of the boat is exposed to the sun. The measurement should be taken on the shaded side of the boat to reduce glare. Where there is no shade, the analyst should keep their back to the sun to block glare and reflection. When taking a measurement the Secchi disk should remain in the water directly below your hand holding the rope attached to the Secchi disk. If the current or wind causes the disk to drift, extra weight should be added to the disk to avoid skewed measurements when read at an angle. Unwind the rope attached to the Secchi disk. Place your foot gently on the end of the rope to prevent loss of Secchi disk overboard. Lower the Secchi disk over the side of the boat into the water until the pattern on the Secchi disk just disappears from view. Read aloud the depth at the water surface from the graduated rope to the nearest 0.5 foot to the second analyst. The second analyst records this number on the datasheet. Lower the disk approximately 0.5ft more and pull the rope, gently out of the water until the pattern on the Secchi disk just reappears. Read aloud the depth at the water surface from the graduated rope to the second analyst. The second analyst records this number, and averages and record the result.

15) Calculations

The depth at which the Secchi disk disappears and the depth at which the Secchi disk reappears is averaged.

16) Method Performance

See Quality Control, section 12

17) Pollution Prevention

This analysis poses little potential for pollution.

18) Data Assessment and Acceptance Criteria for QC Measures

Duplicate Secchi disk depth measurements should vary by no more than 5%. In the case of very shallow Secchi disk depths, this criteria may not be able to be measured, as the rope is graduated in

0.5 ft increments. This acceptance criteria applies both to duplicate measurements taken by the same analyst as well as duplicate measurements taken by the second analyst.

19) Corrective Actions for Out-of-Control Data

If duplicate readings are not within acceptance criteria, a third measurement should be taken. If this measurement is also out of the acceptable range, the boat position on the GPS should be checked to make sure the boat has not drifted. If second analyst measurements are not within acceptance criteria, the analysts should jointly perform a Secchi disk depth measurement to ensure consistent procedures.

20) Contingencies for Handling Out-of-Control Data or Unacceptable Data

Measurements that do not meet acceptance criteria should be identified with an appropriate qualifying QC note on the final report. Consistent issues with acceptance criteria indicate a problem with the procedure. The QC officer should review the procedure with all analysts, and accompany the analysts on the next survey to ensure appropriate procedures are being followed and there is not a problem with the equipment (Secchi disk or boat)

21) Waste Management

This procedure produces no hazardous waste.

22) References

USEPA 2012. *Volunteer Lake Monitoring: A Methods Manual*. Office of Water Regulations and Standards, Washington, D.C. EPA 440-4-91-002
<http://www.epa.gov/volunteer/lake/lakevolman.html>

USEPA 2012. *Using a Secchi Disk or Transparency Tube*.
<http://water.epa.gov/type/rsl/monitoring/155.cfm>

Rhode Island Department of Environmental Management, 2011. *Standard Operating Procedure for Secchi Disk Measurements (SOP WR-W-7)* Office of Water Resources, Providence, RI.

23) Tables, Diagrams, Flowcharts, and Validation Data

None.

Total Suspended Solids (TSS)

Section No. XXIIIC, Revision No. 10, Effective Date June 2017

1) Test Method

Based on Standard Methods, Section 2540 A and D, -97, -11.

2) Applicable Matrix or Matrices

Non-Potable Water.

3) Method Detection Limit

A sample volume that yields a residue between 2.5 and 200 mg should be chosen. Accordingly, the MDL and reporting limit is 2.5 mg/filter (with volume filtered determining the final result). When 2.5 mg net weight of crucible is not achieved, samples results must be qualified as “estimated.”

4) Scope and Application

The Interstate Environmental Commission regularly analyzes environmental samples from municipal and industrial facilities for TSS. This method is applicable to all non-potable surface and saline waters, domestic and industrial influents and effluents. The IEC utilizes this method most often in analyzing industrial and municipal influent and/or effluent samples to assess compliance with regulatory wastewater effluent limitations.

5) Summary of Method

An aliquot of well-mixed sample water is filtered through a weighed gooch crucible containing a standard glass-fiber filter and the residue retained on the filter is dried to a constant weight at 103° to 105°C. The difference in weight of the filter and crucible before and after filtration of the sample represents the total suspended solids.

6) Definitions

Total Suspended Solids- A term applied to the residue retained on a 24 mm glass fiber filter after filtration of a sample and the subsequent drying of the filter and crucible in an oven at a 103-105° C.

7) Interferences

Exclude large, floating particles (e.g. sticks, leaves, bugs) or submerged agglomerates of nonhomogeneous materials from the sample if it is determined that their inclusion is not representative. For samples high in dissolved solids thoroughly wash the filter to ensure removal of dissolved material. Prolonged filtration times resulting from filter-clogging may produce high results owing to increased colloidal materials captured on the clogged filter. Because excessive residue in the dish may form a water-trapping crust, limit sample to no more than 200 mg residue.

8) Safety

There are no hazardous equipment or reagents used in this analysis. As always, wear appropriate personal protective equipment including gloves, goggles, and a labcoat when working with any environmental samples. Transfer crucibles to and from oven using tongs to avoid burns and prevent contaminating sample.

9) Equipment and Supplies

1. Whatman glass microfiber filters (Cat No. 1827-024), 24 mm diameter. Filters must be logged into the Chemical Inventory Logbook. Document received date, opened date, discarded date for each lot of filters received.

2. Drying oven, set and maintained at a temperature of 103 to 105° C.
3. Gooch crucibles, 25 ml to 40 ml capacity.
4. Desiccator, provided with a desiccant containing a color indicator of moisture concentration (e.g. Drierite®).
5. Analytical Balance (Mettler-Toledo Model AX205). 2 mg calibrated weight.
6. Filtration apparatus with a flask or reservoir with vacuum source attachment.
7. Wide-mouth pipets or graduated cylinders of 100 ml capacity.
8. Vacuum source.
9. Tongs.
10. Goggles, latex gloves, and labcoat.

10) Reagents

1. Deionized water for rinsing filter and dilutions.

11) Sample Collection, Preservation, Shipment, and Storage

Use resistant-glass or plastic (polyethylene or equivalent) bottles, provided that the material in suspension does not adhere to container walls. Begin analysis as soon as possible because of the impracticality of preserving the sample. Refrigerate sample at $4\pm 2^{\circ}\text{C}$ up to the time of analysis to minimize microbiological decomposition of solids. Preferably do not hold samples more than 24 hours. In no case hold sample more than 7 days. Bring samples to room temperature before analysis. Minimum sample volume required for analysis is 100 ml, however 400 ml or more of sample may need to be filtered to achieve residue between 2.5 and 200 mg.

12) Quality Control

10% of all samples are analyzed in duplicate (but at least one per batch if batch is less than 10 samples). Duplicate determinations should agree within 5 % of their average. Analyze one blank with each batch. Record the following QC data each time an oven is used: date, starting time, starting temperature and all temperatures taken during analysis, final temperature, analysis performed, ending date and time, initials.

13) Calibration and Standardization

Check accuracy of balance with Class S weights and record before beginning analyses with 2 mg weight to ensure the ability to detect a 2.5 mg change in crucible weight. Check temperature of drying oven with NIST calibrated thermometer to confirm that it is between 103 and 105°C and record before beginning analysis.

14) Procedure

1. Preparation of the gooch crucible: Place one (1) 21 mm glass microfiber-filter in a gooch crucible with the wrinkled surface of the filter facing upward (“gridded”) side down. Be sure to inspect filter to ensure that it is free of holes. Prepare two crucibles for each sample that will be analyzed in duplicate. Rinse each filter and crucible unit with three (3) successive 10 ml portions of deionized water. Heat the crucibles in the 103-105°C oven for one hour. Cool to room temperature in a desiccator. Weigh to the nearest 0.01 mg immediately before use.

2. Choose a sample volume that should yield a residue between 2.5 and 200 mg. Begin Suction. *Stir sample with a magnetic stirrer at a speed to shear larger particles, if practical, to obtain a more uniform (preferably homogeneous) particle size. Centrifugal force may separate particles by size and density, resulting in poor precision when point of sample withdrawal is varied. While stirring, pipet a measured volume of well-mixed sample onto the seated glass-fiber filter. For homogeneous samples, pipet from the approximate mid-point of the container but not in vortex.* For most effluent samples, a 200 ml aliquot is sufficient. Wash with three successive 10 ml portions of deionized water, allowing complete drainage in between washings, and continue suction for about 3 minutes after filtration is complete. Samples with high dissolved solids may require additional washings. Remove the crucible and filter combination from the filter apparatus. Heat the crucibles in the 103-105°C oven for one hour. Cool to room temperature in a desiccator (minimum 30 minutes).

3. Weigh to the nearest 0.01 mg. Repeat this cycle of heating, cooling and weighing until the crucible attains a constant weight or until weight change is less than 4% of previous weight or 0.5 mg, whichever is less. Record all weights in analyst logbook. Use final weight when calculating and reporting final analytical result. When weighing dried sample, be alert to change in weight due to air exposure and/or sample degradation.

15) Calculation

$$\text{mg total suspended solids/L} = \frac{(A-B) \times 1000}{\text{Aliquot volume, ml}}$$

where: A=weight of residue + evaporating dish (final weight, mg)

B=weight of evaporating dish (initial weight, mg)

16) Method Performance

Analyze 10 % of samples in duplicate. Each analyst must perform an initial demonstration of capability (IDC) prior to performing analysis on environmental samples. The IDC consists of analyzing a quality control sample, procured or prepared by the Associate Laboratory Director and analyzed four times by the analyst. Thereafter, each analyst must perform a CDC, consisting of a sample analyzed in duplicate. The results of the IDC and CDC must be within the manufacturer's determined acceptance limits. The laboratory analyzes two proficiency samples per year, provided by the New York State Department of Health (NYSDOH).

17) Pollution Prevention

There are no hazardous reagents used in this analysis. Clean up any spills or leaks from environmental samples promptly with a disinfectant cleanser.

18) Data Assessment and Acceptance Criteria for Quality Control Measures

Duplicate determinations should agree within 5% of their average weight. Successive weighings should be within 4% or 0.5 mg, whichever is less. The blank should be no more than 1 mg/L. Do not subtract the blank value from sample weight, as the deionized water used in the analysis is minimal (for rinsing purposes only).

19) Corrective Actions for Out-of-Control Data

The sample and duplicate should differ in value by no more than 5% of their average weight. In the case of samples with a relatively low analyte concentration, the sample and duplicate may differ by more than 5%. If the sample and duplicate vary by more than 5%, bring the discrepancy to the attention of the associate laboratory director. He or she will determine, based on the analyte concentration, whether the discrepancy is significant. If the blank value exceeds 1 mg/L, re-analyze another blank to determine if there is a consistent error.

20) Contingencies for Handling Out-of-Control Data

Analysis must not proceed on additional samples if any quality control measures, including blank values, duplicates, do not conform to the acceptance criteria outlined above until corrective actions have found the cause and a quality control sample has been analyzed in duplicate with acceptable results. The associate laboratory director will determine whether the data generated with an unacceptable quality control item can be reported. If reported, such data must be qualified with a note specifying the quality control failure. If possible, the analysis should be repeated rather than qualified data reported.

21) Waste Management

Dilute remaining sample (not used in analysis) from 3 to 5 times its original volume with cold water. Open the cold water tap completely and slowly pour the material down the drain. Allow cold water to run for 5 minutes to completely flush the system.

22) References

Standard Methods for the Examination of Water and Wastewater section 2540 A and D. APHA, AWWA, WEF, 2001, 2011.

23) Tables, Diagrams, Flowcharts, Validation Data

None.

BIOCHEMICAL OXYGEN DEMAND

(5 days, 20°C)

Section III, Revision No. 10, Effective Date August 2017

1) Test Method

Based on Standard Methods (18) 5210 B

2) Applicable Matrix or Matrices

Non-Potable Water

3) Method Detection Limit

3 mg/L

4) Scope and Application

The Interstate Environmental Commission analyzes environmental samples for Biological Oxygen Demand routinely in the field at municipal and industrial facilities. The Biochemical Oxygen Demand Test (BOD) is used for determining the relative oxygen requirements of municipal and industrial wastewaters. The BOD test is an empirical bioassay-type procedure which measures the dissolved oxygen consumed by microbial life while assimilating and oxidizing the organic matter present. The standard test conditions include dark incubation at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for a specified time period (in this SOP 5 days). The actual environmental conditions of temperature, biological population, water movement, sunlight and oxygen concentration cannot be accurately reproduced in the laboratory. Results obtained must take into account the above factors when relating BOD results to stream oxygen demands.

5) Summary of Method

The initial dissolved oxygen content of several dilutions of a sample of wastewater is measured using a DO meter or the modified Winkler titration. An aliquot of the same sample is incubated in an airtight bottle, for 5 days at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$, in the dark. The final dissolved oxygen concentration is then measured. The reduction in dissolved oxygen concentration during the incubation period yields a measure of the biochemical oxygen demand.

6) Definitions

Azide Modification- The addition of sodium azide (the alkaline-iodide-azide reagent) prior to acidification in order to suppress any interference from NO_2^- in the sample. Azide prevents any possible reaction of nitrite with iodide.

7) Interferences

Extremes of acidity and alkalinity prevent microorganism growth and lead to underestimates of BOD. Residual chlorine may also cause a low BOD for the same reason. Other toxic substances in industrial wastes may also kill microorganisms and lead to a low BOD. Cold waters may be supersaturated with dissolved oxygen. Samples should be brought to room temperature before beginning analysis.

The azide modification is not applicable for the following types of samples:

- a. samples containing sulfite, thiosulfate, polythionate, appreciable quantities of free chlorine or hypochlorite;
- b. samples high in suspended solids;
- c. samples containing organic substances which are readily oxidized in a highly alkaline solution or which are oxidized by free iodine in an acid solution;
- d. untreated domestic sewage;
- e. biological flocs;
- f. where sample color interferes with end point detection;
- g. samples containing $\geq 5\text{mg/l}$ ferric iron salts

Most organic matter is oxidized partially when the oxidized manganese precipitate is acidified, thus causing negative errors.

Many biological treatment plant effluents contain sufficient numbers of nitrifying organisms to cause nitrification in BOD tests. Because oxidation of nitrogenous compounds can occur in such samples, inhibition of nitrification is recommended for samples of secondary effluent, and for samples of polluted waters. Report results as carbonaceous biochemical oxygen demand (CBOD₅) when inhibiting the nitrogenous oxygen demand. When nitrification is not inhibited, report results as BOD₅.

8) Safety

This method, when the winkler method is used, requires using concentrated sulfuric acid, which is extremely caustic. Use extreme care when adding sulfuric acid and shaking bottles to avoid drips and spills. To protect the analyst from the acid as well as all the other reagents, appropriate personal protective equipment, including goggles, labcoat and gloves must be worn.

9) Equipment and Supplies

1. Incubation bottles, 300 ml capacity, with ground-glass stoppers: Bottles should be cleaned with a good detergent and thoroughly rinsed and drained before use. As a precaution against drawing air into the dilution bottle during incubation, a water seal is recommended. Satisfactory water seals are obtained by adding water to the flared mouth of special BOD bottles and covering them with plastic caps.
2. Air incubator, thermostatically controlled at 20°C ±1°C. All light should be excluded to prevent formation of DO by algae in the sample.
3. Pipets: with elongated tips capable of delivering 1.0 ml of reagent.
4. Class A Buret with a minimum 25ml capacity for titration suspended securely on a ringstand with a reservoir for sodium thiosulfate, for the Winkler titration.
5. 300 ml wide-mouth Ehrlenmeyer flasks.
6. Graduated cylinder graduated in 1 ml increments.
7. Wide-mouth volumetric pipets

10) Reagents

For the Winkler Titration:

1. Manganous sulfate solution: Manufactured by LabChem, Inc., purchased from Fisher Scientific (Catalog #LC16570-4), or Manganese (II) Sulfate Solution (Fisher Scientific Catalog # SM 20-500)
2. Alkaline iodide-azide solution: Manufactured by LabChem Inc, purchased from Fisher Scientific (Catalog # LC10670-2), or Ricca Chemical Company (Catalog # 540-16)
3. Sulfuric acid: concentrated. Purchased from Fisher Scientific (Catalog # A300-212)

4. 6N Sulfuric acid solution
5. Standard potassium bi-iodate solution, 0.0021M. Dissolve 812.4 mg KH (IO₃)₂ in deionized water and dilute to 1000mL.
6. Starch Indicator 1.0%: Manufactured by Lab Chem Inc., purchased from Fisher Scientific (Catalog No LC25310-2), or Ricca Chemical Company (Catalog # 8050-32)
7. Certified Sodium Thiosulfate 0.025N. Manufactured and purchased from Fisher Scientific (Catalog # SS370-01).
8. Potassium Dichromate, 0.025N. Manufactured by Ricca Chemical, purchased from Fisher Scientific (Catalog #6050-16).
9. Potassium Iodide, Granular. Manufactured by and purchased from Fisher Scientific (Catalog # P410-500).
10. L-(+)- Glutamic Acid, Fisher Scientific Catalog # A125-100
11. D (+)-Glucose, Reagent ACS, anhydrous (Acros Cat # 41095-5000)

Dilution Water Reagents/Buffers

12. Distilled water: Water used for solutions and for preparation of dilution water must be of the highest quality, distilled from glass still; it must contain less than 0.01 mg/L copper and be free of chlorine , chloramines, caustic alkalinity, organic material or acids.
13. Phosphate buffer solution: Manufactured by and purchased from Fisher Scientific (Catalog # SP341-1) or Ricca Catalog # 5800-16.
14. Magnesium sulfate solution, 2.25%: Manufactured by and purchased from Fisher Scientific (Catalog #SM109-1).
15. Calcium chloride solution, 2.75%: Manufactured by and purchased from Fisher Scientific (Catalog # SC10-1).
16. Ferric chloride solution, 0.025% w/v: Manufactured by and purchased from Fisher Scientific (Catalog # SF97-1).
17. 1N H₂SO₄: For neutralization of waste samples which are caustic.
18. 1N NaOH: For neutralizing samples which are acidic.
19. Seed Solution (i.e “PolySeed® Solution”): PolySeed® capsules manufactured by InterLab®, purchased through Fisher Scientific. Prepare according to the following manufacturer’s directions: To make the seed solution, place the entire contents of one PolySeed® capsule (discard the gelatin capsule) into 500 mls of buffered dilution water (prepared in section 14a, below). Aerate and stir

the PolySeed® solution for one hour. Finally, decant the supernatant so as not to allow any bran in the biological solution. Pour the decanted PolySeed® solution in a clean 500 ml beaker with a sterile stir bar, place on magnetic stirrer and gently stir for the remainder of the test. Prepare a fresh Polyseed® solution for each day of BOD analysis.

20. Glucose-Glutamic Acid solution: Prepare in the lab or purchase commercially from suppliers such as Hach or Ricca. Because commercially available stock solution may vary in concentration, carefully read the manufacturer's documentation (certificate of analysis) and instructions to ensure the correct preparation of the glucose-glutamic acid solution. Compare any new supplier of G&G solution to a supplier of known quality. If preparing in lab, dry reagent-grade glucose and reagent-grade glutamic acid at 103°C for 1 hour. Add 150 mg glucose and 150 mg glutamic acid to distilled water and dilute to 1 liter. Add 6 ml of glucose-glutamic acid solution in to BOD bottles directly and bring to the 300 ml volume with buffered dilution water. **Prepare fresh immediately before use.**

For Calibration of the pH meter (to measure sample pH):

21. pH 4.0 buffer (Fisher catalog # SB101-500)

22. pH 10.0 buffer (Fisher catalog # SB115-500)

23. pH 7.0 buffer (Fisher catalog # SB 107-500)

11) Sample Collection, Preservation, Shipment and Storage

Samples may be stored in plastic (polyethylene or equivalent) or glass. Cool to 4±2°C. Start analysis of grab samples within 24 hours. Minimum sample volume required is 1000mL.

Limit compositing time to 24 hours. Start the measurement of holding time from end of compositing period.

Sample Pre-treatment:

Samples containing caustic alkalinity or acidity: Neutralize to about pH 7.0 with 1N H₂SO₄ or 1N NaOH, using a pH meter. The pH of the seeded dilution water should not be changed by the preparation of the lowest dilution of sample.

Samples containing residual chlorine compounds: If the samples are allowed to stand for 1 to 2 hours, the residual chlorine will often dissipate. BOD dilutions can then be prepared with properly seeded standard dilution water. Higher chlorine residuals in neutralized samples should be destroyed by adding sodium sulfite. The appropriate quantity of sodium sulfite solution is determined on a 100-1,000 ml portion of neutralized sample by adding 10 ml of 1:1 acetic acid or 1:50 H₂SO₄, followed by 10 ml potassium iodide solution (10 g in 100 ml) per 1000mL portion and titrating with 0.0250N sodium sulfite solution to the starch-iodide end point. Add to a volume of sample the quantity of sodium sulfite solution determined by the above test, mix and after 10-20 minutes, test aliquot samples for residual chlorine to check the treatment. Prepare BOD dilutions with seeded standard dilution water.

Samples containing other toxic substances: Samples such as those from industrial wastes frequently require special study and treatment.

Samples supersaturated with DO: Samples containing more than 9 mg/L DO at 20°C may be encountered during winter months or in localities where algae are actively growing. To prevent loss of oxygen during incubation of these samples, the DO should be reduced to below saturation by bringing the sample to about 20°C in partly filled bottle and agitating it by vigorous shaking or by aerating with compressed air.

12) Quality Control

1. Run duplicate bottles of unseeded dilution water with every setup of BOD analysis. These bottles must be selected at random from the BOD bottle cabinet.
2. Run duplicate bottles of seeded dilution water with every setup of BOD analysis. These bottles must be selected at random from the BOD bottle cabinet.
3. Dilution water bottles are washed with detergent and rinsed and dried before each run to assure that the bottles are not contaminated with materials that may interfere with the test.
4. The normality of the sodium thiosulfate solution must be verified and documented quarterly.
5. Reagents must be prepared and used within six months, with the exception of glucose-glutamic acid, which must be prepared on day of use. Commercially purchased reagents may be used, and must be used by manufacturer's expiration date.
6. Run the glucose-glutamic acid check, at least in duplicate, which each analysis batch.
7. Run parallel winkler and electrode probe methods monthly on one batch of BOD samples.
8. Run one environmental sample in duplicate monthly.
9. Check and RECORD the pH and total residual chlorine of each sample prior to withdrawing aliquots for analysis.
10. Check and RECORD the pH and temperature of prepared buffered dilution water.
11. RECORD the time and date BOD bottles were placed in incubator and time and date BOD bottles were removed from incubator.

13) Calibration and Standardization

1. Calibrate the pH meter according to pH SOP (IEC SOP XIX, Part 13 Calibration and Standardization).
2. Standardization of sodium thiosulfate titrant (if used): Dissolve approximately 2 g KI, free from iodate, in an Erlenmeyer flask with 150 ml deionized water; add 1 ml 6N H₂SO₄, followed by 25.0 ml potassium dichromate (0.025N) solution. Dilute to 200 ml and titrate the liberated iodine with the sodium thiosulfate titrant, adding starch toward the end of the titration, when a pale straw color is reached. Exactly 25.00 ml 0.0250N thiosulfate should be required when the solutions under comparison are of equal strength.
3. If using Membrane Electrode Method- Access the Select mg/L setup page by pressing the **setup** key while in measurement mode until the page displays mg/L. Press **enter** to accept the option of measurement in mg/L Mode and return to measurement mode, OR press **setup** to go to next setup page without making any changes. Determine the standardized DO value by performing a winkler titration on 1 liter of deionized water. Put the DO probe into buffered dilution water to calibrate the meter. Press **std** to enter the standardize screen. Both upper and lower display show the

present measured value. The window provided for the adjustment is $\pm 70\%$ of the present reading. Lowest value that can be set is 2.00 mg/L; highest value is 60.0 mg/L. Manually adjust the measured value to match the DO value of the buffered measured from the Winkler titration. Press **std** key to confirm calibration. Meter blinks the **cal** values for few seconds before returning to measurement mode. Record DO reading. Refer to the Accumet AB40 user manual for more detailed instructions on meter calibration, including alternate calibration methods (% saturation calibration).

4. Check the ATC (automatic temperature compensation probe) annually against the NIST thermometer. Refer to Accumet AB40 user manual for directions for adjusting the ATC temperature offset.

14) Procedure

a. Preparation of buffered dilution water:

Place the desired volume of deionized water (10-20 L depending on volume of samples) in a suitable bottle and add 1 ml each of phosphate buffer, magnesium sulfate, calcium chloride and ferric chloride solutions for each liter of water. Aerate the water thus prepared for 60 minutes so that it will become saturated with oxygen.

Check the pH of the prepared buffered dilution water. The pH should be between 6.5-7.5. Record the pH on BOD record sheet.

b. Seed correction - Determine the oxygen depletion of the seed by setting up a separate series of seed dilutions (15, 20, 25 mls of seed solution diluted in BOD bottles to 300 mls). Ideally make dilutions of seed such that the largest quantity results in at least 50% DO depletion. Select a seed dilution with a result above 50% to calculate the seed correction factor. If there is not a dilution that results in an oxygen depletion in this range, select the dilution that is closest to this range. This dilution is then used to calculate the correction due to the small amount of seed in the dilution water.

c. Dilution water control- Fill two BOD bottles (four if using titration method) with buffered dilution water. Stopper and water-seal two of these for incubation. The other bottles are for determining the DO before incubation. If the membrane electrode method is used for determining DO, use only two bottles. These two bottles will be used to measure the initial DO with a membrane electrode, then stoppered and incubated for five days, when the DO will again be read by using the electrode method to determine oxygen depletion. The DO results on these two bottles are used as a check on the quality of the unseeded dilution water. The depletion obtained should not be used as a blank correction; it should not be more than 0.2 mg/L and preferably not more than 0.1 mg/L.

d. Seeding the dilution water: Add 4 ml of seed solution directly to EACH 300 mL BOD bottle as per Polyseed manufacturer's directions. The seed used is prepared from a PolySeed® capsule (manufactured by InterLab®, purchased through Fisher Scientific. See Section 10, Reagents).

e. Fill two bottles (four if using titration method) with dilution water to use as seeded blanks.

f. Dilution technique-Make several dilutions directly in BOD bottles using wide tip pipette so as to obtain the required depletions. The following dilutions are suggested:

- 0.1-1.0% for strong trade wastes,
- 1-5% for raw and settle sewage,
- 5-25% for oxidized effluents, and
- 25-100% for polluted river waters.

For municipal treatment plant effluents, four sample dilutions are used. Typically, 20, 40, 70, and 100 ml aliquots of sample are diluted to 300 ml. Dilutions may be changed if there is information available (e.g. based on historical sampling) that the sample may be high or low in BOD. For each bottle of sample, add the quantity of carefully mixed sample to make the desired dilution to each of 2 BOD bottles, if the azide modification titration method is used to determine DO. If the membrane electrode method is used, only one bottle per dilution is required since the same bottle is used to determine initial and final DO. Dilute with dilution water. If the titration method is used, stopper tightly one set of bottles (for all dilutions of all samples) and incubate for 5 days at 20°C. The BOD bottles should be water-sealed by filling the flared lip of the BOD bottle with water and covering with a plastic cap.

h. Membrane-electrode method- Follow manufacturer's calibration procedure in section 13 and manufacturer's manual exactly to obtain maximum precision and accuracy. Insert the probe into the BOD bottle, ensuring that the probe is securely lodged. Flip the small red switch on the top of the probe to turn the propeller on. When the meter screen reads "stable" record the DO reading in mg/L. Rinse the DO probe in a BOD bottle containing DI water, by inserting probe in the BOD bottle and turning the propeller on briefly. Remove DO probe and place in next sample and record in same way for all samples, rinsing the probe between each reading.

i. AZIDE modification of the iodometric method- Determine the initial DO in the samples by performing the azide modification of the iodometric method (Winkler titration) on one set of samples, the procedure for which is as follows: To the 300 ml bottle, add 1 ml manganese sulfate solution, followed by 1 ml alkali-iodide-azide reagent, well below the surface of the liquid stopper with care to exclude air bubbles and mix by inverting the bottle at least 15 times. When the precipitate settles, shake again. After at least 2 minutes of settling has produced at least 100 ml of clear supernatant, carefully remove the stopper and immediately add 1.0 ml conc. H_2SO_4 by allowing the acid to run down the neck of the bottle, re-stopper, and mix by gentle inversion until the iodine color is uniform throughout the bottle before decanting the amount needed for titration. **Complete the titration within 15 minutes of adding acid.** Transfer the appropriate volume from the BOD bottle (determined from sodium thiosulfate standardization) into a 300ml wide mouth flask and titrate with standardized 0.025 N sodium thiosulfate solution to a pale straw color. Add 1-2 ml of starch solution and continue to titrate to the first disappearance of the blue color. Record initial DO readings on BOD record sheet. After five days, remove the incubated set of samples from the incubator, add the manganous sulfate and alkali-iodide-azide reagents, followed by the H_2SO_4 as described above and titrate against standardized 0.025N sodium thiosulfate titrant. Complete calculations as described on record sheet.

15) Calculation

a. Because 1 ml 0.025N sodium thiosulfate titrant is equivalent to 0.200 mg DO, if using the Winkler method each milliliter of sodium thiosulfate titrant used is equivalent to 1 mg/L DO when a volume equal to 200 ml of original sample is titrated.

b. Definitions:

D1 = Initial DO of diluted sample immediately after preparation, mg/L

D2 = DO of diluted sample after 5d incubation at 20°C, mg/L.

S= oxygen uptake of seed, Δ DO/mL seed suspension added per bottle

To calculate S, determine the DO uptake per mL of seed using the ratio method. For the ratio method, divide the DO depletion by the volume of seed in milliliters for each seed control bottle having a 2.0 mg/L depletion and greater than 1.0 mg/L minimum residual DO and average the results.

V_s= volume of seed in the respective test bottle, mL and

P = decimal fraction of sample used

c. BOD:

$$\text{mg/L BOD} = [(D1 - D2) - (S)V_s] / P$$

16) Method Performance

The performance of the method is continually reviewed by looking at a number of factors. The average depletion of oxygen in the dilution controls (blanks) should not exceed 0.20 mg/l and ideally should be not more than 0.10 mg/l. Oxygen depletion of more than 0.20 mg/l in any blank indicates contamination of either the dilution water, glassware or buffers, or supersaturated (initial DO > 9.2 mg/l) dilution water. The calculated BOD for the QC sample should be within the manufacturer-determined acceptance criteria. The BOD measured for the Glucose-Glutamic Acid Check must be 198±30.5 mg/L. Also, the laboratory performs two proficiency test samples provided by the NYS DOH each year.

17) Pollution Prevention

After titration, neutralize titrated and extra sample with 1N NaOH. Open cold water tap fully and dispose of samples down drain. Flush for 5 minutes after all samples have been disposed of. Calculate how much dilution water will be necessary for the number of samples to be analyzed. Only prepare as much buffered dilution water as necessary. See Waste Management section of this SOP for more information on minimizing pollution.

18) Data Assessment and acceptance criteria for Quality Control Measures

The depletion of oxygen in the dilution controls (blanks) should not exceed 0.20 mg/l and ideally should be not more than 0.10 mg/l. The calculated BOD for the QC sample should be within the manufacturer-determined acceptance criteria. The BOD measured for the Glucose-Glutamic Acid Check must be 198±30.5 mg/L.

19) Corrective actions for out-of-control data

If contamination is suspected (high blank values), all glassware should be re-cleaned with acid. All reagents should be checked for expiration dates and/or signs of deterioration. Test the dilution water for organic contaminants, specifically Total Organic Carbon. Re-prepare or re-purchase standards. Ensure that the initial DO of the sample set was less than 9.0 mg/l.

20) Contingencies for handling out-of-control or unacceptable data

Unfortunately, due to the short holding time for BOD (48 hours), by the time out-of-control or unacceptable data is produced, at the end of the 5-day incubation period, the analysis cannot be repeated because the sample is out of holding time. If any of the Quality Control measures are outside of the acceptance criteria, the investigation report must include a qualification of the data indicating what Quality Control measure(s) were unacceptable. No more BOD analyses should be initiated until Corrective Actions are taken and the Quality Control officer is confident that the cause of the unacceptable data has been found and rectified.

21) Waste Management

Make every attempt to order chemicals in quantities that will be used up before their expiration date to avoid disposing of extra unusable chemical reagents.

22) References

Standard Methods for the Examination of Water and Wastewater, 21th Edition, sections 5210(B), 4500-O C., and 4500-O G. APHA, AWWA, WEF, 2005.

Accumet® Basic AB40 User Manual. Fisher Scientific.

Fisher Scientific BOD Probe Instructions. Rev. 3, 11/2006. (Part # 7744).

23) Tables, Diagrams, Flowcharts and Validation Data

See “Interstate Environmental Commission B.O.D. Laboratory Record,” attached.

Chlorophyll *a*

Section XXXVII, Revision No. 6, Effective Date January 2018

1) Test Method

Based on EPA 445.0

2) Applicable Matrix or Matrices

Non-potable Water

3) Method Detection Limit

This Method's Detection Limit is 0.7µg/L. The laboratory's reporting level for this method is 2.1µg/L.

4) Scope and Application

This method is useful for estimating phytoplankton biomass in freshwater and marine environments. IEC will use it to analyze marine waters for chlorophyll *a*.

5) Summary of Method

A 400mL, or otherwise specified, aliquot of natural water is filtered in a dark area. Pigment is extracted from the filter through maceration then steeping in a 90% acetone soak, and clarified using a centrifuge. An aliquot of the supernatant is transferred to a glass cuvette and fluorescence is measured before and after acidification to 0.003N HCl with 0.1N HCl. The pigment extract is then analyzed using a fluorometer. Addition of acid results in the loss of the magnesium atom, converting chlorophyll *a* to pheophytin *a*. The change in fluorescence after acidification is used to determine the corrected values for chlorophyll *a*.

6) Definitions

Chlorophyll *a* is a photosynthetic pigment. It is a component of planktonic algae, constituting 1-2% of its dry weight. Chlorophyll *a* is used extensively to estimate phytoplankton biomass.

7) Interferences

Any substance extracted from the filter or acquired from laboratory contamination that fluoresces in the red region of the spectrum may interfere in the accurate measurement of chlorophyll *a*. Fluorescence is temperature dependent. Light, changes in temperature, and exposure to air can also interfere with the test, leading to decreases in chlorophyll *a* concentration. Use care in dealing with samples during the analysis process. Samples, standards, blanks and quality control samples must be at the same temperature to prevent errors and/or low precision. Analyses of samples at ambient temperature is recommended.

All work must be performed in subdued light. QC samples and filters must be stored at -20°C to -70°C to prevent degradation

Excess sample turbidity can interfere with proper fluorescence readings.

8) Safety

Personal protective gear, including gloves, goggles and a lab coat must be worn by analysts during all steps of the analysis. Work under a hood whenever possible. Please refer to the MSDS (on file in the yellow book on wall by micro room door) for specific information on toxicity and safety precautions needed for specific chemicals.

9) Equipment and Supplies

9.1 Turner® Trilogy Laboratory Fluorometer, equipped with chlorophyll *a* acidification module.

9.2 12 x 35 mm or 12 x 75 mm glass test tubes

9.3 Whatman glass microfiber filters GF/F-47 mm or equivalent (0.45-µm porosity)

9.4 47 mm solvent resistant filter funnels,

9.5 1000 mL side-arm filtering flasks or vacuum filtration manifold

9.6 Vacuum pump and tubing

9.7 Graduated Cylinders

9.8 15 mL centrifuge tubes

9.9 stainless steel forceps (for transferring filter)

9.10 1000 µL Brinkmann Eppendorf micropipette with adjustable dispensing volume feature

9.11 1 liter volumetric flask

9.12 freezer

9.13 Analytical balance

9.14 IEC Clinical Centrifuge 120V 1.2 Amp Model 41498

9.15 4°C fridge

9.16 tissue grinder and pestle

10) Reagents

10.1 Deionized Water

10.2 Mix together 90 mL acetone, 10 mL of deionized water

10.3 0.1N Hydrochloric Acid: Add 8.5 mL of concentrated hydrochloric acid to a 1 L flask containing 500 mL of distilled water. Cool and dilute to the mark with distilled water. Stir to mix.

Or use purchased, pre-prepared 0.1N HCL procured with manufacturer's certificate of analysis, which must be retained on file in the laboratory

10.4 Turner Designs (P/N 10-850) Fluorometric Chlorophyll Standards in 90% acetone, low and high concentration standards. They are used to find the lower absorbance detection limit of the spectrophotometer (MDL) and to prepare check standards. Typically have a 1 year shelf life (manufacturer assigns expiration date) and must be stored in the freezer.

10.5 Turner Designs Chlorophyll *a* in 90% acetone, stock standard. Transfer 10 mL from a Turner Designs chlorophyll *a* standard ampoule into a 500 mL flask and dilute to the mark with 90% acetone. Use chilled pipettes and flasks when making transfers. Calculate the stock standard concentration using the original concentration of the ampouled standard. Use until manufacturer-assigned expiration date of original ampoule, if stored frozen.

10.6 Turner Designs Adjustable Solid Secondary Check Standards, P/N 8000-952

11) Sample Collection, Preservation, Shipment and Storage

Samples are collected directly into plastic opaque 500 mL sample bottles using a sludge nabber sampling stick. Bottles are marked with waterproof ink with a specific sample ID number, station identifying number, date and investigation number. On a chain of custody sheet record date and time collected, cruise number (e.g. LISS#1), sampling station, IEC investigation number, personnel, and type of analysis (chlorophyll *a*). Place sample bottles in a cooler containing ice and a cooler thermometer. Minimize the frequency and extent to which the cooler lid is opened, to minimize exposure to light. Upon arrival at the laboratory, record time transferred to laboratory on the chain of custody sheet. Log samples in the laboratory's sample log-in book. Information to be included in the designated columns in the log-in book include: investigation #, # of bottles, bottle condition, date in lab, time in lab, cooler temperature, run #, parameter(s) to be analyzed, and sampler's initials. Turn off laboratory light and transfer samples to sample refrigerator. Filtration must be completed within 48 hours after samples are collected.

12) Quality Control

Blanks are analyzed at a frequency of one per batch at the end of filtration. The method blank consists of a filter placed on the filtration set up with the vacuum turned on for 1 minute to draw air through filter. This blank should be less than the calculated method lower detection limit for the analysis. A Turner Designs secondary check standard must be analyzed at the beginning and the end of each analytical batch.

13) Calibration and Standardization

Calibration should be performed bimonthly (during months when method is being used) or more frequently if an adjustment made to the instrument. Prepare 0.2, 2, 5, 20 and 200 µg/L calibration standards from stock solutions. Direct Calibration Procedure: Turn on the Trilogy. Wait 15 minutes to allow the instrument to warm up. Touch "Chl-A" to select the Chlorophyll *a* Acidification module and confirm by touching "OK." On the home screen, touch "Calibrate" to begin a calibration sequence. Select "Run New Calibration." Select the unit of measurement. Insert calibration "blank" and touch "OK." Enter the concentration for the first standard. If using the Turner Designs Chlorophyll *a* standards, this will be the concentration data supplied with the standard. Follow the screen prompt indicating that the standard before acidification (Fb) should be inserted. Insert sample and touch "OK." Now insert the standard after acidification and press "OK." The (Fa) value will be measured and the ratio of the two readings will be displayed as seen in the next step. If the ratio is in the required range, touch "OK." The ratio will be stored in the Trilogy for use in the measurement of chlorophyll *a*. After the calibration is complete, either select "Proceed with Current Calibration" or select "Enter More Standards," in which case, enter the concentration for the next standard as was done above with the first standard. Name and save the calibration for future use. Measure the solid standard and record the displayed value to enable a

quick calibration verification.

14) Procedure

14.1 Sample Filtration

Conduct work with chlorophyll extracts in subdued light to avoid degradation. Turn off excess laboratory overhead lights and close blinds. Measure 400 mL (or other suitable aliquot) of a well-mixed sample into a 500 mL graduated cylinder and filter. The sample must be kept well-mixed. When pouring a measured volume into a filter funnel, leave a small amount in the cylinder and swirl it well before pouring the final amount. Record volumes filtered for each sample on the chlorophyll *a* data sheet. At least one sample per batch must be filtered in duplicate. Both duplicates must have identical volumes filtered. All graduated cylinders and filtering funnels must be rinsed 2-3 times with deionized water between samples. Changes to the standard 400 mL volume filtered can and should be made if suspended material concentrations are high and the sample is taking 5 minutes or more to filter, however, duplicate filters must have the identical volume of sample filtered through them. Filters must be handled with clean forceps only. Using clean forceps place the filter on the filter funnel by placing the finer mesh side of the filter face down. Be careful to center the filter on the filter holder so that sample does not seep around the filter. Also be careful not to slide the filter off-center when placing the funnel or the clamp on. After filtration, carefully fold and transfer to a 15 mL capped centrifuge tube. Make sure that all tubes are labeled with the correct sample number. Samples on filters taken from water having a pH 7 or higher may be placed in airtight centrifuge tubes and stored frozen in the dark for 3 weeks. Samples from acidic water must be processed promptly to prevent chlorophyll degradation. If samples are to be analyzed immediately go to the next step, 14.2. Place sample filters in the dark in the freezer for analysis at a later date.

14.2 Extraction

If the samples have been placed in a freezer, remove them from the freezer but keep them in the dark. Workspace lighting should be kept to a minimum. Remove a filter from its container and place it into the tissue grinder. The filter may be torn into smaller pieces to facilitate extraction. With a volumetric pipette add 4mL of the 90% acetone solution. Grind the filter until it has been converted to a slurry. Pour the slurry into a 15mL centrifuge tube and using a 6mL volumetric pipette rinse the pestle and the grinder with 90% acetone solution. Add the rinse to the centrifuge tube. Cap the tube and shake vigorously. Place it in the dark before proceeding to the next filter extraction. Before analyzing another sample, use the 90% acetone solution to thoroughly rinse the pestle and tissue grinder. Shake each tube vigorously before placing them to steep in the dark at 4°C. Samples should be allowed to steep for a minimum of 2 h but not to exceed 24 h.

Glass fiber filters of 47mm diameter have dry displacement volumes of 0.10 mL and introduce errors of about 1.0% if a 10 mL extract is used. Clarify by inserting capped centrifuge tubes in centrifuge. Place tubes with similar volumes (within 0.5 mL) opposite each other in centrifuge to maintain centrifuge balance. Centrifuge by incrementally increasing speed to between level 6 and level 7 to the approximation of 675g. Centrifuge for 15 minutes, reduce speed slowly until centrifuge stops completely. Decant clarified extract into a clean, 10 mL cuvette. Record final extract volume by comparing volume to graduated cuvette. Note final extract volume for use as V1 in calculations, below. Remove tubes from freezer and shake. Allow tubes to reach room temperature. Decant 10 mL of clarified extract into a clean 12 x 35 mm or 12 x 75 mm glass test tube.

14.3 Determination of Chlorophyll *a*

Upon completion of the required calibration steps of section 13, insert a blank cuvette containing 90% aqueous acetone solution.

The volume of extract and acid and the time after acidification are critical for accurate, consistent

results. For a test tube that holds 5 mL of extract, 0.15 mL of the 0.1N HCl solution should be used. For a test tube (12mm x 75mm) that holds 10mL of extract, 0.30 mL of the 0.1N HCL solution should be used.

On the Trilogy touchscreen touch “Sample ID” to name your sample

Using the keypad, enter the sample name into the name field and touch “Save”.

Touch “Measure Fluorescence” to make a measurement. The Trilogy will measure the sample for 6 seconds and report the average reading for the sample.

Record the fluorescence measurement of the sample. Remove the test tube from the fluorometer and acidify the extract to a final concentration of 0.003N HCl using the 0.1N HCl. Use a Pasteur pipet to thoroughly mix the sample by aspirating and dispensing the sample into the test tube, keeping the pipet tip below the surface of the liquid to avoid aerating the sample. Wait exactly 90s (using a preset timer!) before measuring fluorescence again. NOTE: Proper acidification, mixing and timing is CRITICAL for precise and accurate results.

15) Calculations

Measure the fluorescence of each standard at sensitivity setting that provide midscale readings.

Obtain response factors for chlorophyll *a* for each sensitivity setting as follows:

$$F_s = C_a/R_s$$

Where:

F_s = response factor for sensitivity setting, S.

R_s = fluorometer reading for sensitivity setting, S.

C = concentration of chlorophyll *a*

Obtain before-to-after acidification response ratios of the chlorophyll *a* calibration standards as follows:

- (1) Measure the fluorescence of the standard, (2) remove the test tube from the fluorometer, (3) acidify the solution to 0.003N HCl with the 0.1N HCl solution, (4) use a Pasteur pipet to thoroughly mix the sample by aspirating and dispensing the sample into the test tube, keeping the pipet tip below the surface of the liquid to avoid aerating the sample, (5) wait exactly 90 s (use preset laboratory timer) and measure the fluorescence of the standard solution again. For a test tube that holds 5 mL of extract, it will be necessary to add 0.15 mL of 0.1N HCl to reach a final acid concentration of 0.003N in the 5 mL (use 0.30mL for a 10 mL extract).

Calculate the ratio, *r*, as follows:

$$r = R_b/R_a$$

Where:

R_b = fluorescence of pure chlorophyll *a* standard before acidification

R_a = fluorescence of pure chlorophyll *a* standard after acidification

For “corrected chlorophyll *a*”, calculate the chlorophyll *a* concentration in the extract as:

$$C_{E,c} = F_s (r/r-1) (R_b-R_a)$$

Where:

$C_{E,c}$ = corrected chlorophyll *a* concentration (µg/L) in the extract solution analyzed,

F_s = response factor for the sensitivity setting, S

r = the before-to-after acidification ratio of a pure chlorophyll *a* solution

R_b = fluorescence of pure chlorophyll *a* standard before acidification

R_a = fluorescence of pure chlorophyll *a* standard after acidification

Calculate the “corrected” concentration of chlorophyll *a* in the whole water sample as follows:

$$C_{s,c} = \frac{C_{E,u} \times \text{extract volume (L)} \times \text{DF}}{\text{sample volume (L)}}$$

Where $C_{s,c}$ = corrected chlorophyll *a* concentration (µg/L) in the whole water sample

Extract volume = volume (L) of extract prepared before dilution

The Relative Percent Difference (RPD) of duplicate determinations should be within 15%. RPD is defined as the absolute value of the difference of duplicate determinations divided by their arithmetic mean and multiplied by 100.

16) Method Performance

Method performance is evaluated by ongoing analysis of QC check standards from Turner Designs for each batch.

17) Pollution Prevention

Dispose of waste as in section 21, Waste Management, to prevent pollution. Store waste in a container within a secondary container to prevent pollution due to spills or container leakage.

18) Data Assessment and Acceptance Criteria for Quality Control Measures

Refer to the Turner Designs certificate of analysis to determine the latest control limits for check standard % recovery.

19) Corrective Actions for Out-of-Control Data

Duplicate determinations should be averaged to determine the reported result. Duplicate determinations should have acceptable RPD values, as noted in Section 15. The deionized water and reagents and supplies may need to be evaluated for possible contamination. If the % recovery for the chlorophyll QC check standard does not meet acceptable limits, the system has to be evaluated for possible errors. Prepare a fresh standard and re-analyze or purchase a new lot to attempt to determine if the standard has degraded or if there is an error in the procedure. If the manufacturer of the QC check standards does not provide acceptance limits, an acceptable recovery of ±15% could be assigned until one is determined by the Laboratory's Directors by spiking samples of known concentration.

20) Contingencies for Handling Out-of-Control or Unacceptable Data

Ideally samples yielding out-of-control or unacceptable QC results should be reanalyzed. If there is insufficient sample for reanalysis or the sample holding time has expired, analytical results must be reported, along with all unsatisfactory quality control measures or reported as "No Result" because of unsatisfactory QC measures. In all cases, the out-of-control result must be recorded in the analyst's logbook and the QA officer notified.

21) Waste Management

Waste is disposed of in an appropriate acetone waste collection bottle. Waste pick-up is arranged for, on an as-needed basis, approximately every 6 months with Environmental Products and Services, Inc.

22) References

USEPA Method 445.0 In Vitro Determination of Chlorophyll *a* and Pheophytin *a* in Marine and Freshwater Algae by Fluorescence

Trilogy Laboratory Fluorometer User's Manual, Version 1.2, September 15, 2010.

23) Tables, Diagrams, Flowcharts and Validation Data

None

Nutrient Filtration

IEC SOP Section No. XXXVII, Revision No. 3, Effective Date May 2018

1) Test Method

General Filtering Procedures for nutrient samples collected from LIS

2) Applicable Matrix or Matrices

Non-potable Water

3) Method Detection Limit

Varies as to method. See current Ambient Water Quality Monitoring in the Far Western Long Island Sound Quality Assurance Project Plan (QAPP) for analyte-specific MDL's provided by contract laboratory.

4) Scope and Application

This method is used for filtering samples collected during annual Summer surveys on the Long Island Sound for nutrient analyses to be analyzed by a contract lab.

5) Summary of Method

Samples collected for nutrients analyses (Ammonia, Nitrate+Nitrite, Orthophosphate (DIP), Total Dissolved Phosphorus, Total Dissolved Nitrogen, Dissolved Organic Carbon, Biogenic and Dissolved Silica, Particulate Phosphorus, Particulate Nitrogen, and Particulate Carbon) are filtered as soon as possible upon arrival at the IEC District Laboratory. After filtration, samples are preserved (refrigerated or frozen) and transported either via overnight carrier to the contract laboratory for analysis.

6) Definitions

N/A

7) Interferences

Many sources could affect the accuracy of the final result. Most of these risks can be eliminated or minimized with careful lab technique and by following the SOP.

Cross-contamination can result from failure to rinse equipment properly or from mishandling samples. Graduated cylinders and filtrate containers are rinsed with sample, and all other filtering equipment is rinsed with deionized water. Filters should never be touched by hand; forceps rinsed with deionized water should be used.

Water samples must be kept well mixed. If not kept mixed, the suspended material settles to the bottom making some samples very concentrated and others very dilute. Neither of these provides an accurate picture of the water sampled. In order to keep the water samples well mixed, the Niskin bottle must be inverted (shaken) end to end several times. This must be done frequently while the water is being used. Water should never be drawn for filtering unless the bottle was just shaken.

8) Safety

Personal protective gear, including gloves, goggles and a lab coat must be worn by analysts during all steps of the analysis. Work under a hood whenever possible. Please refer to the MSDS (on file in the yellow book on wall by micro room door) for specific information on toxicity and safety precautions needed for specific chemicals.

9) Equipment and Supplies

Primary equipment List:

- 25 mm filtering apparatus (Millipore fritted glass filter holder inserted into a fitted stopper with side-arm flask for filtrate collection for dissolved nutrient filtrations- TDN, nitrate-nitrite, Ammonia, TDP, Orthophosphate, DOC)
- 47 mm filtering apparatus (polyethylene for silica filtrations)
- Filtering funnels, bases, and holders (fritted glass for dissolved nutrients other than silica, polyethylene apparatus for silica)
- Vacuum filtration pump with hoses
- Refrigerator, maintained at $\leq 4^{\circ}\text{C}$
- Freezer, maintained at -20°C

Supplies List:

- Field data sheets and chain-of-custody forms
- Deionized water in squirt bottles
- Graduated cylinders (250 ml-glass class A and polycarbonate-for measuring silica samples)
- Foil packets labeled with date and station, for PC/PN storage and delivery
- Centrifuge tubes for BioSi filter storage
- Nalgene poly bottles (125 mL) for dissolved nutrient storage)
- Nalgene poly bottles (125 mL) for dissolved silica filtrate storage-CHILLED)
- Filter forceps
- Permanent marker for labeling
- Latex gloves
- Safety goggles
- Ice and dry ice
- Zip-seal plastic bags
- Kimwipes

Supplies provided by UMCES CBL NASL (subcontract laboratory for nutrients analyses)

- BioSi filters (47mm polycarbonate membrane filter with a pore size of 0.4um)
- PC/PN filters (precombusted 25mm GF/F (glass fiber) filter with a pore size of 0.7um)
- 30 ml Teflon bottles for DOC samples

10) Reagents

10.1 Deionized Water

11) Sample Collection, Preservation, Shipment and Storage

Samples are collected directly into plastic opaque 1000-2000 ml sample bottles using a sludge nabber sampling stick. Bottles are marked with waterproof ink with a specific sample ID number, station identifying number, date and investigation number. On a chain of custody sheet record date and time collected, cruise number (e.g. LISS#1), sampling station, IEC investigation number, personnel, and type of analysis. Place sample bottles in a cooler containing ice and a cooler thermometer. Minimize the frequency and extent to which the cooler lid is opened. Upon arrival at the laboratory, record time transferred to laboratory on the chain of custody sheet. Log samples in the laboratory's sample log-in book. Information to be included in the designated columns in the log-in book include: investigation #, # of bottles, bottle condition, date in lab, time in lab, cooler temperature, run #, parameter(s) to be analyzed, and sampler's initials. Turn off laboratory light and transfer samples to sample refrigerator. Filtration must be completed on the day samples are collected.

12) Quality Control

One field duplicate is collected per survey. The blank for PC/PN is an "air" blank consisting of a filter placed on filtration apparatus. The filtration apparatus is completely set up, however no water is filtered through the apparatus. As with the PC/PN filters, this is done in duplicate. The blank for the Biogenic Silica and Dissolved silica filtration is also an air blank, prepared in duplicate. For the PP blank, which is not required to be performed in duplicate, rinse the filter with three successive 10 ml volumes of DI water. This is done to replicate the rinsing of the PP filter that occurs at the end of sample filtration. This blank should be less than the calculated method lower detection limit for the analysis. See section 14.1.17.

13) Calibration and Standardization

None required for filtration. Class A graduated cylinders are used to measure sample volumes for filtration.

14) Procedure

14.1 General Filtering Procedures

14.1.1 **THE WATER SAMPLES MUST BE KEPT WELL-MIXED.** This is important because the suspended material in the water settles out very fast as the water sits in the water bottles. If not kept mixed, the suspended material settles to the bottom of the bottle making some samples very concentrated with suspended material, and leaving other (later) samples very dilute. Neither of these provides an accurate picture of the water sampled. This must be done frequently while the water is being used. **Water should never be drawn for filtering unless the bottle was just shaken.**

14.1.2 Settling also occurs in the graduated cylinders used to measure out a sample of water to be filtered. When pouring a measured volume into a filter funnel, leave a small amount in the cylinder and swirl it well before pouring this final amount. This will help to resuspend particulate matter that has settled so that it will pour out with the liquid instead of adhering to the inside of the cylinder.

14.1.3 **Record volumes filtered on the filtering information data sheet.** The results of the chemical analyses are on a per volume basis, so an accurate record of the volume filtered is necessary.

14.1.4 Particulate Carbon and Particulate nitrogen are analyzed from the same filter. Filters for particulate carbon, and particulate nitrogen are provided to the laboratory in duplicate. Complete PC and PN filtrations in duplicate. The foil packets that hold these filters should contain two identical filters - an identical amount of the same water sample filtered through each of them.

14.1.5 All graduated cylinders, filtering flasks from which filtrate will be kept for analyses, filtering funnels, and filter holders (frit glass or stainless steel) **must be rinsed 3 times with 10 mls of DI water between samples.** Rinse all apparatus before setting up new filter pads. Discard rinsings.

14.1.6 All graduated cylinders **must be rinsed 3 times with 10 mls of the sample water before being filled with the sample.**

14.1.7 Changes to the standard volume filtered can (and should) be made if suspended material concentrations are high and it is taking 5 minutes or more to filter a sample. In such a case, the pores of the filter clog, changing the relative pore size of the filter and thus changing the size of the material that is being caught by the filter. In addition, if suspended material concentrations are very low and little or no color is visible on the filter, the volume filtered should be increased. Always record the volume filtered on the data

sheet, and be sure that all replicate filters have identical amounts of sample filtered through them. However, a minimum of 125 ml should be filtered whenever possible for PC/PN as the filtrate from these filtrations are used as the sample for the analysis of all dissolved nutrients (total dissolved nitrogen, ammonia, nitrate+nitrite, dissolved organic carbon, total dissolved phosphorus, and orthophosphate)

14.1.8 Filters must be handled only with clean filter forceps. Do not touch filter pads with your fingers.

14.1.9 Be careful to center filter pad on the filter holder so that sample can not get around the filter. Also be careful not to slide the filter off-center when placing the funnel or the clamp on.

14.1.10 Leave the vacuum on for a minute or two after it appears as though all of the water has gone through the filter. A filter should not be saturated when it is removed from the holder and placed in the foil packet or centrifuge tube, let the vacuum remove the excess water.

14.1.11 HANDLE FILTER PADS (with sample on them) BY THE EDGES ONLY and only with clean forceps.

14.1.12 Filters must be carefully folded in half before being placed in foil packets or twice in the case of biogenic silica filters placed in centrifuge tubes. Using filter forceps, grab filter by the edge only, where there is no sample, and gently fold over. With a second forceps, use the flat side to flatten the filter out at the fold. The filter should be folded so that all of the material on the filter is within the fold so there is no loss inside the foil packet.

14.1.13 For PC/PN filtrations, carefully place the two (duplicate) folded filters within the foil packet so that they do not touch one another. Be sure to place them deep enough into the packet so that the filters will not interfere with sealing the packet. Be sure that the label on the foil packet corresponds to the sample that was filtered.

14.1.14 Fold the opening of the foil packets over twice so that there is a complete seal, and place in the freezer.

14.1.15 The two replicate PC/PN filters that are placed in a foil packet together must have had the exact same amount of sample filtered through them. The laboratory can not distinguish between these two filters, so they must be handled in an identical manner.

14.1.16 During each survey at least one sample will be prepared in duplicate. A total of four (4) filters will be prepared for each parameter or parameter group (i.e. PC/PN and biogenic silica) at a duplicate station.

14.1.17 Filter Blanks

During each survey at least one filter blanks are prepared and included with the other samples for analysis. These Blanks provide a way to measure any background contamination on the filters caused by field handling procedures. The Blank filters should be treated in the very same manner as a sample filter, except that no sample is filtered.

a) Set up filters and assemblies as you would to prepare for sample filtering.

b) For all other filter blanks (PC/PN, BioSi): Turn on vacuum pump briefly to draw any DI water off that the filter picked up from rinse water on the holder. DO NOT run any DI water through these filters.

d) Handle filters only by the edges and only with forceps.

e) Place filters in appropriate foil packet or centrifuge tube and freeze.

14.1.18 Check to be sure that the sample label on the foil pack or container corresponds to the sample that was prepared and is recorded on the COC

14.1.19 All filters (in foil packets or centrifuge tubes) should be placed into the freezer immediately. Filtrate samples are chilled (filtrate from silica filter for dissolved silicate analysis) or frozen (filtrate for total dissolved nitrogen, ammonia, nitrate+nitrite, dissolved organic carbon, total dissolved phosphorus, and orthophosphate from the PC/PN filtrations).

Specific Filtering Procedures

14.2) **PC/PN filtration.** The 25mm filtering apparatus (Millipore fritted glass filter holder inserted into a fitted stopper with side-arm flask for filtrate collection) is used for this filtration.

a) This filtering apparatus is used for particulate carbon (PC) and particulate nitrogen (PN). PC and PN are measured from the same filter.

b) The filter pad used is a precombusted 25mm GF/F (glass fiber) filter with a pore size of 0.7 μ m. It is very important that the correct filters be used. Note the labels on the filter packages that come from the analytical laboratory and be careful to use the correct filter. Place filters in filter holders rough side up.

c) Because the filtrate from the PC/PN filtration is being kept rinse the flasks with a small volume (10 ml aliquot) of the sample before filtering the entire sample volume to remove traces of deionized water from flask rinses. Discard rinsings.

d) To avoid leakage around the base of the filter holder, turn on the vacuum and open the valve before pouring sample into the funnel.

e) In general, a volume of 200 ml is used for PC/PN analyses. Sample is filtered in duplicate.

- f) The filtrate from this filtration is used for the analysis of total dissolved nitrogen, ammonia, nitrate+nitrite, dissolved organic carbon, total dissolved phosphorus, and orthophosphate. Sample rinse all containers and lids that will hold filtrate for analysis. When the filtrate is ready to be poured into a laboratory bottle, start by rinsing 2-3 times (bottle and lid) with small volumes of the filtrate before filling the bottle. 125 ml sample bottles used for total dissolved nitrogen, ammonia, nitrate+nitrite, total dissolved phosphorus and orthophosphate and **the 30 ml Teflon bottle used for dissolved organic carbon should be filled only to the shoulder (i.e. not full) because this sample is frozen and if the bottle is overfull, sample expansion when frozen can cause the bottle to burst.**
- g) Watch the water level in the filtrate collection flask. When it approaches the level of the vacuum line the tank must be emptied so that water is not sucked into vacuum line.
- h) Turn off vacuum to the tank.
- g) remove filter with forceps, carefully folding it twice with the assistance of the forceps, and place in labeled foil packet (**see 14.1.8 through 14.1.16**)
- i) Remove tubing from filter flask set up
- j) Remove stopper containing glass filter holder from filter flask set up
- k) Empty flask and re-assemble filter flask set-up.
- l) For filter blanks: Turn on vacuum pump briefly to draw any DI water off that the filter picked up from rinse water on the holder. DO NOT run any DI water through these filters

14.3 Biogenic Silica/Dissolved Silica Filtration: 47mm filtering apparatus (polyethylene filtration set-up): This filtering apparatus is used biogenic (or particulate) silica (BioSi). The filtrate from the BioSi filter is used for dissolved silica (silicate) analysis.

- a) **It is important that the correct filters are used. The filters for Biogenic Silica filtration are 47 mm 0.4µm polycarbonate filters.** The biogenic silica filters are light blue, thin, and provided in between 2 paper dividers. Be sure to use the *filter* and not the paper divider for filtrations! The shiny side of the filter is placed face down on the filtration set up.
- b) Because the filtrate from the BioSi filtration is being kept it is necessary to sample rinse those flasks with a small volume (10 ml) of the sample before filtering the entire sample volume. Discard rinsings from flask. Measure sample volume. (In general 200 ml is filtered for BioSi) **Polyethylene graduated cylinders must be used for biogenic silica sample measurement. No glass should be used in sample measurement, filtration or collection for biogenic/dissolved silica.**
- c) Pour sample slowly into the filtration funnel. Taking care not to overflow the filtration funnel. Swirl sample in the graduated cylinder periodically to resuspend any settling sediment.
- d) Collect filtrate for Dissolved silica analysis. The filtrate from this filtration is used for the analysis of dissolved silica. Sample rinse all containers and lids that will hold filtrate for analysis. Transfer to labeled 125 ml sample bottle.
Chill the dissolved silica filtrate by storing in a refrigerated maintained at <4°C. **DO NOT FREEZE THE DISSOLVED SILICA SAMPLE.**
- e) Fold the biogenic silica filters twice and place individually into centrifuge tubes. **ONE FILTER ONLY PER TUBE.** (Instead of two filters in a single foil packet, these must each be placed into a centrifuge tube. This eliminates some handling of these filters since the digestion step of the analytical procedure can be done right in the tube). Place labeled centrifuge tubes in freezer.
- f) As with other nutrient analyses, one Biogenic silica filtration per survey is performed in duplicate, making sure to filter identical volumes for each duplicate.
- g) For filter blanks: Turn on vacuum pump briefly to draw any DI water off that the filter picked up from rinse water on the holder. DO NOT run any DI water through these filters. Prepare filter blanks in duplicate as is done with the samples.

14.4 Shipping

PC/PN filters and DOC bottles filters are shipped via Federal Express (next business morning-by 10:00am) to

the University of Maryland's Chesapeake Biological Laboratory's Nutrient Analytical Services Laboratory for analysis. Each shipment should consist of 30 ml Teflon bottles (frozen) for the analysis of dissolved organic carbon, twelve (12) foil packets (frozen) each containing two identical duplicate filters, not touching each other, for the analysis of particulate carbon and particulate nitrogen (PC/PN). Quantities of sample bottles and foil packets may vary if additional samples are collected, if additional duplicates are collected, or if fewer samples are collected. The UMCES CBL NASL chain of custody should be completed and included with each shipment. A copy should be made of the COC before shipment. In addition, UMCES CBL NASL has requested that the salinities measured for each sample be supplied with each shipment. The COC and salinity record should be sealed in a large zippered plastic bag and included at the top of the samples in the cooler. Styrofoam coolers are provided by UMCES CBL NASL. Reusable freezer packs are placed below and on top of samples. UMCES CBL NASL requests that samples are only shipped Monday through Wednesday. Ship samples to:

Jerry Frank-Manager Nutrient Analytical Services Laboratory
University of Maryland Center for Environmental Science
Chesapeake Biological Laboratory
146 Williams Street
PO BOX 38
Solomons, MD 20688

15) Calculations

None. Be sure to record volumes filtered on the Chain of Custody sheet and to filter the same volume through each filter for those parameters filtered in duplicate.

16) Method Performance

Method performance is evaluated by ongoing analysis of QC check standards, blanks, and duplicate samples.

17) Pollution Prevention

Dispose of waste as in section 21, Waste Management, to prevent pollution. Store waste in a container within a secondary container to prevent pollution due to spills or container leakage.

18) Data Assessment and Acceptance Criteria for Quality Control Measures

See LISS QAPP for a description of data assessment and acceptance criteria for quality control measures

19) Corrective Actions for Out-of-Control Data

Duplicate determinations should have acceptable recovery values, as noted in LISS QAPP. If blank analysis results are above the reporting level for any parameter, the deionized water and reagents and supplies may need to be evaluated for possible contamination. If the % recovery for the QC checks or duplicate determinations does not meet acceptable limits, the system has to be evaluated for possible errors. Any data associated with unacceptable QC results must be flagged.

20) Contingencies for Handling Out-of-Control or Unacceptable Data

Ideally samples yielding out-of-control or unacceptable QC results should be reanalyzed. If there is insufficient sample for reanalysis or the sample holding time has expired, analytical results must be reported, along with all unsatisfactory quality control measures or reported as "No Result" because of unsatisfactory QC measures. In all cases, the out-of-control result must be recorded in the analyst's logbook and the QA officer notified.

21) Waste Management

Waste is disposed of in an appropriate acetone waste collection bottle. Waste pick-up is arranged for, on an as-needed basis, approximately every 6 months with Environmental Products and Services, Inc.

22) References

Quality Assurance Project Plan: *Ambient Water Quality Monitoring in the Far Western Long Island Sound*, version 5.0 (under review). Prepared by NEIWPCC (IEC District) 2018.

Interstate Environmental Commission Ammonia Standard Operating Procedure, IEC SOP ID XXXIX, Revision No. 3, January 2018

Interstate Environmental Commission Silicate SOP, IEC SOP ID XXXXII, Revision No.2, January 2018.

Interstate Environmental Commission Nitrate (as N), Nitrite (as N), Nitrate-Nitrite (as N) Standard Operating Procedure, IEC SOP ID XXXXI, Revision No. 3, June 2017.

Interstate Environmental Commission Total Phosphorus/Orthophosphate standard operating procedure, IEC SOP ID XXXX, Revision No. 3, December 2017.

Standard Operating Procedure for the Determination of Carbon and Nitrogen in Particulates and Sediments of Fresh/Estuarine/Coastal Waters, Plant and Animal Tissue, and Soils Using Elemental Analysis University of Maryland, Chesapeake Biological Laboratory, Nutrient Analytical Services Laboratory, 2009

Standard Operating Procedure for the Standard Operating Procedure for the Determination of Dissolved Organic Carbon (NPOC), and Total Organic Carbon Fresh/Estuarine/Coastal Waters using High Temperature Combustion and

Infrared Detection. University of Maryland, Chesapeake Biological Laboratory, Nutrient Analytical Services Laboratory, 2013.

23) Tables, Diagrams, Flowcharts and Validation Data

None

Nitrate (as N), Nitrite (as N), Nitrate-Nitrite (as N), and Total Nitrogen
IEC SOP Section XXXXI, Revision No. 3, Effective Date June 2017

1) **Test Method**

This SOP is based on Lachat method 31-107-04-1-C.

2) **Applicable Matrix or Matrices**

Non-Potable Water.

3) **Method Detection Limit**

The MDL and reporting limit for Nitrate (NO₃ as N) is 0.006 mg/L (MDL) and 0.03 mg/L (RL)

The MDL and reporting limit for Nitrite (NO₂ as N) is 0.002 mg/L (MDL) and 0.01mg/L (RL)

The MDL and reporting limit for Nitrate-Nitrite (as N) is 0.004 mg/L (MDL) and 0.02 mg/L (RL)

The MDL and reporting limit for Total Nitrogen is 0.05 mg/L.

4) **Scope and Application**

This method is applicable to surface and saline waters, and domestic and industrial waste waters. This analysis is performed by Flow Injection analysis (FIA).

5) **Summary of Method**

Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl) ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm. Nitrite alone can also be

determined by removing the cadmium column. Total Nitrogen can be determined by adding a persulfate digestion prior to analysis.

6) **Definitions**

In waters and wastewaters, the forms of nitrogen of greatest interest are, in order of decreasing oxidation state: nitrate, nitrite, ammonia and organic nitrogen. All these forms of nitrogen are biochemically interconvertible and are components of the nitrogen cycle. Nitrate generally occurs in trace quantities in surface water. It is an essential nutrient for many photosynthetic autotrophs and in some cases has been identified as the growth-limiting nutrient. Nitrite is an intermediate oxidation state of nitrogen, both in the oxidation state of ammonia to nitrate and in the reduction of nitrate.

7) **Interferences**

7.1 Residual chlorine can produce a negative interference by limiting reduction efficiency. Before analysis, samples should be checked and if required, dechlorinated with sodium thiosulfate. Low results would be obtained for samples that contain high concentrations of iron, copper or other metals. In this method, EDTA is added to the buffer to reduce this interference.

7.2 Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This interference is eliminated by pre-extracting the sample with an organic solvent.

7.3 Sample turbidity may interfere. Remove turbidity first by filtration with 0.45um pore diameter membrane filter prior to analysis

8) **Safety**

8.1 The toxicity and carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be minimized by good laboratory practices, e.g. wear proper protective equipment, safety glasses, gloves, lab coat and working inside hoods whenever possible.

Refer to the Interstate Environmental Commission Laboratory Health and Safety Manual and CSI Chemical Hygiene Plan for specific guidelines.

For detailed explanations consult the Material Safety Data Sheets (MSDS), available in the Laboratory.

The following chemicals have the potential to be highly toxic or hazardous, for detailed explanations consult the MSDS:

Cadmium
Hydrochloric acid

Phosphoric acid
Sulfuric acid

9) **Equipment and Supplies**

9.1 Lachat 8500 QuikChem® Autoanalyzer, manifold reaction unit, colorimetric detector, 520 nm wavelength filter and Omnion software data system.

9.2 Cadmium Reduction Column

9.3 Autoclave

9.4 Miscellaneous laboratory apparatus such as volumetric pipettes, flasks, etc.

10) **Reagents and Standards**

10.1 Digestion Reagent (Total Nitrogen only):

In a 1L volumetric flask dissolve 10 g potassium persulfate, 6.0 g boric acid, 3.0 g sodium hydroxide in approximately 800 mL DI water. Dilute to the mark and mix. Prepare fresh monthly and store in plastic.

Analysis Reagents:

10.2 Sodium Hydroxide, 15N

Slowly add 150 g NaOH to 250 mL DI water with constant stirring. This solution will get very hot. Cool and store in plastic bottle.

10.3 Ammonium Chloride Buffer, pH 8.5

Dissolve 85.0 g ammonium chloride and 1.0 g disodium ethylenediamine tetraacetic acid dihydrate in approximately 800 mL DI water in a 1 L flask. Adjust the pH to 8.5 with 15 N sodium hydroxide. Dilute to the line and mix.

10.4 Sulfanilamide color reagent

Add approximately 600 mL DI water to a 1 L flask. Then add 100 mL of 85% phosphoric acid, 40.0 g sulfanilamide and 1.0 g N-(1-naphthyl) ethylenediamine dihydrochloride. Stir until dissolved, approximately 30 minutes. Dilute to the line and mix. Store in a dark bottle. This solution is stable for 1 month.

OR PREFEREBLY USE HACH LACHAT NITRATE REAGENT SET CAT#52903 as alternative option.

10.5 Dechlorinating reagent

Dissolve 0.35g sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) in 100 ml DI water.

Standard Preparation: Prepare fresh weekly or purchase equivalent from approved vendors (obtain and retain certificates of analysis on file in the laboratory)

10.7 Stock Nitrite Standard, 1000 mg/L

Dissolve 4.93 g sodium nitrite FISHER CAT# S347-500 or 6.07 g potassium nitrite in approximately 800 mL DI water in a 1 L flask. Add 2 mL chloroform, dilute to the line and mix.

10.10 Working Nitrite Standard, 10 mg/L

Dilute 1.0 mL stock nitrite standard up to 100 mL with DI water.

10.11 Cadmium Efficiency Check Standard, Nitrite, 1.0 mg/L Dilute 10 ml of 10 mg/L NO₂ standard up to 100 mL with DI water.

10.12 Working Standards:

Add the following amount of working standard and dilute to 100 mL. The upper and lower value of the standard curve should not be modified. If a project requires a different range the upper and lower standards may be modified but the reporting limit must also be adjusted accordingly.

Samples above the high standard (+10%) must be diluted.

<u>mL Working Nitrite Stock</u>	<u>mg/L NO₃+NO₂, Total Nitrogen</u>
0.50	0.05
1.00	0.10
5.00	0.50
10.00	1.00
15.00	1.50
20.00	2.00

BS/BSD Solution:

Obtain solutions from ERA or other reliable sources. Prepare according to instructions supplied by the manufacturer.

All purchased and prepared standards and reagents are recorded in the chemical inventory logbook. All containers must be labeled with the Name, ID#, concentration, preparation date or date received, and expiration date (where applicable).

11) Sample Collection, Preservation, Shipment and Storage

11.1 Samples are to be collected in plastic or glass containers and must be refrigerated to 4°C. The holding time for Nitrate and Nitrite as separate analytes is 48 hours.

11.2 Samples for Nitrate + Nitrite and Total Nitrogen analysis are to be preserved to pH <2 with sulfuric acid. The holding time for Nitrate + Nitrite and Total Nitrogen is 28 days. Samples collected from Western LIS monitoring surveys may be frozen for up to one year (after filtration) as per QAPP.

12) **Quality Control**

1.2 Calibration Curve

Acceptance Criteria - A minimum of 5 standards and a blank must be used to generate the calibration curve. The correlation coefficient must be >0.995. The % residual for each standard should be $0 \pm 10\%$ with the exception of the lowest standard which is at the reporting limit. The % residual for this standard may be $\pm 30\%$. A calibration curve should be generated at least every six months or following any major change or repair of the instrument. The calibration curve must be verified on each working day by the analysis of an IPC standard and blank.

Corrective Action - If the correlation coefficient of the calibration curve, consisting of at least five standards and a blank, is <0.995, the calibration is disallowed. The analysis must be terminated, and repeated after correcting the problem. % residuals for the standards are monitored so that any possible instrument or dilutor troubleshooting may be performed. If the IPC check does not pass, the instrument should be recalibrated.

12.1.1 Instrument Performance Check (IPC) Standard or Initial Calibration Verification (ICV) and Continuing Calibration Verification (CCV) Standard.

Acceptance Criteria - Analyze the IPC solution for all determinations immediately following each calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution immediately following calibration must verify that the instrument is within $\pm 10\%$ of the true value. Subsequent analyses of the continuing IPC solution must be within $\pm 10\%$ of the true value.

Corrective Action - If the calibration cannot be verified within the specified limits, analysis must be discontinued, the cause determined and/or in the case of drift the instrument re-calibrated. All samples following the last acceptable IPC solution must be reanalyzed.

12.2 Laboratory Reagent Blank (LRB), Prep Blank (PB), Initial Calibration Blank (ICB) and Continuing Calibration Blank (CCB)

Acceptance Criteria - Analyze a blank along with each batch of 20 or fewer samples. All LRB/PB/ICB/CCB results must be < the Reporting Limit.

Corrective Action - If the results of the LRB/PB/ICB/CCB are > the Reporting Limit then all associated samples with a concentration of $\leq 10 \times$ the amount founding the LRB/PB/ICB/CCB should be reprepared and reanalyzed (sample results $\geq 10 \times$ the amount found in the LRB/PB/ICB/CCB are not considered to be affected by the blank contamination or drift).

If the samples cannot be reprepared, then all affected sample results must be either 1) qualified accordingly, or 2) the reporting limit is raised to the amount found in the blank. Check with the team leader/section chief to determine which option should be used.

12.3 Laboratory Fortified Blank (LFB), Blank Spike/Blank Spike Duplicate (BS/BSD) or Quality Control Samples (QCS). (Also referred to as Laboratory Control Samples-LCS)

Acceptance Criteria - Analyze two LFB/BS/BSD/QCS samples with each batch of 20 or fewer samples. Calculate accuracy as percent recovery using the following equation:

$$\% \text{ Recovery} = \frac{\text{LFB/BS/BSD/QCS}}{s} \times 100$$

where:

LFB/BS/BSD/QCS = control sample results determined by laboratory

s = concentration equivalent of analyte added to fortify the LFB/BS/BSD/QCS solution.

The % recovery of the LFB/BS/BSD/QCS for samples analyzed under other programs should be within 85-115%. The relative percent difference (RPD) of the duplicates should not exceed 20% for aqueous standards.

Corrective Action - If the % recovery or RPD results are outside the required control limits, the affected samples should be reprepared and reanalyzed. If the samples cannot be reprepared, then all affected sample results must be qualified accordingly.

12.4 Laboratory Fortified Matrix (LFM) or Matrix Spike (MS) Recovery Acceptance Criteria. For samples analyzed under other programs, e.g. Ambient Water, prepare one LFM/MS per matrix for an analytical batch of 20 samples or less regardless of the number of different projects that comprise the analytical batch. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. Calculate the percent recovery, corrected for background concentration measured in the unfortified sample aliquot as per the equation below. The recovery should be 80- 120%. Calculate percent recovery using the following equation:

$$R = \frac{C_s - C}{C_s} \times 100$$

where: R = percent recovery,
 C_s = fortified sample concentration,
 C = sample background concentration, and
 s = conc. equivalent of spike added to sample.

Corrective Action - If the % recovery of the LFM/MS is outside the required control limits, and the laboratory performance is shown to be in control, the recovery problem encountered is judged to be matrix related, not system related. The native sample result of the sample used to produce the LFM/MS must be qualified accordingly.

Note: The % recovery of the LFM/MS is not evaluated if the result of the unfortified sample concentration is >1X the level used to fortify the sample.

13) **Calibration and Standardization**

See Quality Control Section 12.1 for the procedure for preparing the calibration curve.

14) **Procedure**

14.1 Sample Preparation

Sample preparation is documented in the Sample Preparation Log Book

Screen all samples received for chlorine prior to analysis. If chlorine is present, dechlorinate with sodium thiosulfate.

For samples requiring Total Nitrogen, pipet 10mL of sample into a culture tube. Add 5 mL of digestion solution, cap and vortex. Digest the samples in the autoclave for 30 minutes at 15 psi.

Note: If the final value of a sample or BS/BSD is higher than 2.0 mg/L, make an initial dilution prior to digestion since above this level there will be insufficient persulfate for complete oxidation.

14.2 Instrument Set-up

Turn on the Lachat instrument and install the proper manifold for the analysis to be performed. If nitrate and/or nitrite are to be reported separately, install both the nitrate + nitrite manifold and the nitrite only manifold. If only $\text{NO}_3 + \text{NO}_2$ or Total Nitrogen is to be reported, use only the $\text{NO}_3 + \text{NO}_2$ manifold. Consult the Lachat methods manual for installation details if necessary. The heating coil is not used for this analysis. As per Lachat Tech Support recommendation, install 100 cm Backpressure coil. Place the tube from the autodilutor into a container with DI water. Click on configuration, autosamplers and prime dilutor.

Make sure the cadmium column on the $\text{NO}_3 + \text{NO}_2$ manifold is off line, then pump Milli-Q water through the system and check for leaks and smooth flow. Perform any routine maintenance if necessary. Once instrument is stable, remove reagent lines from the water and place in the proper reagent receptacle. Turn on the cadmium reduction column once all reagents are flowing. **Do not**

run water thru the cadmium column!

Instrument Maintenance and Repair records are entered into the Lachat Instrument Maintenance Log.

14.3 Sample Analysis

Click on the Omnion icon. From file, open the method file for the analysis being performed. Update the run worksheet with the correct sample IDs. Make any changes to the method that may be necessary, i.e. changes in standard concentrations, etc.

All Lachat methods have been configured to alert the user if the QC criteria Method Performance section 16 has been met. If the criteria are not met, follow the corrective action in the appropriate section.

Place standards and samples into the autosampler as per the run worksheet protocol. Enter any necessary dilutions into the worksheet such as those done during the distillation step or those due to high level standards or samples.

When ready to start analysis, click on the start button at the top of the Windows screen.

Once the run is finished, click on tools, custom report and open format. Choose standard report and click OK. Preview the report and make any required changes before printing. Print the report and exit from the reporting area.

Data from the Lachat instrument is backed up monthly.

Close the flow to the cadmium column, then remove all reagent lines and place into a DI water receptacle. Flush system with water for at least 15 minutes. Remove lines from water and allow air to pump through system. Release tension on pump tubing by lowering arms on pump.

15) Calculations

15.1 Integration

Since the peak expectation window may shift within a method if any of the instrument conditions change, an analyst may need to reintegrate data after the run is finished. Some of the instrument conditions that affect timing are ones that will either speed up or slow down the flow of sample between the autosampler and detector such as changing pump tubing or removing a clog from a reagent line. If the peak expectation window is adjusted for any sample in a run, the adjustment is made to each sample and standard in the entire run. When setting the peak expectation window, the entire area of the subject peak and only the area of the subject peak should be integrated. If any analytical run is altered after

analysis such as modifying the peak expectation window, save the data file using the original date/time stamp and add the word reprocessed to the end so that the original and modified data file are saved. Refer to the Lachat User Manual for specific instructions on operating this software.

Sample results are calculated by the Omnion 3.0 software supplied with the Lachat autoanalyzer. The calculations are performed by determining the area of each sample or standard peak that falls within a set peak expectation window. The peak expectation window is determined separately for each method based upon the time needed for a sample to travel from the autosampler to detector and also the width of each peak.

The efficiency of the cadmium column must be calculated each time Nitrate, Nitrite and Nitrogen are analyzed. The efficiency should be between 90-110%. If the efficiency is outside of this range, stop the analysis and perform required system or column maintenance before continuing.

% cadmium efficiency:
$$\frac{1.0 \text{ mg/L NO}_3 \text{ result}}{1.0 \text{ mg/L NO}_2 \text{ result}} \times 100$$

16) **Method Performance**

A demonstration of capability (DOC) should be performed each time there is a significant change in the chemistry of the method, a major modification to an existing instrument, or a new instrument is installed. A DOC is performed by each analyst designated to analyze samples using this method. An annual check must subsequently be performed and documented for each analyst using this method.

16.1 Demonstration of Capability

A demonstration of capability study must be performed and documented for each analyst using this method. The study should consist of the analysis of four standards which are from a source independent of the standard curve. The results of the standards must be within the acceptance criteria for BS/BSD samples in section 14. If samples are analyzed under more than one program, the acceptance criteria used should be the tighter limits. The % RSD should be within 20%. The results of the accuracy and precision study (true value, % recovery, standard deviation and % RSD) are maintained by the Quality Assurance Officer for each analyst and are located in the IEC Laboratory Active Employee Training Manual.

Continuing Demonstration of Capability

An annual continuing demonstration of capability study must be performed and documented. It may consist of either successfully analyzing a PT sample or analyzing 2 sets of BS/BSD standards to within control limits as stated in section 12. The results of the continuing accuracy and precision study (true value, % recovery, standard deviation and % RSD or final report from the PT provider) are maintained by the Quality Assurance Officer for each analyst and are located in the

IEC Laboratory Active Employee Training Manual.

16.2 Method Detection Limit (MDL)

An MDL Study was conducted for this method. The study is based on the requirements listed in 40 CFR Part 136 Appendix B. The MDL Study comprised the analysis of seven reagent grade water samples fortified at a level between 2-3x the detection limit. The results of the MDL determination (true value, average concentration, standard deviation and calculated MDL) are maintained by the Quality Assurance Officer for each method and are located in the Lachat analysis Log Book.

16.3 Limit of Quantitation (LOQ)

The Laboratory performs a Limit of Quantitation (LOQ) study on an annual basis for analytes associated with chemistry methods. The validity of LOQ is confirmed by successful analysis of a Laboratory Fortified Blank (LFB) at approximately 2X the reporting limit. The acceptance criteria for each analyte is $\pm 30\%$ of the true value. After this study is completed, it is reviewed and approved by the Laboratory Management. A summary of all LOQ study performance is maintained.

17) **Pollution Prevention**

Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the recycling as the next best option.

The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

For information about pollution prevention consult the IEC Laboratory's Health and Safety Manual

18) **Data Assessment and Acceptance Criteria for Quality Control Measures**

See Quality Control, Section 12, for a discussion of QC acceptance criteria and corrective actions.

19) **Corrective Actions for Out-of-Control Data**

See Quality Control, Section 12, for a discussion of QC acceptance criteria and corrective actions.

20) **Contingencies for Handling Out-of-Control or Unacceptable Data**

See Quality Control, Section 12 for a description of contingencies for handling out-of-control or unacceptable data (corrective actions)

21) **Waste Management**

The IEC requires that laboratory waste management practice be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. IEC urges staff to protect the air, water and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any water discharge permit and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions.

22) **References**

EPA Methods for the Determination of Inorganic Substances in Environmental Samples,
Rev. 2.0 August 1993, Method 353.2

Lachat QuikChem Automated Ion Analyzer Methods Manual, Method 10-107-04- 1-C. July 2008.
Standard Methods for the Examination of Water and Wastewater, Method 4500- NO₃⁻ I
Cadmium Reduction Flow Injection Method, 21st Edition, 2000

23) **Tables, Diagrams, Flowcharts and Validation Data**

None.

See Lachat Manual QuickChem Method 31-107-04-1-C (above).

TOTALPHOSPHOROUS/ORTHOPHOSPHATE

SOP ID XXXX, Revision No.3, Effective Date December 2017

1. **Test method**

1.1 This SOP is based on LACHAT Methods 10-115-01-1-A (Orthophosphate) and
10-115-01-1-E (Total Phosphorus)

2. **Applicable Matrix or Matrices**

This method is applicable to surface and saline waters, and domestic and industrial waste waters.

3. **Method Detection Limits**

MDL Orthophosphate is 0.001 mg/L Reporting Limit 0.005 mg/L

MDL Total Phosphorus is 0.334 mg/L Reporting Limit 1.67 mg/L

4. Scope And Application

- 4.1 This analysis is performed by Flow Injection analysis (FIA).
- 4.2 The orthophosphate method determines total orthophosphate or if the sample is filtered through a 0.45 micron pore size filter, the result is termed dissolved orthophosphate. The difference between the result of a sample determined directly and filtered is termed insoluble (particulate) orthophosphate
- 4.3 The Total Phosphorus method determines total phosphorus, or if the sample is filtered through a 0.45micron pore size filter, the result is termed total dissolved phosphorus. The method are based on reactions that are specific for the orthophosphate (PO_4^{3-}) ion.

5. Summary of Method

The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample. Polyphosphates may be converted to the orthophosphate form by sulfuric acid digestion and organic phosphorous may be converted to orthophosphate by persulfate digestion. The determination of Total phosphorus (or total dissolved phosphorus) is performed by performing this method on digested samples, and utilization of a Total Phosphorus manifold. Samples are digested in an autoclave for 30 minutes at 121° C 15-20 psi with sodium persulfate. Following the autoclave digestion, samples are injected onto a Phosphate manifold.

6. Definitions

Phosphorus is a nutrient that occurs in natural waters and in wastewaters almost solely as phosphates. Small amounts of orthophosphate or certain condensed phosphates are added to some water supplies during treatment. Larger quantities are added through use of detergents and fertilizers. Phosphorus is essential to the growth of organisms and can be the limiting nutrient for primary productivity of an organism.

7. Interferences

- 7.1 Orthophosphate: Only orthophosphate forms a blue color in this test. Polyphosphates and organic phosphorus compounds are not recovered. The sulfuric acid in the molybdate reagent does not have enough contact time with polyphosphates to hydrolyze them. The PO_4^{3-} ion reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form an

antimony-phosphomolybdate complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880nm. The absorbance is proportional to the concentration of PO_4^{3-} in the digested sample. Sample turbidity must be removed by filtration immediately after sampling for orthophosphate samples

- 7.2 Total Phosphorus: Samples for dissolved total phosphorous should be filtered only after digestion. Turbidity is removed by filtration. Silica forms a pale blue complex, which also absorbs at 880nm. This interference is generally insignificant as a silicate concentration of approximately 30 mg SiO_2/L would be required to produce a 0.005 mg P/L positive error in orthophosphate. Glassware contamination is a problem in low level phosphorus determinations. Glassware should be washed with 1:1 HCl and rinsed with deionized water. Commercial detergents should rarely be needed but, if they are used, use special phosphate-free preparations for lab glass.
- 7.3 High concentrations of iron may interfere by consuming some of the reducing agent, resulting in low recoveries. This interference is eliminated by the addition of sodium bisulfite. Sample color that absorbs in the photometric range used for analysis will also interfere
- 7.4 Arsenate is a positive interference. If it is known to be present it can also be eliminated by the addition of sodium bisulfite.
- 7.5 This method is very sensitive to contamination. Detergent can contain extremely high concentrations of phosphorous. All glassware must be scrupulously cleaned and acid washed before use.

8. **Safety**

- 8.1 The toxicity and carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be minimized by good laboratory practices, e.g. wear proper protective equipment, safety glasses, gloves, lab coat and working inside hoods whenever possible.
- 8.2 Refer to the IEC Laboratory Health and Safety Manual and College of Staten Island Chemical Hygiene Plan for specific guidelines.
- 8.3 For detailed explanations consult the Material Safety Data Sheets (MSDS), available in the Laboratory.
- 8.4 The following chemicals have the potential to be highly toxic or hazardous, for detailed explanations consult the MSDS.
 - 8.4.1 Sulfuric Acid
 - 8.4.2 Dodecyl Sulfate

9. Equipment and Supplies

- 9.1 Balance
- 9.2 Glassware—Class A volumetric flasks, pipettes or plastic containers as required. Samples may be stored in plastic or glass. All glassware used in the determination of phosphate should be washed with hot 1:1 HCl and rinsed with distilled water. Preferably, this glassware should be used only for the determination of phosphate and after use it should be rinsed with distilled water and kept covered until needed again. If this is done, the treatment with 1:1 HCl is only required occasionally.
- 9.3 LACHAT Autoanalyzer, manifold reaction unit, heating unit, colorimetric detector, 880 nm wavelength filter and Omnion 4.0 software data system. PVC pump tubes must be used with this method.
- 9.4 Autoclave at 121° C (15-20 psi).
- 9.5 Miscellaneous laboratory apparatus such as volumetric pipettes, flasks, digestion tubes, etc.

10. Reagents and Solutions

10.1 Reagents

10.1.1 Stock Ammonium Molybdate Solution

Dissolve 40.0 g ammonium molybdate tetrahydrate in approximately 800 mL of water in a 1 liter flask. Dilute to the line and mix. Store in plastic and refrigerate. This reagent is stable for 2 month.

10.1.2 Stock Antimony Potassium Tartrate Solution

Dissolve 3.0g antimony potassium tartrate (potassium antimonyl tartrate hemihydrate) or 3.22 g antimony potassium tartrate (potassium antimonyl tartrate trihydrate) in approximately 800 mL of water in a 1 liter flask. Dilute to the line and mix. Store in a dark bottle and refrigerate. This reagent is stable for 2 month.

10.1.3 Molybdate Color Reagent

Add approximately 500 mL water to a 1 liter flask. Slowly add 21.0 mL of concentrated sulfuric acid while mixing. When the solution can be comfortably handled, add 72.0 mL of Stock Antimony Potassium Tartrate Solution and 213 mL of Ammonium Molybdate Solution. Dilute to 1 liter and mix. This reagent is stable for 1 week. **Use HACH LACHAT Color reagent CAT# 52002 as alternative.**

10.1.4 Ascorbic Acid Reducing Solution, 0.33 M

Dissolve 60.0 g granular ascorbic acid (FISHER CAT# A62-500) in approximately 700 mL of water in a 1-liter flask. Dilute to the mark and

mix. Add 1.0 g sodium dodecyl sulfate and mix. Prepare fresh weekly and discard if the solution becomes yellow. **Use HACH LACHAT Ascorbic Acid reagent CAT# 52020 as alternative.**

10.1.5 Carrier,

To 1L volumetric flask containing about 900 ml of DI water, add 1.0g dodecyl sulfate ($\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na}$) FISHER CAT#O2674-25. Dilute to the mark and stir to mix. **For orthophosphate analysis use DI water as a carrier.**

10.1.6 Digestion Solution

Dissolve 50.0g of sodium persulfate($\text{Na}_2\text{S}_2\text{O}_8$) FISHER CAT#O6114-500 in about 800 mL DI water. Stir until dissolved. Dilute to the mark, and invert to mix. Prepare fresh monthly. Store in plastic.

10.1.7. 4 M H_2SO_4 (for digestion)

To 1L volumetric flask containing about 600 mL of DI water, carefully and with stirring, add 222 mL concentrated sulfuric acid (H_2SO_4). Allow to cool. Dilute to the mark. Always add acid to the water.

10.1.8. Diluent. This solution is used as diluent for standards and over-range samples.

To a 1L volumetric flask containing about 900 mL of DI water, add 10 mL 4M H_2SO_4 and 8.33g of sodium persulfate ($\text{Na}_2\text{S}_2\text{O}_8$). Stir to mix. Dilute to the mark.

If necessary, prevent bubble formation by degassing all prepared solutions except the standards with helium. Use He at 140kPa (20 lb/in²). Preferably, purchase equivalent solutions which should not require degassing.

Use HACH LACHAT Phosphates reagent set CAT#52902 as alternative.

10.2 Standard Preparation

10.2.1 Stock Phosphorous Standard, 100 mg/L

In a 1-liter volumetric flask, dissolve 0.4396 g anhydrous potassium phosphate monobasic (KH_2PO_4) FISHER CAT# P382-500, that has been dried for one hour at 105°C in approximately 800 mL DI water. Dilute to the mark with DI water and invert to mix. When refrigerated, this standard may be stored in a glass for up to one month.

10.2.2 Working Standards

Prepare 5 working standards.

Dilute the following mL of working stock standard to 100 mL. The upper and lower value of the standard curve should not be modified. If a project requires a different range the upper and lower standards may be modified but the reporting limit must also be adjusted accordingly. Samples above the high standard (+10%) must be diluted. The mid-range standards listed below are recommended but may be modified by the analyst.

<u>mL Working Stock</u>	<u>mg/L Phosphorous</u>
0.1	0.1
0.50	0.50
1.00	1.00
2.00	2.00
5.00	5.00

Note: Select and prepare the required standards according to the analysis and linear range being used.

10.2.3 BS/BSD Solution

Obtain solutions from ERA, EMSL or other reliable sources. Prepare according to instructions provided by the supplier.

10.3 Complex Nutrients LCS sample (Environmental Resources Associates Catalog # 525)

10.4 All purchased and prepared standards and reagents are recorded in the chemical inventory logbook (purchased) or the standards preparation logbook (prepared) which assigns a unique ID# to each. All containers must be labeled with the Name, ID#, concentration, preparation date and expiration date (where applicable).

11. Sample Collection, Preservation, Storage and Holding Time

11.1 Sample containers may be of plastic or glass. All bottles must be thoroughly cleaned and acid rinsed. The volume collected should be sufficient to insure a representative sample and allow for replicate analysis if required. Samples for orthophosphate should be filtered immediately upon collection, with a maximum holding time of 48 hours.

11.2 Samples must be refrigerated to 4°C.

11.3 Samples for total phosphorous are preserved by the addition of sulfuric acid to a pH of <2 and analyzed within 28 days of collection. This is accomplished by adding no more than 2 mL concentrated H₂SO₄ per liter and verifying that the pH is less

than 2. If the pH is still greater than 2, more sulfuric acid is added until the pH is <2. Samples collected from Western LIS monitoring survey may be frozen for up to one year (after filtration) as per QAPP.

11.4 Samples for orthophosphate are not preserved and must be analyzed within 48 hours of collection.

11.5 Digested samples may be store up to one month in screw cap tubes.

12. Quality Control

a. Calibration Curve

Acceptance Criteria - A minimum of 5 standards and a blank must be used to generate the calibration curve. The correlation coefficient must be >0.995 . The % residual for each standard should be $0 \pm 10\%$ with the exception of the lowest standard which is at the reporting limit. The % residual for this standard may be $\pm 30\%$. A calibration curve should be generated at least every six months or following any major change or repair of the instrument. The calibration curve must be verified on each working day by the analysis of an IPC standard and blank. Analyze at least three standards and a blank using a first order calibration curve. If any verification data exceeds the initial values by $\pm 10\%$, reanalyze the standards. If any portion of the range is shown to be nonlinear, sufficient standards must be used to clearly define the nonlinear portion. The LCR must be verified every six months or whenever a significant change in instrument response is observed.

Corrective Action - If the correlation coefficient of the calibration curve, consisting of at least five standards and a blank, is <0.995 , the calibration is disallowed. The analysis must be terminated, and repeated after correcting the problem. % residuals for the standards are monitored so that any possible instrument or dilutor troubleshooting may be performed. If the IPC check does not pass, the instrument should be recalibrated.

b. Instrument Performance Check (IPC) Standard or Initial Calibration Verification (ICV) and Continuing Calibration Verification (CCV) Standard.

Acceptance Criteria - Analyze the IPC solution for all determinations immediately following each calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution immediately following calibration must verify that the instrument is within $\pm 10\%$ of the true value. Subsequent analyses of the continuing IPC solution must be within $\pm 10\%$ of the true value.

Corrective Action - If the calibration cannot be verified within the specified limits, analysis must be discontinued, the cause determined and/or in the case of drift the instrument re-calibrated. All samples following the last acceptable IPC solution must be reanalyzed.

c. Laboratory Reagent Blank (LRB), Prep Blank (PB), Initial Calibration Blank (ICB) and Continuing Calibration Blank (CCB)

Acceptance Criteria - Analyze a blank along with each batch of 20 or fewer samples. All LRB/PB/ICB/CCB results must be < the Reporting Limit.

Corrective Action - If the results of the LRB/PB/ICB/CCB are > the Reporting Limit then all associated samples with a concentration of <10x the amount found in the LRB/PB/ICB/CCB should be reprepared and reanalyzed (sample results $\geq 10x$ the amount found in the LRB/PB/ICB/CCB are not considered to be affected by the blank contamination or drift).

If the samples cannot be re-prepared, then all affected sample results must be either 1) qualified accordingly, or 2) the reporting limit is raised to the amount found in the blank. Check with the team leader/section chief to determine which option should be used.

d. Laboratory Fortified Blank (LFB), Blank Spike/Blank Spike Duplicate (BS/BSD) or Quality Control Samples (QCS)

Acceptance Criteria - Analyze two LFB/BS/BSD/QCS samples (complexed phosphorus sample) with each batch of 20 or fewer samples. Calculate accuracy as percent recovery using the following equation:

$$\% \text{ Recovery} = \frac{\text{LFB/BS/BSD/QCS}}{s} \times 100$$

where:

LFB/BS/BSD/QCS = control sample results determined by laboratory

s = concentration equivalent of analyte added to fortify the LFB/BS/BSD/QCS solution.

The % recovery of the LFB/BS/BSD/QCS for samples analyzed under NPDES program should be within 10% of true value. The relative percent difference (RPD) of the duplicates should not exceed 20% for aqueous standards.

Corrective Action - If the % recovery or RPD results are outside the required control limits, the affected samples should be reprepared and reanalyzed. If the samples cannot be reprepared, then all affected sample results must be qualified accordingly.

e. Laboratory Fortified Matrix (LFM) or Matrix Spike (MS) Recovery Acceptance

Criteria:

For samples analyzed under NPDES program, prepare one LFM/MS per matrix for an analytical batch of 10 samples or less regardless of the number of different projects that comprise the analytical batch. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. Calculate the percent recovery, corrected for background concentration measured in the unfortified sample aliquot as per the

equation below. The % recovery for NPDES or DW samples should be 90-110%. The recovery for all other programs should be 80- 120%.

Corrective Action - If the % recovery of the LFM/MS is outside the required

$$R = \frac{C_s - C}{C} \times 100 \text{ s}$$

where: R = percent recovery,
Cs = fortified sample concentration,
C = sample background concentration, and
s = conc. equivalent of spike added to sample.

control limits, and the laboratory performance is shown to be in control, the recovery problem encountered is judged to be matrix related, not system related. The native sample result of the sample used to produce the LFM/MS must be qualified accordingly.

Note: The % recovery of the LFM/MS is not evaluated if the result of the unfortified sample concentration is >1X the level used to fortify the sample.

13. Calibration and Standardization

- 13.1. Prepare reagents and standards as describe in section 10.
- 13.2. Set up manifold according LACHAT manifold diagram, input data system parameters.
- 13.3. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow system to equilibrate until a stable baseline is achieved.
- 13.4. Place samples and standards in the sampler. Input the information required by the data system, such as concentration, replicates, etc.
- 13.5. Calibrate the instrument by injecting the standards. The data system will then associate the concentration with the instrument responses for each standard.
- 13.6. If samples concentration are greater than the high standard, the digested sample should be diluted with diluent (Reagent 10.1.8.). **Do not dilute digested samples or standards with DI water.**

14. Procedure

14.1 Sample Preparation

- 14.1.1 Sample preparation is documented in the Sample Digestion Log Book.

Digestion procedure for total phosphorous

- 14.1.2 Check sample pH before digestion and record on digestion bench sheet. Samples should be preserved to a pH of <2.

14.1.3 Measure 10 mL of each standard and sample into a digestion tube and add 0.1 mL of 4M H₂SO₄ and 2.0 mL of persulfate digestion solution. Vortex. All samples should be carried throughout this procedure. Standards are prepared in digestion matrix. Digestion of a “blank” is a good test of digestion reagent contamination.

14.1.4. Loosely screw the caps onto digestion tubes. Caps must be loose enough that air/steam can escape.

14.1.5. Digest the samples in the autoclave for 30 minutes at 121°C and 15-20 psi. Digestion time begins when the pressure has reach 15 psi.

14.1.6. Vent the pressure from the autoclave. Remove the samples, and allow 15 minutes for cooling, then remix on vortex mixer. Screw caps on tightly until samples are analyzed. Digested samples may be stored up to one month. If the samples are turbid after digestion, filter prior to analysis.

14.2 Instrument Operating Conditions

14.2.1 Turn on the LACHAT instrument and install the proper manifold for the analysis to be performed. Use the 650 cm heater coil (175 cm for orthophosphate) and set the temperature to 40° C (37° C for orthophosphate). Use 880 nm filter for total phosphate and orthophosphate. Place the sample tube into a container with DI water. Click on manual run pump button. Consult the LACHAT methods manual for installation details if necessary.

14.2.2 Pump Milli-Q water through the system and check for leaks and smooth flow. Perform any routine maintenance if necessary. Once instrument is stable, remove reagent lines from the water and place in the proper reagent receptacle.

14.2.3 Instrument Maintenance and Repair records are entered into the Instrument Maintenance Log.

14.3 Sample Analysis

14.3.1 Click on the Omnion icon. From file, open the method file for the analysis being performed. Update the run worksheet with the correct sample IDs. Make any changes to the method that may be necessary, i.e. changes in standard concentrations, etc.

14.3.2 Place standards and samples as per the run worksheet protocol. Enter any necessary dilutions into the worksheet such as those done during the distillation step or those due to high level standards or samples. When diluting samples, use the appropriate volume of digestion solution so that the matrix matches that of the standard curve.

14.3.3 When ready to start analysis, click on the start button at the top of the Windows screen.

14.3.4 All LACHAT methods have been configured to alert the user if the QC criteria in sections 14.1-14.3.5 has been met. If the criteria are not met, follow the corrective action in the appropriate section.

14.3.5 Once the run is finished, click on tools, custom report. Choose standard report and click OK. Preview the report and make any required changes before printing. Print the report and exit from the reporting area.

14.3.6 Data from the LACHAT instrument is backed up periodically.

14.3.7 Remove all reagent lines and place into a DI water receptacle. Flush system with water for at least 15 minutes. Remove lines from water and allow air to pump through system. Release tension on pump tubing by lowering arms on pump.

15. Calculations

15.1 Integration

Since the peak expectation window may shift within a method if any of the instrument conditions change, an analyst may need to reintegrate data after the run is finished. Some of the instrument conditions that affect timing are ones that will either speed up or slow down the flow of sample between the sampler and detector such as changing pump tubing or removing a clog from a reagent line. If the peak expectation window is adjusted for any sample in a run, the adjustment is made to each sample and standard in the entire run. When setting the peak expectation window, the entire area of the subject peak and only the area of the subject peak should be integrated. If any analytical run is altered after analysis such as modifying the peak expectation window, save the data file using the original date/time stamp and add the word reprocessed to the end so that the original and modified data file are saved. Refer to the LACHAT User Manual for specific instructions on operating this software.

15.2 Calculations

Sample results are calculated by the Omnion 4.0 software supplied with the LACHAT analyzer. The calculations are performed by determining the area of each sample or standard peak that falls within a set peak expectation window. The peak expectation window is determined separately for each method based upon the time needed for a sample to travel from the sampler to detector and also the width of each peak.

16. Method Performance

A demonstration of capability (DOC) should be performed each time there is a significant change in the chemistry of the method, a major modification to an existing instrument, or a new instrument is installed. A DOC is performed by each analyst designated to analyze samples using this method. An annual check must subsequently be performed and documented for each analyst using this method.

a. Accuracy and Precision

i. Demonstration of Capability

A demonstration of capability study must be performed and documented for each analyst using this method. The study should consist of the analysis of four standards which are from a source independent of the standard curve. The results of the standards must be within the acceptance criteria for BS/BSD samples in section 14. If samples are analyzed under more than one program, the acceptance criteria used should be the tighter limits. The % RSD should be within 20%. The results of the accuracy and precision study (true value, % recovery, standard deviation and % RSD) are maintained by the Quality Assurance Officer for each analyst and are located in the IEC Laboratory Active Employee Training Manual.

ii. Continuing Demonstration of Capability

An annual continuing demonstration of capability study must be performed and documented. It may consist of either successfully analyzing a PT sample or analyzing 2 sets of BS/BSD standards to within control limits as stated in section

13.1.1. The results of the continuing accuracy and precision study (true value, % recovery, standard deviation and % RSD or final report from the PT provider) are maintained by the Quality Assurance Officer for each analyst and are located in the IEC Laboratory Active Employee Training Manual.

b. Method Detection Limit (MDL)

An MDL Study was conducted for this method. The study is based on the requirements listed in 40 CFR Part 136 Appendix B. Specific procedures for conducting an MDL study can be found in SOP # G-8. The MDL Study comprised the analysis of seven reagent grade water samples fortified at a level between 2-3x the detection limit. The results of the MDL determination (true value, average concentration, standard deviation and calculated MDL) are maintained by the Quality Assurance Officer for each method and are located in the Laboratory.

c. Limit of Quantitation (LOQ)

The Laboratory performs a Limit of Quantitation (LOQ) study on an annual basis for analytes associated with chemistry methods. The validity of LOQ is confirmed by successful analysis of a Laboratory Fortified Blank (LFB) at approximately 2X the reporting limit. The acceptance criteria for each analyte is $\pm 30\%$ of the true value. After this study is completed, it is reviewed and approved by the Laboratory Management. A summary of all LOQ study performance is maintained in the Laboratory.

17. Pollution Prevention

17.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The IEC has established a preferred hierarchy of

environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation.

17.2 The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

18. Data Assessment and Acceptance Criteria for Quality Control Measures

18.1 Laboratory Spike Sample Matrix (LSM) – The laboratory must spike, in duplicate, minimum of 10% of routine samples. In each case the LSM aliquots must be duplicate of the aliquot used for sample analyses. The spiking level shall be at 1 to 5 times higher than the background concentration of the sample.

Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range 90-110%. Percent recovery may be calculated using the following equation.

$$R = \frac{C_s - C}{s} \times 100$$

Where R= percent recovery, C_s = fortified sample concentration, C= sample background concentration, s= concentration equivalent of analyte added to sample.

18.2 If the recovery of any analyte falls outside the designated LSM recovery range and the laboratory performance for that analyte is shown to be in control (sect. 18.3), the recovery problem encountered with the LSM is judge to be either matrix or solution related, not system related.

18.3 Computer the relative percent difference (RPD) between the two LSM results and compare the value to the designated RPD recovery range of 10%. The RPD may be calculated using the following equation:

$$RPD = \frac{(D_1 - D_2)}{(D_1 + D_2) / 2} \times 100$$

Where D1= concentration of analyte in the sample, D2= concentration of analyte in the second (duplicate) sample.

18.4 If the RPD falls outside the designated recovery range and the laboratory performance for that analyte is shown to be in control (sect 18.3), the recovery problem encountered with the LSM is judge to be either matrix or solution related, not system related.

18.5 Where reference materials are available, they should be analyzed to provide additional

performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

See Quality Control, Section 12, for a discussion of QC acceptance criteria and corrective actions.

19. Corrective Actions for Out-of-Control Data

See Quality Control, Section 12, for a discussion of QC acceptance criteria and corrective actions

20. Contingencies for Handling Out-Of-Control Data

See Quality Control, Section 12, for a discussion of QC acceptance criteria and corrective actions

21. Waste Management

The IEC requires that laboratory waste management practice be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The agency urges laboratories to protect the air, water and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any water discharge permit and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions.

22. References

LACHAT QuikChem Automated Ion Analyzer Methods Manual, Method 10-115-01-1-E (2001) Determination of Total Phosphorus by Flow Injection Analysis (Acid Persulfate Digestion Method)

LACHAT QuikChem Automated Ion Analyzer Methods Manual, Method 10-115-01-1-A (2007) Determination of Ortho Phosphate in Waters by Flow Injection Analysis Colorimetry

Standard Methods for the Examination of Water and Wastewater, Method 4500-P G. Flow Injection Analysis for Orthophosphate, 21st Edition, 1999.

Standard Methods for the Examination of Water and Wastewater, Method 4500-PH. Flow Injection Analysis for Total Phosphorous (Proposed), 20th Edition, 1998

23. Tables, Diagrams, Flowcharts, And Validation Data

See LACHAT Methods 10-115-01-1-E (2001) and LACHAT Method 10-115-01-1-A (2007) in LACHAT methods manual

Ammonia
SOP ID XXXIX, Revision No. 3, January 2018

1. TEST METHOD

Based on LACHAT Method 10-107-6-1B (2001).

2. APPLICABLE MATRIX OR MATRICES

The method is applicable for non-potable water (ambient surface water and wastewater).

3. METHOD DETECTION LIMIT

The method detection limit is 0.020 mg/L as NH₃. The reporting limit is 0.1 mg/L.

4. SCOPE AND APPLICATION

4.1 This method covers the determination of ammonia in surface waters, and domestic and industrial wastes. IEC will be using for the analysis of samples collected in western LIS (brackish samples) as well as periodic samples collected from wastewater treatment plants, as applicable.

5. SUMMARY OF METHOD

5.1 This method is based on the Berthelot reaction. Ammonia reacts with alkaline phenol, and sodium hypochlorite to form indophenol blue to form monochloramine, which, in the presence of phenol, catalytic amounts of nitroprusside (nitroferricyanide), and excess hypochlorite gives indophenol blue. The formation of monochloramine requires a pH between 8 and 11.5. At higher pH, ammonia may begin to oxidize to nitrate. At pH greater than 9.6, some precipitation of calcium and magnesium as hydroxides and carbonates occurs in seawater. EDTA added to the buffer prevents this from occurring. The indophenol blue measured at 630 nm is proportional to the original ammonia concentration.

The method is calibrated using standards prepared in deionized water. Once calibrated, samples of varying salinities (0 to 35 ppt) may be analyzed. The determination of background absorbance is necessary only for samples which have color absorbing at 630 nm.

If distillation is required, the sample is buffered at pH of 9.5 with a borate buffer to decrease hydrolysis of cyanates and organic nitrogen compounds, and is distilled into a solution of boric acid.

6. DEFINITIONS

The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

6.1 CALIBRATION BLANK (CB) – A volume of reagent water in the same matrix as the

calibration standards, but without the analyte.

6.2 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

6.3 INSTRUMENT PERFORMANCE CHECK SOLUTION (ICP) -- A solution of one or more method analytes used to evaluate the performance of the instrument system with respect to a defined set of criteria.

6.4 LABORATORY SPIKED BLANK (LSB) -- An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LSB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements

6.5 LABORATORY SPIKED SAMPLE MATRIX (LSM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LSM is analyzed exactly like sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM correct for background concentrations.

6.6 LABORATORY REAGENT BLANK (LRB) – An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, except that it is not taken to the sampling site. The purpose is to determine if the analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

6.7 LINEAR CALIBRATION RANGE (LCR) – The concentration range over which the instrument response is linear.

6.8 MATERIAL SAFETY DATA SHEETS (MSDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

6.9 METHOD DETECTION LIMIT (MDL) – The lowest level at which an analyte can be detection with 99 percent confidence that the analyte concentration is great than zero.

6.10 LIMIT OF QUANTITATION (LOQ) – The Laboratory performs a Limit of Quantitation (LOQ) study on an annual basis for analytes associated with chemistry methods. The validity of LOQ is confirmed by successful analysis of a Laboratory Fortified Blank (LFB) at approximately 2X the reporting limit. The acceptance criteria for each analyte is + 30% of the true value. After this study is completed, it is reviewed and approved by the Laboratory Management. A summary of all LOQ study performance is maintained.

6.11 QUALITY CONTROL SAMPLE (QCS) – A solution of method analytes of known concentrations that is used to spike an aliquot of LRB or sample matrix. THE QCS is obtained for a source external to the laboratory or is prepared from standards obtained from a different source

than the calibration standards. The purpose is to check laboratory performance using test materials that have been prepared independently from the normal preparation process.

6.12 STOCK STANDARD SOLUTION (SSS) – A concentrated solution containing one or more method analytes prepared in the laboratory using assay reference materials or purchased from a reputable commercial source.

7. INTERFERENCES

7.1 Calcium and magnesium ions may precipitate if present in sufficient concentration. EDTA is added to the sample in-line to prevent this problem.

7.2 Color, turbidity, and certain organic species may interfere. Turbidity is removed by manual filtration. Sample color may be corrected for by running the samples through the manifold without color formation. See System Note 11 for specific instructions.

7.3 Sulfide may interfere at levels greater than 2 mg H₂S/L. Samples containing concentrations greater than this should be diluted.

7.4 Salinity does not normally interfere in this method. This may be verified by running the samples through the manifold with all reagents pumping, except hypochlorite, which is replaced by deionized water. The resulting concentrations are then compared to those obtained for samples determined with color formation.

7.5 The salt effect (salinity influence on absorbance) is less than 2%.

See System Note 11 for specific instructions.

8. SAFETY

8.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials.

8.2 Each laboratory is responsible for maintaining a current awareness file of the Occupational Health and Safety Act (OSHA) regulation regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) is made available to all personnel involved in the chemical analysis.

8.3 The following chemicals have the potential to be highly toxic or hazardous, for detailed explanation consult the MSDS.

5.3.1. Sodium hydroxide

5.3.2. Phenol

9. EQUIPMENT AND SUPPLIES

9.1 Balance – analytical, capable of accurately weighing to the nearest 0.0001 g.

9.2 Glassware – Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

9.3 Flow injection analysis equipment (LACHAT 8500 series 2) designed to deliver and react sample and reagents in the required order and ratios.

9.3.1. Sampler

9.3.2. Multichannel proportioning pump

9.3.3. Reaction unit or manifold

9.3.4. Colorimetric detector

9.3.5. Data system

9.4 Special Apparatus

6.4.1. Seawater Accessories Kit LACHAT Part No. 50969 – RAS or 50970 – ASX510 (for brackish samples)

6.4.2. PVC PUMP TUBES MUST BE USED FOR THIS METHOD.

10. REAGENTS AND STANDARDS

10.1 PREPARATION OF REAGENTS

Use deionized water (10 megaohm) for all solutions.

Degassing with helium:

To prevent bubble formation, DO NOT DEGAS USING AN INVASIVE PROCEDURE SUCH AS A WAND TO AVOID CONTAMINATION. Degas by vacuum or sonication. DO NOT DEGAS PHENATE, HYPOCHLORITE, OR STANDARDS.

Reagent 1. Buffer Chelating Agent

By Volume: In a 1 L volumetric flask, dissolved 50.0 g disodium ethylenediamine tetraacetate (Na_2EDTA) and 11.0 g sodium hydroxide (NaOH) in about 900 mL DI water. Stir to mix and dilute to the mark with DI water. Degas as above. Prepare fresh monthly sample.

By Weight: To a tared 1 L container, add 966 g DI water. Add 50.0 g disodium ethylenediamine tetraacetate (Na_2EDTA) and 11.0 g sodium hydroxide (NaOH). Stir to mix. Degas as above. Prepare fresh monthly sample. **Or use HACH Cat# 52017** as alternative option.

Reagent 2. Phenate Reagent

CAUTION: Wear gloves. Phenol causes skin burns and is rapidly absorbed into the body through the skin. Do not degas this reagent.

By Volume: In a 1 L volumetric flask, dissolved 83 g crystalline phenol (C_6H_5OH) in approximately 500 mL DI water. While stirring, slowly add 32 g sodium hydroxide ($NaOH$). Cool, dilute to the mark with DI water, and invert to mix. The color of this reagent darkens with age, increasing the baseline absorbance. Prepare fresh reagent after 72 hours. Prepare fresh every 3-5 days and discard when turns dark brown.

By Weight: To a tared 1 L container, add 888 g DI water. Add 83 g crystalline phenol (C_6H_5OH). While stirring, slowly add 32 g sodium hydroxide ($NaOH$). Cool and stir to mix. The color of this reagent darkens with age, increasing the baseline absorbance. Prepare fresh every 3-5 days and discard when turns dark brown. **Or use HACH Cat#52005** as alternative option.

Reagent 3. Sodium Hypochlorite

By Volume: Dilute 250 mL sodium hypochlorite (SS290-1 hypochlorite solution, 4-6% $NaOCl$ from Fisher) to 500 mL with DI water. Prepare fresh daily.

By Weight: Dilute 250 g sodium hypochlorite (SS290-1 hypochlorite solution, 4-6% $NaOCl$ form Fisher) to 500 g with DI water. Prepare fresh daily. **Or use HACH Cat# 52007** as alternative option.

Reagent 4. Sodium Nitroprusside

By Volume: Dissolve 1.75 g sodium nitroprusside in 500 mL DI water. Prepare fresh every 1-2 weeks.

By Weight: Dissolve 1.75 g sodium nitroprusside in 500 mL DI water. Prepare fresh every 1-2 weeks. **Or use HACH Cat# 52006** as alternative option.

OR PREFEREBLY USE HACH LACHAT AMMONIA REAGENT SET CAT # 52904 as alternative option.

10.2 PREPARATION OF STANDARDS

To prepare the stock and working standards, the following containers will be required:

By Volume: One 1 L and four 200 mL volumetric flasks.

By Weight: One 1 L and four 200 mL containers.

Standard 1. Stock Standard 100.0 mg/L

By Volume: In a 1 L volumetric flask dissolve 0.3818 g ammonium chloride (NH_4Cl) FISHER CAT #3384-12 (Macron) in about 800 mL DI water. Dilute to the mark with DI water and invert to mix.

Standard 2. Working Standards preparation (Prepare fresh daily)

By Volume: In a 100 mL volumetric flask add following amount of stock standard 100.0 mg /L

(Standard 1). Dilute to the mark with DI water and invert to mix.

Concentration (mg/L)	ml of 100 mg/L Stock standard
4 mg/L	4 ml
2 mg/L	2 ml
1 mg/L	1 ml
0.5 mg/L	0.5 ml
0.1 mg/L	0.1 ml

11. SAMPLE COLLECTION, PRESERVATION AND STORAGE

11.1 There is no single preservation method that may be recommended of all types of samples. The analyst must examine each situation critically and treat this information as a guide.

11.2 If samples must be chemically preserved, samples should be cooled, and adjusted to pH < 2 with H₂SO₄. And store at 4 °C in glass or polyethylene. Analyze within 28 days. Samples collected from western Long Island Sound sampling and stored in polyethylene bottles may be stored frozen up to 1 year. If samples are not run within 24 hours a storage stability study should be done.

11.3 Researchers have found serious errors when investigating the effects of filtration. The analyst should examine sample preparation and preservation techniques before routine testing.

12. QUALITY CONTROL

12.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

12.2 INITIAL DEMONSTRATION OF PERFORMANCE

12.2.1. The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.

12.2.2. Linear Calibration Range (LCR) --- The LCR must be determined initially and verified every 6 months or whenever a significant change in instrument response is observed and expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards. If any verification data exceeds the initial values by +/- 10%, linearity must be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

12.2.3. Quality Control Sample (QCS) or Laboratory Control Sample (LCS)– When

beginning the use of this method, and as required to meet data-quality needs, verify the calibration standards are acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within +/- 10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with ongoing analyses.

12.2.4. Method Detection Limit (MDL) – MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate MDLs as follows:

$$MDL = t \times S$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t= 3.14 for seven replicates, t = 2.528 for twenty-one replicates]. S= standard deviation of the replicate analyses.

MDLs should be determined every six months, when a new operator begins work, or whenever there is a significant change in the background or instrument response. The reporting limit is 5X the calculated MDL and must be verified at least annual, or as required for data-quality needs.

13. CALIBRATION AND STANDARDIZATION

13.1 Prepare a series of standards, covering the desired range, and a blank by dilution suitable volumes of standard solution (suggested range in Section 10.2).

13.2 Calibrate the instrument as described in Section.

13.3 Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. Acceptance or control limits should be established using the difference between the measured value of the calibration solution and the “true value” concentration.

13.4 After the calibration has established, it must be verified by the analysis of a suitable quality control sample (QCS). If measurements exceed +/- 10% of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.

14. PROCEDURE

14.1 CALIBRATION PROCEDURE

14.1.1 Prepare reagents, blank and standards covering the desired range, as described in section 10. Select 630 nm interference filter.

14.1.2 Set up manifold as shown in LACHAT Ammonia Manual.

14.1.3 Input data system parameters as shown in LACHAT Ammonia Manual.

14.1.4 Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

14.1.5 Place samples and/or standards in the sampler. Input the information required by the data system, such as concentration, replicates and QuikChem scheme (see section 14.2).

14.1.6 Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the instrument responses for each standard.

14.2 SYSTEM NOTES

14.2.1 For information on system maintenance and troubleshooting refer to the Troubleshooting Guide in the System Operation Manual. This guide is also available on request from LACHAT.

14.2.2 Allow 15 minutes for heating module to warm up to 60 ° C.

14.2.3 Allow 15 minutes with reagents pumping for the system to equilibrate.

14.2.4 To check for gross carrier contamination:

- A. Monitor the baseline on the system unit screen.
- B. Equilibrate the pumping system with DI water in all reagent lines.
- C. Equilibrate the pumping system with reagents in the appropriate lines.
- D. The increase in absorbance should be 0.02 V or less.

14.2.5 A backpressure coil (100 cm x 0.5 mm (0.022”) i.d. Teflon tubing) is used to prevent air bubble formation.

14.2.6 All reagent containers should be covered with Parafilm after insertion of the transmission lines to prevent contamination from airborne ammonia.

14.2.7 Reagent recipes from other automated wet chemistry analyzers should not be substituted.

14.2.8 If sample tube or standard container materials other than polystyrene are used, standards and samples in these containers should be analyzed to investigate absorption or contamination.

14.2.9 The blank in this method should not give a peak. If the blank peak is negative, the carrier is contaminated. If the blank peak is positive, the blank is contaminated.

14.2.10 If the detection limit is greater than that specified in the method the following outline should be followed.

- A. Verify standards preparation procedures.
- B. Verify that a 630 nm filter is being used.
- C. Verify that the sample loop is completely filled by running dye.
- D. Verify that the reagents are being added in the correct order.
- E. Verify that the reagents are flowing smoothly. If not, check for obstructions in the manifold and replace worn tubing.
- F. Prepare fresh reagents. Take care to be sure that the EDTA is completely

dissolved.

14.2.11 If the samples are colored or are suspected to show a background absorbance, this interference should be subtracted. This can be done by using the following procedure:

- A. Calibrate the system in the normal manner.
- B. Disable the check standards or DQM features and analyze the samples.
- C. Place reagent and carrier lines in DI water and allow the baseline to stabilize.
- D. Inject samples again without recalibrating.
- E. Subtract the “background” concentration from the original concentration to give the correct concentration.

$$\text{Corrected Concentration} = \text{Original Concentration} - \text{Background}$$

Concentration

14.2.12 It is critical that the peak be detected on the “flat top” of the standard peaks. If the window is not on the “flat top”, the peak start time should be adjusted.

14.2.13 To ascertain that the standards peak is properly positioned in the window, observe the peaks on the system unit screen. The baseline trace should be flat for the duration of the baseline window. If the peak is not properly positioned, change the peak detection parameters. Low nutrient or artificial seawater, as is and spiked at 1 or 2 low levels, can be used to set timing parameters.

14.2.14 For low level analysis it is recommended that samples be analyzed in replicate from each sample cup. This is done by entering Replicates = 2 when entering sample information.

14.2.15 Use consumer bleaches with caution. Proprietary additives may contribute to staining of tubing and data quality.

14.2.16 Add reagents in the order that they appear on the manifold to reduce staining.

Data from LACHAT instrument is back up periodically.

15. CALCULATIONS

15.1 Calibration is done by injecting standards. The data system will then prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation.

15.2. Report only those values that fall between the lowest and highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.

15.3 Report results in mg/L or µg/L.

16. METHOD PERFORMANCE

16.1. Laboratory Reagent Blank (LRB) – The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be

suspected and corrective actions must be taken before continuing the analysis.

16.2. Laboratory Fortified Blank (LFB) – The laboratory must analyze at least one LFB (also referred to as a Quality Control Sample, QCS, or Laboratory Control Sample, LCS, with each batch of samples. Calculate accuracy as percent recovery (Section 18). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judge out of control, and the source of the problem should be identified and resolved before continuing analyses.

16.3. The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90-110%.

16.4. Instruments Performance Check Solution (IPC) – For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within +/- 10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within +/- 10%. If the calibration cannot be verified within the specific limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with sample analyses data.

A demonstration of capability (DOC) should be performed each time there is a significant change in the chemistry of the method, a major modification to an existing instrument, or a new instrument is installed. A DOC is performed by each analyst designated to analyze samples using this method. An annual check must subsequently be performed and documented for each analyst using this method.

17. POLLUTION PREVENTION

17.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the recycling as the next best option. The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

For information about pollution prevention consult the IEC Laboratory's Health and Safety Manual.

18. DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES

18.1 Laboratory Spike Sample Matrix (LSM) – The laboratory must spike, in duplicate, minimum of 10% of routine samples. In each case the LSM aliquots must be duplicate of the aliquot used for sample analyses. The spiking level shall be at 1 to 5 times higher than the background concentration of the sample.

Calculate the percent recovery for each analyte, corrected for concentrations measured in the

unfortified sample, and compare these values to the designated LFM recovery range 90-110%. Percent recovery may be calculated using the following equation.

$$R = \frac{C_s - C}{s} \times 100$$

Where R= percent recovery, C_s = fortified sample concentration, C= sample background concentration, s= concentration equivalent of analyte added to sample.

18.2 If the recovery of any analyte falls outside the designated LSM recovery range and the laboratory performance for that analyte is shown to be in control (sect. 18.3), the recovery problem encountered with the LSM is judge to be either matrix or solution related, not system related.

18.3 Computer the relative percent difference (RPD) between the two LSM results and compare the value to the designated RPD recovery range of 10%. The RPD may be calculated using the following equation:

$$RPD = \frac{(D_1 - D_2)}{(D_1 + D_2) / 2} \times 100$$

Where D1= concentration of analyte in the sample, D2= concentration of analyte in the second (duplicate) sample.

18.4 if the RPD falls outside the designated recovery range and the laboratory performance for that analyte is shown to be in control (sect 18.3), the recovery problem encountered with the LSM is judge to be either matrix or solution related, not system related.

18.5 Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

See Quality Control, Section 12, for a discussion of QC acceptance criteria and corrective actions.

19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

See Quality Control, Section 12, for a discussion of QC acceptance criteria and corrective actions.

20. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

See Quality Control, Section 12 for a description of contingencies for handling out-of-control or unacceptable data (corrective actions).

21. WASTE MANAGEMENT

The USEPA requires that laboratory waste management practice be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. IEC urges staff to protect the air, water and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any water discharge permit and regulations, and by complying with all solid

and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult IEC's Health and Safety Manual and the College of Staten Island's Chemical Hygiene Plan.

22. REFERENCES

- 22.1. LACHAT Instruments, September 2008. Determination of Ammonia (Phenolate) By Flow Injection Analysis Colorimetry. QuikChem® Method 10-107-06-1-B
- 22.2. U.S. Environmental Protection Agency, methods for Chemical Analysis of Water and Wastewater, EPA-600/4-79-020, Rev 2; August 1993, Method 350.1.

23. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA:

NONE.

See LACHAT Manual QuickChem Method 10-107-06-1-B (above).

DETERMINATION OF SILICATE IN BRACKISH OR SEAWATER BY FLOW INJECTION ANALYSIS COLORIMETRY IEC SOP ID XXXXII, Revision No. 2, Effective Date January 2018

1. SCOPE AND APPLICATION

- 1.1. This method covers the determination of silicate in brackish or seawaters.
- 1.2. The applicable range is 1.0 to 100 μM SiO_2 (60.09 - 6009 μg SiO_2/L). The method detection limit is 0.9 $\mu\text{g}/\text{L}$ SiO_2 . The reporting limit is 10 $\mu\text{g}/\text{L}$ SiO_2 .

2. SUMMARY OF METHOD

Soluble silica species react with molybdate at 37°C and a pH of 1.2 to form a yellow silicomolybdate complex. This complex is subsequently reduced with ascorbic acid to form molybdenum blue. The absorbance of the molybdenum blue, measured at 660 nm, is linearly proportional to the concentration of silicate in the sample. Oxalic acid is added to reduce the interference from phosphate.

Though the method is written for Brackish water and Seawater, it is also applicable to non-saline sample matrices.

The method is calibrated using standards prepared in deionized water. Once calibrated, samples of varying salinities (0 to 35 ppt) may be analyzed.

3. DEFINITIONS

The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

- 3.1. ANALYTICAL BATCH -- The set of samples extracted/distilled/or digested at the same time to a maximum of 10 samples.
- 3.2. CALIBRATION BLANK (CB) -- A volume of reagent water in the same matrix as the calibration standards, but without the analyte.
- 3.3. CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.
- 3.4. FIELD BLANK (FMB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, including exposure to a sample bottle holding time, preservatives, and all preanalysis treatments. The purpose is to determine if the field or sample transporting procedures and environments have contaminated the sample.
- 3.5. FIELD DUPLICATE (FD) -- Two samples taken at the same time and place under identical circumstances which are treated identically throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.
- 3.6. LABORATORY BLANK (LRB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, except that it is not taken to the sampling site. The purpose is to determine if the if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.
- 3.7. LABORATORY CONTROL STANDARD (LCS) -- A solution prepared in the laboratory by
dissolving a known amount of one or more pure compounds in a known amount of reagent water. The purpose is to assure that the results produced by the laboratory remain within the acceptable limits for precision and accuracy. (This should not be confused with a calibrating standard).
- 3.8. LABORATORY DUPLICATE (LD) -- Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation, or storage procedures.
- 3.9. QUALITY CONTROL CHECK SAMPLE (QCS) -- A sample containing analytes of interest at known concentrations (true values) of analytes. The QCS is obtained for a source external to the laboratory or is prepared from standards obtained from a different source than the calibration standards. The purpose is to check laboratory performance using test materials that have been prepared independently from the normal preparation process.
- 3.10. METHOD DETECTION LIMIT (MDL) -- The lowest level at which an analyte

can be detected with 99 percent confidence that the analyte concentration is greater than zero.

4. INTERFERENCES

- 4.1. The interference due to phosphate is reduced by the addition of oxalic acid. A solution of 1000 µg P/L was determined as 20 µg SiO₂/L and 10 mg P/L was determined as 0.1 mg/L SiO₂/L. The extent of phosphate interference should be verified by determining a solution of phosphate at the highest concentration that is expected to be encountered. If the 7 cm reaction coil after the oxalic acid does not sufficiently reduce phosphate interference, a longer coil can be used.
- 4.2. Tannin and large amounts of iron or sulfides are interferences. Sulfides can be removed by boiling and acidifying the sample. Addition of disodium EDTA will eliminate the interference due to iron. Treatment with oxalic acid decreases interference from tannin.
- 4.3. Sample color and turbidity can interfere. The presence of these interferences can be determined by analyzing samples without the presence of molybdate.
- 4.4. Silica contamination may be avoided by storing samples, standards, and reagents in plastic. Deionize glass distilled water before use to remove silica.

5. SAFETY

- 5.1. The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials.
- 5.2. Each laboratory is responsible for maintaining a current awareness file of the Occupational Health and Safety Act (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.
- 5.3. The following chemicals have the potential to be highly toxic or hazardous, for detailed explanation consult the MSDS.
 - 5.3.1. Oxalic Acid
 - 5.3.2. Sulfuric Acid

6. EQUIPMENT AND SUPPLIES

- 6.1. LACHAT QuikChem 8500 automated ion analyzer with Silica manifold
- 6.2. Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.
- 6.3. Glassware -- Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

- 6.4. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.
 - 6.4.1. Sampler
 - 6.4.2. Multichannel proportioning pump
 - 6.4.3. Reaction unit or manifold with 660 nm filter.
 - 6.4.4. Colorimetric detector
 - 6.4.5. Data system
- 6.5. Special Apparatus
 - 6.5.1. Heating Unit.
 - 6.5.2. Plastic Reagent and Standard Preparation Containers.
 - 6.5.3. All sample tubes must be non-glass (polystyrene or polypropylene).

7. REAGENTS AND STANDARDS

7.1. PREPARATION OF REAGENTS

Use deionized water (10 megohm) for all solutions.

Degassing with helium:

To prevent bubble formation, degas all solutions except the standards with helium. Use He at 140kPa (20 lb/in²) through a helium degassing tube (LACHAT Part No. 50100.) Bubble He through the solution for one minute.

Reagent 1. Ammonium Molybdate Solution

By Volume: In a **500 mL** plastic volumetric flask completely dissolve **20.0 g ammonium molybdate tetrahydrate** [(NH₄)₆Mo₇O₂₄ · 4H₂O] **FISHER Cat# A674-500** in approximately **400 mL water**. Ensure that the molybdate is dissolved before adding the acid. Then add **8.0 mL concentrated sulfuric acid** (H₂SO₄). Dilute to the mark and invert three times. Store in plastic container and refrigerate. Prepare this reagent fresh monthly and discard if precipitate or blue color is observed.

By Weight: To a tared **500 mL** plastic container completely dissolve **20.0 g ammonium molybdate tetrahydrate** [(NH₄)₆Mo₇O₂₄ · 4H₂O] and **486 g water**. Ensure that the molybdate is dissolved before adding the acid. Then add **14.8 g concentrated sulfuric acid** (H₂SO₄). Stir or shake until completely dissolved. Store in plastic and refrigerate. Prepare this reagent fresh monthly and discard if precipitate or blue color is observed.

Reagent 2. Oxalic Acid Solution

By Volume: In a **500 mL** plastic volumetric flask, dissolve **50.0 g oxalic acid** (HO₂CCO₂H · 2H₂O) **FISHER Cat# A219-250** in approximately **450 mL water**. Dilute to the mark and invert three times. Store in plastic.

By Weight: To a tared **500 mL** plastic container add **50.0 g oxalic acid**

($\text{HO}_2\text{CCO}_2\text{H} \cdot 2\text{H}_2\text{O}$) and **490 g water**. Stir or shake until dissolved. Store in plastic.

Reagent 3. Ascorbic Acid Reducing Solution

By Volume: In a **500 mL** plastic volumetric flask add about **450 mL water**, then dissolve 20 g **granular ascorbic acid FISHER Cat# A62-500**. Dilute to the mark and invert three times. Store in plastic and prepare fresh weekly. Discard if the solution becomes yellow.

By Weight: To a tared **500 mL** plastic container add 20 g **granular ascorbic acid** and **500 g water**. Store in plastic and prepare fresh weekly. Discard if the solution becomes yellow.

Reagent 4.

Carrier DI Water

Note: For the most accurate results prepare all fresh reagents daily.

7.2. PREPARATION OF STANDARDS

To prepare working standards from **Certified QC ERA Silica 1000 mg/L stock standard Cat# 064** follow the instructions below.

By volume: In 100 ml plastic volumetric flask add following amount of QC ERA 1000 mg/L stock standard. Dilute to the mark with DI water and invert to mix. Prepare fresh working standard daily.

Concentration (mg/L)	ml of 1000 mg/L Stock standard
10 mg/L	1.0 ml
5 mg/L	0.5 ml
2 mg/L	0.2 ml
1 mg/L	0.1 ml
0.5 mg/L	0.05 ml

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

- 8.1. Since there is no single preservation method that may be recommended for all types of samples the analyst must examine each situation critically.
- 8.2. Samples should be collected in screw cap polyethylene bottles (ca. 250 mL). Samples may be frozen at -20°C but some loss of silicate may occur at high concentrations (e.g. deep samples). In high diatom blooms, some regeneration of soluble silicate can occur if plankton is not removed by filtration. Ideally, analysis should be commenced within 24 hours of sample collection.
- 8.3. Frozen samples should be thawed by immersion in warm water, with occasional mixing to ensure uniform sample temperature. Do not warm samples above ambient temperature. Since the analyte is in the liquid portion of the thawing sample, care should be taken to ensure complete thawing. Samples should be at room temperature when they are run.

9. QUALITY CONTROL

9.1. Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.1.1. Analyses of matrix spike and matrix spike duplicate samples are required to demonstrate method accuracy and precision and to monitor matrix interferences (interferences caused by the sample matrix). The procedure and QC criteria for spiking are described in section 9.3.

9.1.2. Analyses of laboratory blanks are required to demonstrate freedom from contamination.

9.1.3. The laboratory shall, on an ongoing basis, demonstrate through calibration verification and analysis of the ongoing precision and recovery sample that the analysis system is in control.

9.1.4. The laboratory should maintain records to define the quality of data that is generated.

9.2. INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1. Method Detection Limit (MDL) --To establish the ability to detect the analyte, the analyst shall determine the MDL per the procedure in 40 CFR 136, Appendix B using the apparatus, reagents, and standards, that will be used in the practice of this method. An MDL less than or equal to the MDL in section 1.2 must be achieved prior to the practice of this method.

9.2.2. Initial Precision and Recovery -- To establish the ability to generate acceptable precision results, the operator shall perform 10 replicates of a mid-range standard, according to the procedure beginning in Section 11.

9.2.2.1. Using the results of the replicates compute the average percent recovery (X) and the standard deviation (s) for the analyte. Use the following equation for the calculation of the standard deviation.

$$s = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}}$$

Where, n = Number of samples, x = concentration in each sample

9.2.2.2. Compare s and x results with the corresponding data in Section 17. If the results meet the acceptance criteria, system performance is acceptable and analysis of samples may begin. If however, s and x do not match the data in Section 17, system performance is unacceptable. In this event correct the problem, and repeat the test.

- 9.3. Matrix spikes- The laboratory must spike, in duplicate, a minimum of 10 percent of all samples (one sample in each batch of ten samples) from a given sampling site or if for compliance monitoring, from a given discharge. The two sample aliquots shall be spiked with the stock standard (section 7.2).
- 9.3.1. The concentration of the spike in the sample shall be determined as follows:
- 9.3.1.1. If, as in compliance monitoring, the concentration of the analyte in the sample is being checked against a regulatory concentration limit, the spiking level shall be at that limit or at 1 to 5 times higher than the background concentration of the sample (determined in Section 9.3.2), whichever is higher.
 - 9.3.1.2. If the concentration of the analyte in a sample is not being checked against a limit, the spike shall be at the concentration of the precision and recovery standard used in Section 9.2.5 or at 1 to 5 times higher than the background concentration, whichever concentration is higher.
- 9.3.2. Analyze one sample aliquot out of each set of ten samples from each site or discharge according to the procedure beginning in Section 11 to determine the background concentration of (B) of the analyte.
- 9.3.2.1. If necessary, prepare a standard solution appropriate to produce a level in the sample at the regulatory compliance limit or at 1 to 5 times the background concentration (per Section 9.3.1).
 - 9.3.2.2. Spike two additional sample aliquots with the spiking solution and analyze these aliquots to determine the concentration after spiking (A)
- 9.3.3. Calculate the percent recovery (P) of the analyte in each aliquot using the following equation.

$$P = \frac{(A - B)100}{T}$$

Where, A = Measured concentration of analyte after spiking, B = measured background concentration of analyte, T = True concentration of the spike

- 9.3.4. The percent recovery of the analyte should meet current laboratory acceptance criteria.
- 9.3.4.1. If the results of the spike fail the acceptance criteria, and the recovery of the QC standard in the ongoing precision and recovery test of the analytical batch is within the current laboratory acceptance criteria, an interference is present. In this case, the results may not be reported for regulatory compliance purposes and the analyst must assess the potential cause for the interference. If the interference is attributable to sampling, the site or discharge should be resampled. If the interference is attributable to a method deficiency, the analyst must modify the method, repeat the test required in Section 9.1.2 and repeat the analysis of the sample and the matrix spike.
 - 9.3.4.2. If the results of both the spike and ongoing precision and recovery test fail the acceptance criteria, the analytical system is judged to be out of

control, and the problem shall be identified and corrected, and the sample reanalyzed.

- 9.3.5. Compute the relative percent difference (RPD) between two sample results using the following equation:

$$RPD = \frac{(D_1 - D_2)}{(D_1 + D_2) / 2} \times 100$$

Where, D1 = Concentration of analyte in the sample, D2 = Concentration of analyte in the second (duplicate) sample.

- 9.3.6. The RPD for duplicates shall meet the current laboratory acceptance criteria. If

the criteria are not met, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected and the analytical batch reanalyzed.

- 9.4 Laboratory blanks - Laboratory reagent water blanks are analyzed to demonstrate freedom from contamination.
 - 9.4.1. Analyze a laboratory reagent water blank initially (with the test in Section 9.2) and with each analytical batch. The blank must be subjected to the same procedural steps as a sample.
 - 9.4.2. If analyte is detected in the blank at a concentration greater than the Minimum Level (Section 1.6), analysis of the samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination. All samples must be associated with an uncontaminated method blank before the results may be reported for regulatory compliance purposes.
- 9.5. Calibration Verification Verify calibration using the procedure described in Section 10
- 9.6. On-going Precision and Recovery (OPR) - With every analytical batch, a midrange standard must be prepared using the procedure described in Section 11.
 - 9.6.1. Compare the results with the current laboratory acceptance criteria. If the criteria are not met, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected and the analytical batch reanalyzed.
- 9.7. Quality Control Samples (QCS) It is suggested that the laboratory obtain and/or prepare a quality control sample using a source different from the source routinely used in section 7. The QCS is used to verify the concentrations of the calibration standards.
- 9.8. Depending on the specific program requirements, field replicates and field spikes of the analytes of interest into samples may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

10.CALIBRATION PROCEDURE

- 10.1. Prepare reagents and standards as described in Section 7.
- 10.2. Set up manifold as shown in Section 17.
- 10.3. Input data system parameters as shown in Section 17.
- 10.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.
- 10.5. Place standards in the sampler. Input the information required by the data system.
- 10.6. Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the peak area for each standard to determine the calibration curve.
- 10.7. Verify calibration using a midrange calibration standard every ten samples or every analytical batch. Compute the percent recovery using the following equation:

$$\%recovery = \frac{D}{K} \times 100$$

Where, D = Determined concentration of analyte in the calibration standard, K = Actual concentration of the analyte in the calibration standard

- 10.8. If % recovery exceeds $100 \pm 10\%$, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected and the analytical batch reanalyzed

11. PROCEDURE

11.1. SAMPLE PREPARATION PROCEDURE

- 11.1.1. Filter samples through a 0.45 μm membrane filter before placing in the sample cups.
- 11.1.2. If molybdate unreactive silica is present and its inclusion in the determination is desired, digest the samples as follows:
- 11.1.2.1. Place 50 mL, or a smaller portion diluted to 50 mL, of filtered sample in a 100 mL platinum dish.
- 11.1.2.2. Add 200 mg silica-free NaHCO_3 and digest on a steam bath for 1 hour. Cool.
- 11.1.2.3. Add slowly and with stirring 2.4 mL 0.5 M H_2SO_4 .
- 11.1.2.4. Immediately transfer to a 50 mL Nessler tube, dilute to the mark with distilled water and proceed with the determination without delay.

11.2. SYSTEM NOTES

- 11.2.1. For information on system maintenance and troubleshooting refer to the Troubleshooting Guide in the System Operation Manual. This guide is also available on request from LACHAT.
- 11.2.2. Allow 15 minutes for heater module to warm up to 37°C.
- 11.2.3. Reagent recipes from other automated wet chemistry analyzers should not be substituted.
- 11.2.4. It is important to avoid using glassware. This means that the preparation of standards and deionized water used to prepare the standards, carrier and reagents should be done in plastic containers. Plastic autosampler cups must be used for both standards and samples.
- 11.2.5. To avoid contamination problems it is recommended that the helium degassing be dedicated to this method and stored wrapped in plastic.
- 11.2.6. The blank in this method should not give a peak. If the blank peak is negative, the carrier is contaminated. If the blank peak is positive, the blank is contaminated.
- 11.2.7. If the detection limit is greater than that specified in the method the following outline should be followed:
- 11.2.7.1. Verify standards preparation procedures.
- 11.2.7.2. Verify that a 660 nm filter is being used.

- 11.2.7.3. Verify that the sample loop is completely filled by running dye.
- 11.2.7.4. Verify that the heater is set to 37°C.
- 11.2.7.5. Prepare fresh reagents. Take extra care to be sure that all the reagents are completely dissolved.
- 11.2.7.6. Verify that reagents are flowing smoothly. If not, check for obstruction in the manifold and replace worn pump tubing.
- 11.2.8. For low level analysis it is recommended that samples be analyzed in duplicate from each sample cup. This is done by entering Replicates = 2 when entering sample information.

12. DATA ANALYSIS AND CALCULATIONS

- 12.1. Calibration is done by injecting standards. The data system will then prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation.
- 12.2. Report only those values that fall between the lowest and highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.
- 12.3. Report sample results for silica in mg SiO₂/L to two significant figures for samples above the MDL. Report results below the Reporting Limit as less than the reporting limit.
- 12.4. Report results in µM SiO₂ or µg SiO₂/L.

13. METHOD PERFORMANCE

- 13.1. The method support data are presented in section 17. This data was generated according to a LACHAT Work Instruction during development of the method.
- 13.2. Although LACHAT Instrument publishes method performance data, including MDL, precision, accuracy and carryover studies, we cannot guarantee that each laboratory will be capable of meeting such performance. Individual laboratory and instrument conditions, as well as laboratory technique, play a major role in determining method performance. The support data serves as a guide of the potential method performance. Some labs may not be able to reach this level of performance for various reasons, while other labs may exceed it.

14. POLLUTION PREVENTION

- 14.1. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The USEPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the United States Environmental Agency (USEPA) recommends recycling as the next best option.
- 14.2. The quantity of chemicals purchased should be based on expected usage during their

shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

- 14.3. For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waste Reduction," available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N. W., Washington D. C. 20036, (202) 872-4477.

15. WASTE MANAGEMENT

- 15.1. It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water and land by minimizing and controlling all releases from fume hoods and bench operation. Compliance with all sewage discharge permits and regulations is also required.
- 15.2. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N. W., Washington D.C. 20036, (202) 872-4477.

16. REFERENCES

- 16.1. Yamane, T. Saito, M., "Simple Approach for Elimination of Blank Peak Effects in Flow Injection Analysis of Samples Containing Trace Analyte and Excess of Another Solute", Talanta, 39, No. 3, (1992) 215-219.
- 16.2. Parsons, T.R., Maita, Y. Lalli, C.M., "A Manual of Chemical and Biological Methods for Seawater Analysis", 1.7, Determination of Silicate, 25-27.
- 16.3. Grasshoff, K. "Methods of Seawater Analysis", Verlag Chemie, Second Edition, 1976.
- 16.4. U.S. Environmental Protection Agency, Methods for the Determination of Dissolved Silicate in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis, September 1997, Method 366.0

APPENDIX D

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CHESAPEAKE BIOLOGICAL LABORATORY
NUTRIENT ANALYTICAL SERVICES LABORATORY
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**Standard Operating Procedure for Determination of Carbon and Nitrogen in
Particulates and Sediments of Fresh/Estuarine/Coastal Waters, Plant and Animal
Tissue, and Soils Using Elemental Analysis.**

(Reference Method: EPA 440.0)

Document #: NASLDoc-033

**Revision 2018-1
Replaces Revision 2017-4
Effective May 1, 2018**

**I attest that I have reviewed this standard operating procedure and agree to comply
with all procedures outlined within this document.**

_____ Employee (Print)	_____ Employee (Signature)	_____ Date
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Revised by: _____ Date: _____

Reviewed by: _____ Date: _____

Laboratory Supervisor: _____ Date: _____

Revisions 2018

Section 1.2: Changed the PN MDL

Section 1.3: Added quantitation limits for sediment samples and changed the quantitation limit for PN.

Section 3.10.2: Added K-factor spread for nitrogen.

Section 11.2.2: Changed sample tray set-up in Table 4 to reflect current operating procedures.

Updated Table 5 to include a newer version of the PC/PN preparation bench sheet.

1. SCOPE and APPLICATION

- 1.1. Elemental analysis is used to determine particulate carbon (PC), and particulate nitrogen (PN) in fresh, estuarine and coastal waters and sediments as well as for plant and animal tissue and soils. The method measures the PC and PN irrespective of source (organic or inorganic.)
- 1.2. A Method Detection Limit (MDL) of 0.0633 mg C/l and 0.0263 mg N/l, for filtered samples, and 0.130 %C and 0.008% N for sediment samples, were determined using the Student's *t* value (3.143) times the standard deviation of seven replicates. If more than seven replicates are used to determine the MDL, refer to the Student's *t* test table for the appropriate n-1 value.
- 1.3. The quantitation limit was set at 0.1899 mg C /L and 0.0789 mg N/l for filtered samples, and 0.39 %C and 0.024% N for sediment samples. These values are three times the method detection limit set for each parameter.
- 1.4. This procedure should be used by analysts experienced in the theory and application of elemental analysis. A minimum of 3 months experience with an elemental analyzer is recommended.
- 1.5. This method is for use by all programs that require analysis of particulate carbon and nitrogen in water and sediment, soils and tissues. The need to determine the organic fraction of the total particulate carbon and nitrogen in samples depends on the data-quality objectives of the study. Section 11.2.5 outlines the procedure used to ascertain the organic fraction.

2. SUMMARY

- 2.1. In the Exeter Analytical, Inc. Model CE-440 Elemental Analyzer, the carbon and nitrogen content in organic and inorganic compounds can be determined. Combustion of the sample occurs in pure oxygen under static conditions. The combustion train and analytical system are shown below in the CE-440 flow diagram. Helium is used to carry the combustion products through the analytical system to atmosphere, as well as for purging the instrument. Helium was selected for this purpose because it is chemically inert relative to tube packing chemicals, and it has a very high coefficient of thermal conductivity. The products of combustion are passed over suitable reagents in the combustion tube to assure complete oxidation and removal of undesirable by-products such as sulfur, phosphorus and halogen gases. In the reduction tube, oxides of nitrogen are converted to molecular nitrogen and residual oxygen is removed. In the mixing volume the sample gasses are thoroughly homogenized at precise volume, temperature, and pressure. This mixture is released through the sample volume into the thermal conductivity detector. Between the first of three pairs of thermal conductivity cells an absorption trap removes water from the sample gas. The differential signal read before and after the trap reflects the water concentration and, therefore, the amount of hydrogen in the original sample. A similar measurement is made of the signal output of a second pair of thermal conductivity cells, between which a trap removes carbon dioxide, thus determining the carbon content. The remaining gas now consists only of helium and nitrogen. This gas

passes through a thermal conductivity cell and the output signal is compared to a reference cell through which pure helium flows. This gives the nitrogen concentration.

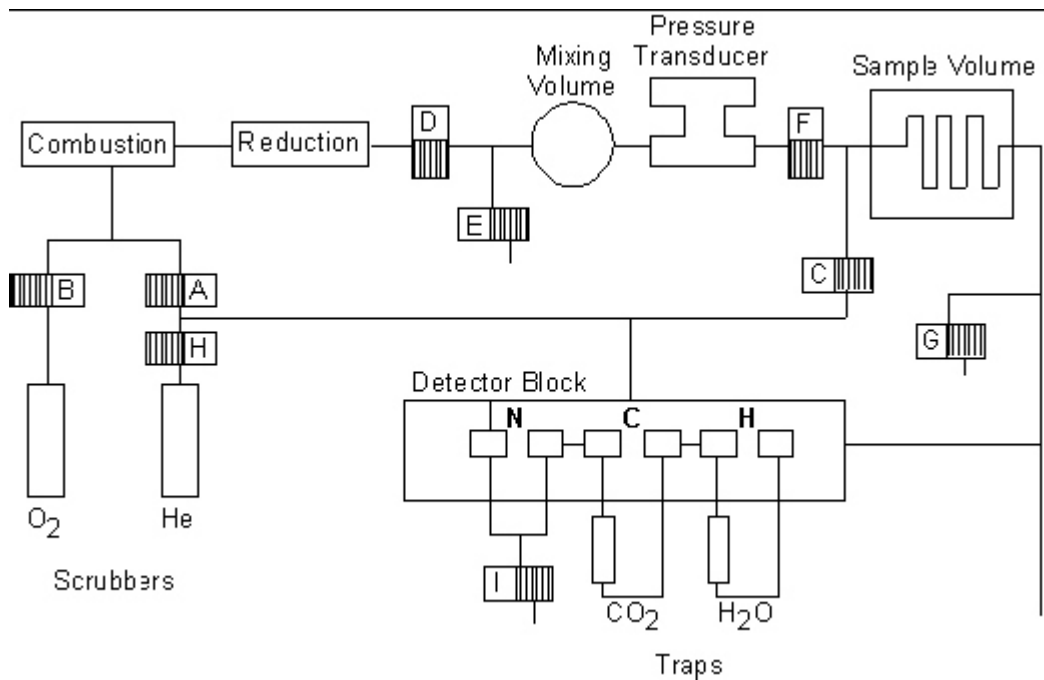


FIGURE 1. Schematic diagram of the Exeter Analytical, Inc. CE-440 Elemental Analyzer

3. DEFINITIONS

- 3.1. **Acceptance Criteria** - Specified limits placed on characteristics of an item, process, or service defined in a requirement document. (ASQC)
- 3.2. **Accuracy** - The degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations; a data quality indicator. (QAMS)
- 3.3. **Aliquot** - A discrete, measured, representative portion of a sample taken for analysis. (EPA QAD Glossary)
- 3.4. **Batch** - Environmental samples, which are prepared and/or analyzed together as a group using the same calibration curve or factor with the same process and personnel using the same lot(s) of reagents. An analytical batch is composed of approximately 50 environmental samples meeting the above mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 6-10 hours. An analytical batch defined by NELAC can

- include samples originating from various environmental matrices and can exceed 20 samples. (NELAC/EPA)
- 3.5. **Blank** - A sample that has not been exposed to the analyzed sample stream in order to monitor contamination during sampling, transport, storage or analysis. The blank is subjected to the usual analytical and measurement process to establish a zero baseline or background value and is sometimes used to adjust or correct routine analytical results. (ASQC)
- 3.5.1. **Blank value** = blank read minus blank zero. An indicator of the stability of the system. (Exeter)
- 3.6. **Bridge** - Electrical configuration of the thermal conductivity filaments.(Exeter)
- 3.7. **Calibrate** - To determine, by measurement or comparison with a standard, the correct value of each scale reading on a meter or other device, or the correct value for each setting of a control knob. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)
- 3.8. **Calibration** - The set of operations which establish, under specified conditions, the relationship between values indicated by a measuring device. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)
- 3.9. **Calibration Method** - A defined technical procedure for performing a calibration. (NELAC)
- 3.10. **Calibration Standard** - A substance or reference material used to calibrate an instrument. (QAMS)
- 3.10.1. **Initial Calibration Standard (CAL)** - An accurately weighed amount of a certified chemical used to calibrate the instrument response with respect to analyte mass. For this procedure the calibration standard is acetanilide, 99.9%+ purity. It has known percentages of C, H, and N.
- 3.10.2. **Initial Calibration Verification (ICV)** - An individual standard, analyzed initially, prior to any sample analysis, which verifies acceptability of the calibration curve or previously established calibration curve. To verify that the instrument is running well, all the individual K-Factor values should be within +/- 0.08 of the mean for carbon and +/- 0.22 of the mean for nitrogen. This may be a substitute for the ICV.
- 3.10.3. **Continuing Calibration Verification (CCV)** - An individual standard which is analyzed after 25 samples and at the end of the analysis run cycle. This standard may be a certified reference material or internal standard such as Atropine.
- 3.11. **Capsule** - Aluminum container. Used for containing samples and standards with an accurate weight and maintains integrity prior to combustion.
- 3.12. **Certified Reference Material** - A reference material one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body. (ISO 17025)
- 3.13. **Combustion Time** - Time for sample to fully combust in an oxygen environment.
- 3.14. **Combustion Tube** - Quartz tube packed with reagents and used for sample combustion.

- 3.15. **Conditioner** - A standard chemical which is not necessarily accurately weighed that is used to coat the surfaces of the instrument with the analytes (water vapor, carbon dioxide, and nitrogen).
- 3.16. **Corrective Action** - Action taken to eliminate the causes of an existing nonconformity, defect or other undesirable situation in order to prevent recurrence. (ISO 8402)
- 3.17. **Deficiency** - An unauthorized deviation from acceptable procedures or practices. (ASQC)
- 3.18. **Demonstration of Capability** - A procedure to establish the ability of the analyst to generate acceptable accuracy. (NELAC)
- 3.19. **Detection Limit** - The lowest concentration or amount of the target analyte that can be determined to be different from zero by a single measurement at a stated degree of confidence.
- 3.20. **Detector** - Consists of three bridges and determines the percentages of carbon, hydrogen, and nitrogen in the sample via thermal conductivity.
- 3.21. **Detector Oven** - Keeps the temperature of the detector, pressure transducer, mixing volume, and sample volume constant.
- 3.22. **Double Drop** - Two samples are dropped for one run - used for filter and inorganic applications. Sample requires a + prefix.
- 3.23. **External Standard (ES)** - A pure analyte (atropine) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard is used to calibrate the instrument response for the corresponding analyte(s). The instrument response is used to calculate the concentrations of the analyte(s) in the unknown sample.
- 3.24. **Field Duplicates (FD1 and FD2)** - Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.
- 3.25. **Fill Time** - Time required to build-up the pressure in the mixing volume to 1500 mm Hg.
- 3.26. **Filtered Sample** - An accurately measured amount of water from fresh, estuarine or coastal samples, concentrated on a filter pad by filtering through a 25 mm Whatman GF/F filter or equivalent, which has been pre-combusted at 500° C for 90 minutes.
- 3.27. **Furnace** - Heats the reduction and combustion tubes to operating temperature.
- 3.28. **Heated Line** - Connects the reduction tube outlet to the inlet of the mixing volume. Heated to prevent condensation of gases on tube walls.
- 3.29. **Holding Time** - The maximum time which samples may be held prior to analysis and still be considered valid. (40 CFR Part 136) The time elapsed from the time of sampling to the time of extraction or analysis, as appropriate.
- 3.30. **Inject Solenoid** - Solenoid used on the automated injection system to actuate the rotation of the sample wheel.
- 3.31. **Injection** - Moving the ladle containing a sleeve with the sample into the combustion furnace.

- 3.32. **Injector Box** - The box assembly that houses the sample wheel.
- 3.33. **Instrument Detection Limit (IDL)** - The minimum quantity of analyte or the concentration equivalent which gives an analyte signal equal to three times the standard deviation of the background signal at the selected wavelength, mass, retention time, absorbance line, etc.
- 3.34. **K-Factor** - Instrument sensitivity factor in microvolts per microgram, calibrated using a calibration standard.
- 3.35. **Laboratory Duplicates (LD1 and LD2)** - Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.
- 3.36. **Laboratory Reagent Blank (LRB)** - A matrix blank (i.e., a pre-combusted filter or sediment capsule) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.
- 3.37. **Laboratory Control Sample (LCS)** - A sample matrix, free from the analytes of interest, with verified known amounts of analytes from a source independent of the calibration standards or a material containing known and verified amounts of analytes. The LCS is generally used to establish intra-laboratory or analyst-specific precision and bias or to assess the performance of all or a portion of the measurement system. (NELAC)
- 3.38. **Ladle** - Transports the capsule with the sample into a combustion furnace
- 3.39. **Limit of Detection (LOD)** - The lowest concentration level that can be determined by a single analysis and with a defined level of confidence to be statistically different from a blank. This is also referred to as the MDL. (ACS)
- 3.40. **Limit of Quantitation (LOQ)** - The minimum levels, concentrations, or quantities of a target variable (target analyte) that can be reported with a specified degree of confidence. The LOQ is set at 3 to 10 times the LOD, depending on the degree of confidence desired. This is also referred to as the Quantitation Limit.
- 3.41. **Linear Dynamic Range (LDR)** - The absolute quantity over which the instrument response to an analyte is linear. This specification is also referred to as the Linear Calibration Range (LCR).
- 3.42. **Material Safety Data Sheet (MSDS)** - Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.
- 3.43. **May** - Denotes permitted action, but not required action. (NELAC)
- 3.44. **Method Detection Limit (MDL)** - The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.
- 3.45. **Mixing Volume** - Spherical bottle in which sample gases become homogenous.
- 3.46. **Mother Board** - The main printed circuit board. All CE-440 power supplies are located here.
- 3.47. **Must** - Denotes a requirement that must be met. (Random House College Dictionary)

- 3.48. **Precision** - The degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves; a data quality indicator. Precision is usually expressed as standard deviation, variance or range, in either absolute or relative terms. (NELAC)
- 3.49. **Preservation** – Refrigeration, freezing and/or reagents added at the time of sample collection (or later) to maintain the chemical and or biological integrity of the sample.
- 3.50. **Pressure Transducer** - Used to check for leaks in the system and to monitor pressure in the mixing volume.
- 3.51. **P Valve** - The valve on the injector box of the horizontal auto-injector (HA) used to automatically purge the box.
- 3.52. **Profile** - Generated by the bridge signal. Used to help determine if a leak or malfunction occurs in the system.
- 3.53. **Quality Control Sample (QCS)** - A sample of analytes of known and certified concentrations. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials. Also referred to as CRM.
- 3.54. **Reduction Tube** - Quartz tube with reduced copper that removes excess oxygen from the sample gas and reduces oxides of nitrogen to free nitrogen.
- 3.55. **Response Factor (RF)** - The ratio of the response of the instrument to a known amount of analyte.
- 3.56. **Run** - One full sample analysis from start to finish, including printout.
- 3.57. **Run Cycle** - Typically a day or half day of operation - the entire analytical sequence of runs from the first run to the last run on the Sample Wheel.
- 3.58. **Sample Volume** - Tube where sample gas is exhausted from the mixing volume prior to entering the detector.
- 3.59. **Sample Wheel** – Sample holding device which contains up to 64 blanks, standards and samples. One wheel equals roughly 6 hours of run time, which is called the Run Cycle.
- 3.60. **Scrubber** - Removes water and carbon dioxide from the gas supplies.
- 3.61. **Sediment (or Soil) Sample** - A fluvial, sand, or humic sample matrix exposed to a marine, estuarine or fresh water environment.
- 3.62. **Sensitivity** - The capability of a test method or instrument to discriminate between measurement responses representing different levels (concentrations) of a variable of interest.
- 3.63. **Shall** - Denotes a requirement that is mandatory whenever the criterion for conformance with the specification requires that there be no deviation. (ANSI)
- 3.64. **Should** - Denotes a guideline or recommendation whenever noncompliance with the specification is permissible. (ANSI)
- 3.65. **Sleeve** - Nickel - to maintain integrity of the sample capsule and to protect the quartz ware from devitrification (to destroy the glassy qualities by prolonged heating).
- 3.66. **Standard Reference Material (SRM)** - Material which has been certified for specific analytes by a variety of analytical techniques and/or by numerous laboratories using similar analytical techniques. These may consist of pure chemicals, buffers, or compositional standards. The materials are used as an

indication of the accuracy of a specific analytical technique. Also referred to as CRM.

- 3.67. **Trap** - Used for removing water and CO₂ from the sample gas.
- 3.68. **Tissue sample** – Plant or animal tissue dried and ground ready for weighing.
- 3.69. **Zero Value** - Bridge signal with only pure helium flowing through the detector.

4. INTERFERENCES







- 4.1. There are no known interferences for fresh, estuarine or coastal water or sediment samples. The presence of C and N compounds on laboratory surfaces, on fingers, in detergents and in dust necessitates the utilization of careful techniques (i.e., the use of forceps and gloves) to avoid contamination in every portion of this procedure (EPA.)

5. SAFETY

- 5.1. Safety precautions must be taken when handling reagents, samples and equipment in the laboratory. Protective clothing including lab coats and safety glasses and enclosed shoes must always be worn. In certain situations it may also be necessary to use gloves and goggles. If solutions or chemicals come in contact with eyes, flush with water continuously for 15 minutes. If solutions or chemicals come in contact with skin, wash thoroughly with soap and water. Contact Solomons Rescue Squad (911) if emergency treatment is needed. Inform the CBL Associate Director of Facilities and Maintenance of the incident if additional treatment is required.
- 5.2. The toxicity or carcinogenicity of each reagent used in this procedure may not have been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials and procedures.
- 5.3. High current and voltages are exposed near the furnaces, furnace control card, and mother board even while the CE-440 is OFF. If non-electrical trouble shooting is desired, remove the CE-440 line cord from the wall receptacle.
- 5.4. The combustion tube is brittle since it is fused quartz. Do not put any unnecessary stress on it.
- 5.5. The exterior of the furnace becomes extremely hot; do not touch it or the heat shield unless wearing appropriate gloves.
- 5.6. Do not wear any jewelry if electrically troubleshooting. Even the low voltage points are dangerous and can injure if allowed to short circuit.
- 5.7. The following hazard classifications are listed for the chemicals regularly used in this procedure.

TABLE 1.

Chemical	Health Hazard	Fire Hazard	Instability Hazard	Specific Hazard	
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Acetanilide	1	1	0		
Atropine	4	0	0		
Magnesium Perchlorate	2	0	2	OX	 
Ascarite (Sodium Hydroxide)	3	0	0	ALK, COR	
Silver vanadate on Chromosorb	3	0	0		
Silver oxide/Silver tungstate on Chromosorb	1	0	0		
Silver tungstate/Magnesium	0	0	0		
Copper wire	0	0	0		
<p>On a scale of 0 to 4 the substance is rated on four hazard categories: health, flammability, reactivity, and contact. (0 is non-hazardous and 4 is extremely hazardous)</p> <p><u>HAZARD RATING</u></p> <p>Health Hazard - Blue: 4 – deadly, 3 – extreme danger, 2 – hazardous, 1 – slightly hazardous, 0 – normal material</p> <p>Fire Hazard - Red: Flash Points: 4 – below 73° F, 3 – below 100° F, 2 – below 200° F, 1 – above 200° F, 0 – will not burn</p> <p>Instability Hazard - Yellow: 4 – may detonate, 3 – Shock and heat may detonate, 2 – violent chemical change, 1 – unstable if heated, 0 – stable</p>					

6. EQUIPMENT AND SUPPLIES

- 6.1. An elemental analyzer capable of maintaining a combustion temperature of 980°C and analyzing particulate and sediment samples for elemental carbon and nitrogen. The Exeter Model CE-440 is used in this laboratory.
- 6.2. A gravity convection drying oven, capable of maintaining 47°C ± 2°C for extended periods of time.
- 6.3. Muffle furnace, capable of maintaining 900°C +/- 15°C.
- 6.4. Ultra-micro balance that is capable of accurately weighing to 0.1 ug.
- 6.5. Vacuum pump or source capable of maintaining up to 10 in. Hg of vacuum.
- 6.6. Freezer, capable of maintaining -20°C ± 5°C.
- 6.7. 25-mm vacuum filter apparatus made up of a glass or plastic filter tower, fritted glass or plastic disk base and 2-L vacuum flask.
- 6.8. Flat blade forceps.

- 6.9. Labware - All reusable labware (glass, quartz, polyethylene, PTFE, FEP, etc.) must be sufficiently clean for the task objectives. Clean glassware by rinsing with reagent water; soaking for 4 hours or more in 10% (v/v) HCl and then rinsing with reagent water. Store clean. All traces of organic material must be removed to prevent carbon and nitrogen contamination.

7. REAGENTS AND STANDARDS

- 7.1. **Purity of Water** – Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to ASTM Specification D 1193, Type I.
- 7.2. **Purity of Reagents** – Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.
- 7.3. **Acetanilide, 99.9% + purity, C₈H₉NO (CASRN 103-84-4)** - ACS grade acetanilide; primary standard used to calibrate the instrument
- 7.4. **Blanks** – Three blanks are used for the analysis. Two blanks are instrument related. The *instrument zero* response (ZN) is the background response of the instrument without sample holding devices such as capsules and sleeves. The *instrument blank* response (BN) is the response of the instrument when the sample capsule, sleeve and ladle are inserted for analysis without standard or sample. The is also the laboratory reagent blank (LRB) for standards and sediment or other weighed samples. The LRB for water samples includes the sleeve, ladle and a pre-combusted filter without standard or sample. These blanks are subtracted from the uncorrected instrument response used to calculate concentration. The third blank is the *laboratory fortified blank* (LFB.) For all sample analysis, a weighed amount of atropine or other certified standard is placed in an aluminum capsule and analyzed. The LFB may need to be recalculated with the correct instrument blank; this is dependent on the type of samples being analyzed and the original instrument blank type.
- 7.5. **Quality Control Sample (QCS)** – For this procedure, the QCS can be any assayed and certified sediment or particulate sample which is obtained from an external source. PACS-2 from the National Research Council of Canada is used by this laboratory. The laboratory fortified blank may also be considered a QCS.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

- 8.1. **Water Sample Collection** – Samples collected for PNC analysis from fresh, estuarine and coastal waters are normally collected from a boat or pier using one of two methods; hydrocast or submersible pump systems. Follow the recommended sampling protocols associated with the method used. Whenever

possible, immediately filter the samples as described in Section 11.1.1. Store the filtered sample in a labeled aluminum foil pouch and freeze at -20°C or store in a low temperature (47°C) drying oven after drying at 47°C ± 2°C, until use. If storage of the unfiltered water sample is necessary, place the sample into a clean bottle and store at 4°C until filtration is performed. Filter samples within 24 hours. Dry samples in a low temperature (47°C+/-2°C) drying oven prior to analysis.

- 8.2. The volume of water sample collected will vary with the type of sample being analyzed. Table 2 below provides a guide for a number of matrices of interest. If the matrix cannot be classified by this guide, collect 1 L of water from each site.
- 8.3. Sediment, Tissue, or Soil Sample Collection – Sediment samples are collected with benthic samplers. The type of sampler used will depend on the type of sample needed by the data-quality objectives. Tissue and soil samples are collected by a variety of methods. Store the wet sample in a clean labeled jar and freeze at -20°C until ready for analysis. Dry samples in a low temperature (47°C+/-2°C) drying oven, and grind to a homogenous powder with a mortar and pestle, prior to analysis.
 - 8.3.1. The amount of solid material collected will depend on the sample matrix. A minimum of 1 g is recommended.
 - 8.3.2. Filtration Volume Selection Guide

TABLE 2.

Sample Matrix	
	25mm Filter
Open Ocean	500 – 1000 ml
Coastal	400 – 500 ml
Estuarine (Low particulate)	250 – 400 ml
Estuarine (High Particulate)	25 – 200 ml

9. QUALITY CONTROL

- 9.1. The laboratory is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the continued analysis of laboratory instrument blanks, field duplicates, and standards analyzed as samples as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data generated.

9.2. Initial Demonstration of Capability

- 9.2.1. **The initial demonstration of capability (DOC)** – is used to characterize instrument performance (MDLs) and laboratory performance (analysis of QC samples) prior to the analyses conducted by this procedure.
- 9.2.2. **Quality Control Sample (QCS/SRM)** – When using this procedure, a quality control sample is required to be analyzed at the middle and end of the run (along with CCV standards) to verify data quality and acceptable instrument performance. If the determined concentrations are not within $\pm 3\sigma$ of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before proceeding with the initial determination of MDLs. It is possible the QCS/SRM may fall above or below control limits but still passes within $\pm 10\%$ of the certified values. This is still within acceptance limits.
- 9.2.3. **Method Detection Limits (MDLs)** – MDLs should be established for aqueous particulate carbon and nitrogen using a low level natural water sample. The same procedure should be followed for sediments or other weighed samples. To determine the MDL values, analyze seven replicates and process through the entire analytical procedure. Calculate the MDL as follows:

$$\text{MDL} = St_{(n-1, 1-\alpha=0.99)}$$

Where,

S = Standard deviation of the replicate analyses.

n=number of replicates

$t_{(n-1, 1-\alpha=0.99)}$ = Student's *t* value for the 99% confidence level with n-1 degrees of freedom ($t=3.14$ for 7 replicates.)

- 9.2.4. MDLs should be determined annually, whenever there is a significant change in instrumental response or a new matrix is encountered.

9.3. Assessing Laboratory Performance

- 9.3.1. **Laboratory Reagent Blank (LRB)** – The laboratory must analyze at least one LRB (Section 3.37) with each batch of samples. For sediment samples the LRB consists of the ladle, sample sleeve and sample capsule, as there are no reagents involved in this procedure. For aqueous samples, the LRB consists of the ladle, sample sleeve and a pre-combusted filter of the same type and size used for samples. LRB data are used to assess contamination from the laboratory environment. For sediment samples, the blank value for carbon should not exceed $150\mu\text{v}$ and the blank value for nitrogen should not exceed $15\mu\text{v}$. For aqueous samples, the blank value for carbon should not exceed $300\mu\text{v}$ and the blank value for nitrogen should not exceed $15\mu\text{v}$.

- 9.3.1.1. If the nitrogen blank during a BLANK analysis is in excess of 2000% the nitrogen blank in memory the “COPPER APPEARS SPENT” warning is printed. If the nitrogen blank increased over $100\mu\text{v}$ over BN in memory and the first STANDARD KC/KN is more than any following STANDARD KC/KN by $0.2\mu\text{v}/\mu\text{g}$, then a “COPPER APPEARS SPENT” warning will be printed either during a BLANK analysis or a STANDARD analysis. Maintenance will be required.

9.3.1.2. The Reagent Blank Control Chart is constructed from the average and standard deviation of Reagent Blank measurements recorded annually. This includes both filter pad blanks and capsule blanks. The accuracy chart includes upper and lower warning levels ($WL=\pm 2s$) and upper and lower control levels ($CL=\pm 3s$). The standard deviation (s) is specified relative to statistical confidence levels of 95% for WLs and 99% for CLs. Enter Reagent Blank results on the chart each time the Reagent Blank is analyzed.

9.3.2. **Quality Control Sample (QCS)/ Standard Reference Material (SRM)** - When using this procedure, a quality control sample is required to be analyzed at the middle and end of the run, to verify data quality and acceptable instrument performance. The QCS will be obtained from a source external to the laboratory and different from the source of calibration standards. If the determined concentrations are not within $\pm 3\sigma$ of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with the analyses. Corrective action documentation is required for all data outside $\pm 3\sigma$. The standard deviation data should be used to establish an on-going precision statement for the level of concentrations included in the QCS. This data must be kept on file and be available for review. Values for QCSs should be plotted with the other control data. The sample weight of the SRM should mirror that of the unknown samples (~10 mg).

9.3.2.1. The Accuracy Control Chart for QCS/SRM samples is constructed from the average and standard deviation of QCS/SRM measurements recorded annually. The accuracy chart includes upper and lower warning levels ($WL=\pm 2s$) and upper and lower control levels ($CL=\pm 3s$). These values are derived from stated values of the QCS/SRM. The standard deviation (s) is specified relative to statistical confidence levels of 95% for WLs and 99% for CLs. Set up an accuracy chart by using $\pm 10\%$ in addition to $\pm 3s$ since the concentration of the QCS/SRM varies. Enter QCS/SRM results on the chart each time the sample is analyzed.

9.4. **Assessing Analyte Recovery** - Percent recoveries cannot be readily obtained from particulate samples. Consequently, accuracy can only be assessed by analyzing check standards as samples (CCV, QCS/SRM).

9.5. Data Assessment and Acceptance Criteria for Quality Control Measures

TABLE 3.

QC INDICATOR	ACCEPTANCE LIMITS	ACTION	FREQUENCY (BATCH)
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K-factor	KC = 18 to 25 18 to 25 $\mu\text{v}/\mu\text{g}$ is manufacturer's recommended limits. KN = 7 to 10 $\mu\text{v}/\mu\text{g}$	The k-factors must be within the specified limits or the standard must be reanalyzed. (see 10.3)	3 per batch to acquire an acceptable K-factor calibration range.
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	7 to 10 $\mu\text{V}/\mu$ is manufacturer's		
Initial Calibration Verification (ICV)	$\pm 10\%$	Qualify data if not within acceptance limits. Rejection criteria for batch.	At the beginning of a run immediately following the calibration.
Quality Control Sample (QCS)/ Certified Reference Material (CRM)	$\pm 10\%$	If QCS is outside acceptance limits, qualify the data for all samples back to last acceptable QCS.	After every 25 samples.
Continuing Calibration Verification (CCV)	$\pm 10\%$	Qualify data if not within acceptance limits. Rejection criteria for batch. May be CRM or Atrazine standard.	After every 25 samples.
Method Blank/Laboratory Reagent Blank (LRB)	Filter Pad Blanks: BC < 300 μV BN < 15 μV Capsule Blanks: BC < 150 μV BN < 15 μV	If the blank value is greater the acceptable value, replace and rerun the blanks.	At the beginning and end of a run. LRB may be a filter pad or capsule blank.
Field Duplicate	$\pm 30\%$	Duplicate sample data must be within $\pm 30\%$ or be qualified. All duplicates for this procedure are field duplicates and are more a measure of field collection and filtration techniques.	

9.6. Corrective Actions for Out-Of-Control Data

- 9.6.1. All samples must be qualified when external QC samples are out of control.
- 9.6.2. All samples between QCSs that are out of control must be qualified.
- 9.6.3. All problems with analytical runs must be documented on the bench sheet.

9.7. General Operation

- 9.7.1. To assure optimal operation and analytical results, it is advisable to track the stability of the instrument. Of primary importance is the precision and repeatability of standard and blank values during the course of a day of operation. Thus, a standard (as an unknown) should be inserted approximately every twenty five samples. Try to use different standards for QA in order to assure the validity of the calibration values over the entire operating range of the instrument.

10. CALIBRATION, STANDARDIZATION and CALCULATIONS

- 10.1. Calibration - Daily calibration procedures must be performed and evaluated before sample analysis may begin. Single point calibration is used with the Exeter Model CE-440 Analyzer.
- 10.2. Establish single calibration factors (K) for each element (carbon, hydrogen, and nitrogen) by analyzing three weighed portions of calibration standard (acetanilide). The mass of the calibration standard should provide a response within 20% of the

response expected for the samples being analyzed. Calculate the (K) for each element using the following formula:

$$Kfactor (\mu v / \mu g) = \frac{RN - ZN - BN}{M (T)}$$

Where: RN = Instrument response to standard (μv)
ZN = Instrument zero response (μv)
BN = Instrument blank response (μv)
M = Mass of standard matter in μg
T = Theoretical % C, N, or H in the standard. For acetanilide %C = 71.09, %N = 10.36 and %H = 6.71.

- 10.3. The detector generates a signal directly proportional to the compound of interest in the sample. The following formula is used to calculate carbon, nitrogen and hydrogen concentrations in unknown samples.

$$\% = \frac{1}{K} \times \frac{1}{W} \times (R - Z - B) \times 100$$

Where

K = calibration factor for the 440 instrument
W = sample weight
R = read signal of sample gas
Z = zero reading or base line of instrument
B = blank signal generated by instrument itself, including ladle and capsules

- 10.4. The K-factor is established by running samples of a known standard. The default value is for acetanilide, which is used as the standard:

Acetanilide	C = 71.09%	H = 6.71%	N = 10.36%
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If another standard is used, the values will need to be entered into the computer using the Edit Standards function in the Customizing Menu.

- 10.4.1. Once the blank values have been established and entered into memory, proceed to run known standards to arrive at the calibration factors for carbon and nitrogen for the instrument.
- 10.4.2. Run a minimum of three standards, average the results, and enter into computer memory, or use the automatic enter mode. During the run, standards may be entered as samples to verify the K-factors and blanks.
- 10.4.3. Any time a STD1 is entered as sample ID the computer calculates and enters a new set of operating Ks based on a weighted formula using the last three sets of Ks in memory. This occurs only if all three Ks fall within the following windows:

New KC = KC in memory ± 1.0
KN = KN in memory ± 0.5

10.4.3.1. It is important that the Ks in memory be close to expected values or new Ks generated will not be within the window and therefore will not be accepted for automatic insertion.

10.4.3.2. The weighted formula for calculating the Ks:

$$K = k^1 + 0.5 \times k^2 + \frac{(0.25 \times k^3)}{1.75}$$

where:

k^1 = k found in this run

k^2 = Next k in memory

k^3 = Last k in memory

10.5. **Conditioner** - Before injecting any samples or blanks, it is necessary to run one or more conditioners. The purpose of the conditioner runs is to coat the walls of the system surfaces, especially the mixing and sample volume, with water vapor, carbon dioxide and nitrogen which simulates actual sample running conditions. To simulate this condition as closely as possible, it is advisable to use conditioners of approximately the same weight as the samples. Always inject a conditioner before a standard.

10.6. **Blanks** - The blank value used in the calculation is the total signal generated by the system including the ladle and sample capsule. This blank should always be analyzed immediately after a weighed conditioner to represent a true blank of the instrument. Never use the blank value from an empty wheel since the system dries up and the blank value would be lower than normal. The instrument program will only accept blanks if they fall within the following:

New BC < 500

New BN < 250

10.7. **K-Factors** - Once the blank values have been established and entered into memory, proceed to run known standards (acetanilide) in order to establish the calibration factors for carbon, hydrogen and nitrogen. The computer will calculate K-factors as long as STD# has been entered as the sample ID. Run a minimum of three (3) standards, average the results, and enter into the computer memory, or use the automatic enter mode. To verify that the instrument is running well, all the individual K-Factor values should be within +/- 0.08 of the mean. The instrument is now ready to analyze samples. Acetanilide standards may be analyzed as unknowns verify the K-factors and blank values; atropine and PACS-2 already serve as a calibration check and are analyzed throughout the run cycle.

11. PROCEDURE

11.1. Aqueous Sample Preparation

11.1.1. Water Sample Filtration

Pre-combust 25-mm GF/F glass fiber filters at 500°C for 1.5 hours. Store filters covered, if not immediately used. Place a pre-combusted filter on a fritted filter

base of the filtration apparatus and attach the filtration tower. Thoroughly shake the sample container to suspend the particulate matter. Measure and record the

required sample volume using a graduated cylinder. Pour the measured sample into the filtration tower. Filter the sample using a vacuum no greater than 10 in. of Hg. Vacuum levels greater than 10 in. of Hg can cause cell rupture. Do not rinse the filter following filtration. It has been demonstrated that sample loss occurs when the filter is rinsed with an isotonic solution or the filtrate. Air dry the filter after the sample has passed through by continuing the vacuum for 30 sec. Using flat-tipped forceps, fold the filters in half while still on the base of the filter apparatus. Store filters as described in Section 8.1.

- 11.1.2. If the sample has been stored frozen in foil pouches, place in a drying oven at $47^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 24 hours before analysis. Slightly open the pouch to allow drying. When ready to analyze, fold, and insert the filter into a pre-combusted nickel sleeve using forceps. Tap the filter pad down into the nickel sleeve using a clean stainless steel rod. The sample is ready for analysis.

11.2. Sample Analysis

- 11.2.1. As the filters are packed into the nickel sleeves they are placed into the 64 position sample wheel. The calibration series must be placed at the beginning of the batch. The sample schedule consists of a conditioner, a blank, a conditioner and three standards. ACS grade acetanilide 99.9% + purity must be used to calibrate the instrument.

- 11.2.2. Set up the sample tray in the following manner (used for aqueous samples):

TABLE 4.

Position #	Contents	Notes	Type	Weight, ug
1	Conditioner	Acetanilide (1500-2500 μg)	Conditioner	Weight of Acetanilide
2	Capsule + sleeve	Blank	Blank	0
3	Capsule + sleeve	Blank	Blank	0
4	Conditioner	Acetanilide (1500-2500 μg)	Conditioner	Weight of Acetanilide
5	Standard	Acetanilide (1500-2500 μg)	STD1 ^a	Weight of Acetanilide
6	Standard	Acetanilide (1500-2500 μg)	STD1	Weight of Acetanilide
7	Standard	Acetanilide (1500-2500 μg)	STD1	Weight of Acetanilide
8	Sleeve +capsule+ standard	Atropine (1500-2500ug)	LFB	Weight of Atropine
9	Sleeve + filter pad	Filter Blank	LRB	0
10-34	Samples			Volume filtered/10
35	Sleeve +capsule+ standard	PACS-2 (8000-15,000ug)	QCS/SRM	Weight of PACS-2
36-60	Samples			Volume filtered/10
61	Capsule + Sleeve	Blank	Blank	0
62	Sleeve + capsule+ standard	Atropine (1500-2500ug)	LFB	Weight of Atropine
63	Sleeve + capsule+ standard	PACS-2 (8000-15,000ug)	QCS/SRM	Weight of PACS-2
64	Capsule + Sleeve	Blank	Blank	0

^a Always use STD1 in the Standard position. The system recognizes this as acetanilide and makes the appropriate calculations for the K factor.

- 11.2.3. By entering volume filtered/10 for the weight of the aqueous filtered samples, results printed out represent micrograms of carbon or nitrogen per liter. This corresponds directly to the known amount of liquid that has passed through the filter. The maximum sample capacity per run is approximately 4,000 to 5,000 micrograms of carbon on the filter pad. Filters containing more than that amount may be cut in half and analyzed separately and the results added.
- 11.2.4. Filter Preparation for Analysis
 - 11.2.4.1. Work on a clean, non-contaminating surface.
 - 11.2.4.2. Using two pairs of clean forceps, fold the filter in half so that the exposed surface is inside. Continue folding the filter in half until you have a compact package.
 - 11.2.4.3. Place a pre-combusted 7 x 5 mm nickel sleeve into the filter loading die, which functions as a holding device. Use the clean 4 mm loading rod to force the compressed filter through the clean loading funnel and into the nickel sleeve.
 - 11.2.4.4. Make sure no excess filter protrudes above the lip of the sleeve.
 - 11.2.4.5. Place loaded sleeve in the 64-sample wheel.
- 11.2.5. Determination of Particulate Organic and Inorganic Carbon
 - 11.2.5.1. Thermal Partitioning is the method used to partition organic and inorganic carbon. The difference found between replicate samples, one of which has been analyzed for total PC and PN and the other of which was muffled at 550°C for three hours to drive off organic compounds, and then analyzed for PC and PN, is the particulate organic component of that sample. This method of thermally partitioning organic and inorganic PC may underestimate slightly the carbonate minerals' contribution in the inorganic fraction since some carbonate minerals decompose below 500°C, although CaCO₃ does not. This method is used for filtered samples where at least two filters per sample must be supplied. For sediment samples at least 1 g of sample is required and at least 0.5g of sample is weighed into a crucible of known weight. The weight is recorded. The crucible is then muffled as above, and weighed again. The percent remaining of the ash is calculated and multiplied times the %C in the ash which is then determined by the CE-440.

12. DATA ANALYSIS AND CALCULATIONS

- 12.1. Raw results are printed. These data are then exported from the instrument computer to the analyst computer by a flash drive. The data is then entered into an Excel spreadsheet. Results are reported in mg/L for aqueous samples, and in % for sediment or other weighed samples, standards and SRMs or QCSs.
- 12.2. Recalculation of data (if necessary)
 - 12.2.1. The software gives the analyst the opportunity to recalculate values generated by the run. This option can be useful for adjusting the values of the data due to explained or unexpected changes in the blank or calibration (K) factor

during an analytical run cycle. Blanks can change due to sample handling, different capsules or sleeves, small leaks in the system and contamination. K factors should remain stable but can drift due to flow changes caused by variable pressure drops in the traps or helium scrubber, or by changing delivery pressure at the helium regulator.

- 12.2.2. Before the analyst can change calibration values and recalculate the results, there must be a valid reason. When data is recalculated, always document the incident.

12.3. Example of Excel spreadsheet of results:

	A	B	C	D	E	F	G
1	3/31/2017						
2	ANALYST NAME						
3	CLIENT						
4	RECEIVED DATE						
5							
6	SAMPLE ID	PC, mg C/L	PN, mg N/L	ERROR			
7	SAMPLE1	0.8340	0.1420				
8	SAMPLE2	0.7070	0.1090				
9	SAMPLE3	0.6600	0.1010				
10	SAMPLE4	0.6890	0.1030				
11	SAMPLE5	0.7720	0.0869				
12	SAMPLE6	0.2600	0.0513				
13	SAMPLE7	0.2720	0.0435				
14	SAMPLE BLANK	0.00	0.00	ug/pad			
15							
16	LAB DUPS	PC, mg C/L	PN, mg N/L				
17	SAMPLE ID	DUP 1	DUP 2	DUP 1	DUP 2		
18	SAMPLE4	0.7000	0.6780	0.1070	0.0993		
19							
20	BLANKS C= 235		K VALUES C= 22.097				
21	N= 9		N= 8.055				
22							
23	QC NAME	ACTUAL		EXPECTED		% RECOVERY	
24		%C	%N	%C	%N	%C	%N
25	ATROPINE	69.21	4.81	70.56	4.84	98.1	99.4
26	ATROPINE	70.40	4.93	70.56	4.84	99.8	101.9
27	PACS2	3.24	0.29	3.21	0.29	100.9	100.0

- 12.3.1. Cell 1A - Analysis date
 12.3.2. Cell 2A – Analyst’s name
 12.3.3. Cell 3A – Sample source or client
 12.3.4. Cell 4A – Sample or Received date
 12.3.5. Cell 6A – Column heading for Sample
 12.3.6. Cell 6B – Column heading for C concentration
 12.3.7. Cell 6C – Column heading for N concentration
 12.3.8. Cell 6D – Column heading for errors and qualifiers
 12.3.9. Cells 7A to 14D – Sample Results table

- 12.3.10. Cells 16A to 18E – QC table for field duplicates. The mean of these values is reported in the sample results table
- 12.3.11. Cells 20A to 21D – Instrument values for the Blanks and Ks.
- 12.3.12. Cells 23A to 27G- Values for CCV/QCS/SRM samples and percent recoveries for each day of analyses.
- 12.4. Sample data should be reported in units of mg/L as carbon or nitrogen for aqueous samples, and as percent carbon or nitrogen for sediment samples.
- 12.5. Report analyte concentrations to three significant figures for both aqueous and sediment samples.
- 12.6. For aqueous samples, calculate the sample concentration using the following formula:
$$\text{Concentration (mg / L)} = \frac{\text{Corrected sampler response}(\mu\text{g / L})}{1000\mu\text{g / mg}}$$
- 12.7. For weighed samples, % N or %C are already calculated by the instrument software.

13. POLLUTION PREVENTION

- 13.2. Pollution prevention encompasses any technique that reduces or eliminates the quantity of toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The USEPA has established a preferred hierarchy of environmental management techniques that places pollution as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the agency recommends recycling as the next best option.
- 13.3. For information about pollution prevention that may be applicable to laboratories and research institutions, consult “Less is Better: Laboratory Chemical Management for Waste Reduction”, available from the American Chemical Society, Department of Government Relations and Science Policy, 1155 16th Street N. W., Washington, D.C. 20036.

14. WASTE MANAGEMENT

- 14.1. The reagents used in this procedure are minimal and are not hazardous with the exception of the ascarite and magnesium perchlorate. Due to the small quantity of ascarite and magnesium perchlorate used, the spent reagent can be flushed down the drain with running water.
- 14.2. For further information on waste management consult The Waste Management Manual for Laboratory Personnel, available from the American Chemical Society.

15. REFERENCES

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- 15.8. Zimmermann, C. F., Keefe, C. W., and Bashe, J. 1997. Method 440.0. Determination of Carbon and Nitrogen in Sediments and Particulates of Estuarine/Coastal Waters Using Elemental Analysis. USEPA

TABLE 5

PC/PN BENCH SHEET							
WHEEL LABEL: ANALYST:				PREP DATE: ANALYSIS DATE:			
HOLE NO	ID	VOL OR WT	ADDITIONAL	HOLE NO	ID	VOL OR WT	ADDITIONAL
1	COND			51			
2	CAP BL			52			
3	CAP BL			53			
4	COND			54			
5	STD1			55			
6	STD1			56			
7	STD1			57			
8	ICV ATROPINE			58			
9	ICB FP BL			59			
10				60			
11				61	CAP BL		
12				62	CCV ATROPINE		
13				63	CRM PACS2		
14				64	CAP BL		
15							
16							
17					ACETANILIDE LOT#	B0137476	
18					ATROPINE LOT#	YF0611	
19					CRM LOT#	PACS2	
20							
21							
22							
23							
24							
25							
26							
27							
28							
29							
30							
31							
32							
33							
34							
35	CRM PACS2						
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Appendix

Initial Start-Up

The following sequence should be followed when initially starting up the Exeter CE-440 Elemental Analyzer or when restarting after a shutdown. Make sure the power switches on the computer and on the CEC- 490 (Interface) are off. Remove the CE-440 cover from instrument. Check that the helium regulator is set at 18 psig and oxygen at 20 psig and open the in-line gas valves. If restarting, check that the combustion and reduction tubes, scrubber and traps are not exhausted. Turn the selector switch to SYSTEM. Turn on the CEC-490 and the computer. If this is a cold re-start, set combustion and reduction furnace temperature controls to values previously established. Wait until the reduction furnace has reached operating temperature. **DO NOT PUSH DETECTOR RESET BUTTON AT THIS TIME!** If the tubes need to be replaced, go to “Tube Replacement” in the Service Menu, then follow the directions under “Combustion Tube Replacement” to purge the helium and oxygen regulators twice. This will also serve the purpose of conditioning the reduction and combustion tubes. Then go to Main Menu and install the end connectors.

After allowing the CE-440 oven to reach operating temperature (about one hour) go to the Service Menu and select Calibrate CEC-490. Calibrate all and follow instructions. Run 2 to 3 blanks to establish a fill time of about 20 to 40 seconds. If the fill time has been exceeded, increase the helium pressure and repeat this step until fill time is achieved. Helium pressure should be at about 12-18 psig. (This is dependent on regulator type.) If the system still aborts, go through the leak test mode.

Make certain that helium gas is flowing by checking that the tank is open and the regulator is set to the correct pressure before pressing the DETECTOR RESET button. After the first accepted blank, push DETECTOR RESET. High concentrations of air or oxygen in the system will damage the filaments in the detectors if power is applied. To protect the detectors, a detector safety circuit is provided which shuts off power when the helium carrier gas becomes contaminated with air or oxygen at levels generating an imbalance of about 450 μ v or higher. The safety circuit will activate should leaks develop or when the helium supply is depleted. The safety circuit monitors the gross imbalance between the two sides of the nitrogen bridge. If air or oxygen is present on both sides of the bridge, the safety circuit may not activate and damage to the detectors may occur. The safety circuit is also activated when accidental or deliberate power interruption occurs. If power has been interrupted for more than 5 minutes, do not push DETECTOR RESET until the system has performed a blank run. Do not hold the DETECTOR RESET button in more than one second. If the light stays on when the button is released, continue with additional blank runs as necessary before pushing the button again. Go through one blank run before turning on the detector.

Once the detector has been reset, go to the Service Menu and monitor the bridge readings. Adjust the “zero” reading to approximately 2500 μ v by turning the respective potentiometers on the Bridge Balance Card located in the left rear corner of the Motherboard. (To access these systems, the instrument cover must be removed.) Typically the bridges should be set well above negative or zero (approximately + 2500 μ v). This is after the instrument has stabilized. Stability is based on furnace and oven temperatures being steady for a period of

not less than 1 hour. Check the furnace and oven temperatures. If these have reached operating levels, let the instrument go through another three sets of blanks in order to purge the system and condition the reagents. This can be done through the CHN Run Mode (Run Menu). Make sure the B-valve is ON to run oxygen and run blanks until (a) BC and BN are less than 200 μ v and BH is less than 1500 μ v, and (b) consecutive BC/BN agrees to 10 μ v and BH to 50 μ v. It may take time for BH to settle. Go to the Service pull down Menu and calibrate all of the CEC-490 again. The instrument is now ready for system calibration with known standards.

Standby Mode

To reduce helium consumption and minimize wear on the terminal screen, the overnight standby mode is used. Select the overnight standby mode (in the Run pull- down menu). To return to normal operation, select Stop Overnight Standby.

Powering Down

It is preferable for the system to remain powered up at all times since this will extend the life time of the glassware, reagents, and electronics. However, helium and power will be consumed during this standby and it might be necessary to power down the CE-440 instrument. To assure minimum disruption for a future start up after a power down, proceed as follows:

- Turn the furnace temperature controllers to zero.
- Allow several hours for the furnace temperatures to drop below 100°C.
- Turn off the power to the instrument as well as gas valves between the instrument and the regulators.
- Turn off the gas on the cylinder.

To Start a Run

Select “Run” from the menu and continue as described below.

- Select “Carbon, Hydrogen, Nitrogen Run”. Select “Yes” for a new run
- Check “Enter the Ks and Blanks automatically”. Enter date followed by AM or PM as appropriate for the message of this run series. Select “Enter Data”.
- Sample Entry Screen: Enter Weight (μ g): when entering the weight of the sample press [ENTER] to use the present weight or enter a new weight. If a weight of zero [0] is entered then the ID is assumed to be a blank. If a weight of 100 has been entered the results will be reported in micrograms (μ g). When analyzing aqueous samples, enter the volume filtered (milliliters)/10 as the weight. The results will be reported in μ g /L. When analyzing sediment samples or weighed QC samples, enter the weight in μ g. The result will be reported in %.
- Enter Sample ID: enter the sample ID as either STD1, blank, or any other text. If STD1 is entered as the first three letters, then Ks will be calculated on the result report. If blank is entered, then blanks will be calculated.
- Use the PC/PN bench sheet to set up a wheel. (See Table 5)
- Press “Start Run”

To Insert a Wheel

This mode opens the A, D, F and C valves allowing helium to enter the injection box and minimize air in this area while installing the sample wheel for the 64 sample automatic injector. The pressure will build up and eventually equilibrate to the helium tank pressure if the instrument is left in this mode for a long period of time. This is not recommended; therefore, do not delay carrying out the following steps.

- Open the manual purge valve on the injector box (right side, behind the P valve) to relieve the internal pressure. Loosen the 4 cover screws and lift the lid. Remove the empty wheel from the sample chamber.
- Blow out with canned air any material that might be in the box from previous run.
- Insert the loaded sample wheel with the locking pin in place. Tilt the wheel slightly, line up the scribe mark on the wheel with the ratchet in the housing, lower the wheel and make sure that it is properly seated. Place the locking pin in the center hold. Check that the o-ring of the cover is clean and well seated in the groove.
- Close the cover and tighten equally on all four screws. This should be performed in an alternating sequence to achieve a uniform seal. Never over-tighten or use any tools on the screws.
- Open and remove any spent capsules in the capsule receiver located under the sample chamber. Re-grease the gasket if needed and re-install cover.
- Close the purge valve, let pressure build up for about 30 seconds. Re-open the purge valve for about 5 seconds and then close again.
- Select “OK” to continue operation.

Sample Run

The sample is automatically injected into the combustion tube at the appropriate time. Upon completion of the fill time the ladle is retracted and allowed to cool. At the end of the run the results are printed and the soft key commands are followed if any have been selected.

Once the run begins, the screen displays the following information:

Run number, Sample Weight and ID., the operating K and B values, the preset combustion and purge times, valve status, and the elapsed time in minutes:seconds. Temperatures and Pressure are also displayed near the bottom of the screen. These numbers may not be updated all of the time as time critical sections of a run occur. Run counters for the various tubes are displayed above the valve status diagram. The run counters will change from white to red when they approach 10% within the thresholds set by the user.

During the run the analyst has various options available through the buttons at the top of the screen (accessed via simply selecting one). If a key is actuated, the button changes from grey to white. The buttons are for the following functions:

- **Ks & Bs** - To access the Ks and Bs table at the end of the current run. This allows the operator to change the operating values.
- **PARAMETERS** - Goes to parameters table at the end of the current run.
- **LEAK TEST** - The leak test program is activated at the end of the run cycle.

- **STANDBY** - At the end of the run cycle the instrument will go into standby.
- **DATALOG** - At the end of the run cycle a datalog is printed every half hour. A, D, and F valves are turned on, as in the overnight standby mode.
- **SSI** – A function to activate the single sample inject program after the completion of the current run. The program will automatically resume after the SSI run (unless SSI is pressed again).
- **MENU NEXT** - Goes to the Analytical Menu at the end of the current run. The data will be stored on the data disc at that point.
- **STOP** - Aborts the current operation and goes to the Analytical Menu. This is typically only used during emergency operations. If you exit an HA run cycle prematurely and you wish to start over or resume the HA run with the sample IDs and weights already in memory, then DO NOT exit the Analytical Menu. If you exit or reboot the Analytical Menu then the IDs and weights will be erased.
- **NONE** – Nothing at end of run or run cycle.

Tube Replacement

This mode is used when one or more of the reagent tubes in the CE-440 need to be changed, as indicated by the maintenance schedule, poor analytical results or in the case of a cold restart. See instrument manual for additional details on tube replacement beyond this appendix.

Go to the Service Pull-down Menu. Select “Tube Replacement.” “Select CHN Analysis.” Another menu will be displayed that will contain options for tube packing information or for replacement of any tubes used for that analysis. If a new gas cylinder or regulator is to be replaced, select the appropriate item from the menu for changing a tank.

In the individual tube replacement options, follow the step by step instruction shown on the screen. If the procedure is followed correctly and to its conclusion, the Maintenance Schedule Information for that tube will be reset. You can return to the Service Menu at any point by pressing “End.”

Combustion Tube

Hold the tube vertically with the short end from the indentation up. Roll up a piece of platinum gauze so that it will fit snugly into the combustion tube. Slide the gauze plug into the tube and up against the indentation. Add a small plug of quartz wool. (Quartz wool may be muffled for one hour at 850 °C to remove any residual carbon). Add 1½ inches of silver tungstate/magnesium oxide on Chromosorb. Gently tap the tube to prevent the reagent from channeling. Add a small plug of quartz wool. Add 2 inches of silver oxide/silver tungstate on Chromosorb. Tap the tube and add another small plug of quartz wool. Add 2 inches of silver vanadate on Chromosorb. Tap the tube and add another plug of quartz wool. Slide a rolled-up piece of silver gauze into the tube and pack against the quartz wool. Make sure that there is no less than ½ inches of space between the end of the tube and the silver gauze since the silver gauze will conduct heat and damage the o-ring on the end connector. There is rarely such a thing as a “too tightly” packed combustion tube. Loosely packed combustion tubes can cause non-linearity.

- Silver Vanadate on Chromosorb: Reacts with and removes chlorine, bromine, iodine and sulfur contained in the combustion gases. When absorbing sulfur, it changes color from yellow to dark brown when saturated. In absorbing halogens, exhaustion of the silver vanadate is indicated by color changes on the surface of the silver gauze at the end of the combustion tube. Each element forms a distinctively colored salt deposit – silver chloride is gray, silver bromide is brown, and silver iodide is purple.
- Silver Tungstate/Magnesium Oxide on Chromosorb: Removes fluorine, phosphorus, and arsenic.
- Silver Oxide/Silver Tungstate on Chromosorb: Removes sulfur and halogens (except fluorine).
- The silver gauze can be cleaned with water and a small amount of dish soap; swirl for about five minutes, rinse the gauze multiple times with tap water then rinse again with laboratory reagent water. Rinse the gauze with acetone for 30 seconds then dump the waste. Air-dry the gauze thoroughly. Finish by muffling at 550°C for 30 minutes.

Reduction Tube

Pack about ¾ inches of quartz wool into the bottom of the tube from the opposite end. Fill the tube with copper wire while gently tapping to tightly settle the copper and avoid channeling. Pack another plug of quartz wool into the tube against the copper. Insert a rolled-up piece of silver gauze into each small diameter tube end.

Perform a leak test of the combustion-reduction area after tube replacement.

Carbon Dioxide Trap and Gas Scrubbers (2)

These three tubes are identically packed even though the scrubbers are a larger diameter. Pack a ¼ inches plug of quartz wool into one end of the tube. Add 3½ inches Ascarite (Colorcarb) while gently tapping the tube. Add ¼ inches plug of quartz wool. Add 1½ inches magnesium perchlorate while gently tapping the tube. Add ¼ inches plug of quartz wool. There should be about ¼ inches of free space at each end of the tube. Gas scrubbers should be loosely packed to allow for the high gas flows associated with the CE-440. Note the orientation (in the instrument) of the helium and oxygen scrubbers versus the CO₂ trap. The orientation is reversed for the CO₂ trap.

Water Trap

Pack a ¼ inches plug of quartz wool into one end of the tube. Add magnesium perchlorate while gently tapping the tube. Add ¼ inches plug of quartz wool. There should be about ¼ inches of free space at each end of the tube.

Fill time should always be checked after replacing traps and scrubbers. The instrument should be conditioned after replacing maintenance by running two blanks before proceeding to a sample run.

Important Factors for Proper CE-440 Operation

- Oxygen pressure should be at ~ 20 psig.
- Helium pressure should be at ~ 12-18 psig and the fill time for a run should be between 20 and 40 seconds. (This is dependent on regulator type.)
- When greasing o-rings or gaskets, it is recommended to use Krytox (R) by Dupont.
- The furnace temperatures reach set temperature very quickly.
- Never set the combustion temperature above 1100 °C.
- Never set the reduction temperature above 900 °C.

University of Maryland Center for Environmental Science
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**Standard Operating Procedure for
Determination of Dissolved Organic Carbon/Non-Purgeable Organic Carbon
(DOC/NPOC), and Total Organic Carbon (TOC) in Fresh/Estuarine/Coastal
Waters using High Temperature Combustion and Infrared Detection.**

(References: SM5310B)

NASLDoc-014
Revision 2018-1
Replaces Revision 2017-4
Effective May 1, 2018

**I attest that I have reviewed this standard operating
procedure and agree to comply with all procedures
outlined within this document.**

Employee (Print)

Employee (Signature)

Date

Employee (Print)

Employee (Signature)

Date

|

Quality Assurance Project Plan
Ambient Water Quality Monitoring in Western Long Island Sound,
May 21st, 2018—Version 5.0—Page 179 of 232

Employee (Print)

Employee (Signature)

Date

Revised by: _____ Date: _____

Reviewed by: _____ Date: _____

Laboratory Supervisor: _____ Date: _____

Changes affecting Revision 2018

Section 1.2: Changed MDL definition to reflect new EPA Federal Register changes

Section 3.35: 99% confidence changed to 99th percentile

Section 8.2: PETG was added as an acceptable material for sample bottles.

Section 9.2.4: Changed MDL procedures to match EPA changes. Added sub sections 9.2.4.1 through 9.2.4.6.

Table 2: Correlation Coefficient, if curve is < 0.995 , rerun curve.

Section 10.1.2: added absolute value of y intercept to calculation.

Under Procedures:

 Loading samples: revised sample bottle volumes and added PETG bottles as acceptable.

 QAQC section: analyze a CRM after every 10 samples along with a blank and CCV.

 Changed wording to say there is enough volume in the sample vial to sample 3 times.

 Added: Load the LFB after the clean check blank.

 Updated Bench Sheet example.

1. SCOPE and APPLICATION

- 1.1 High temperature combustion (680°C) is used to determine dissolved organic carbon (DOC), also known as non-purgeable organic carbon (NPOC), total organic carbon (TOC), and total carbon (TC), using a non-dispersive infrared detector (NDIR). The method is used to analyze all ranges of salinity.
- 1.2 A Method Detection Limit (MDL) of 0.16 mg/L DOC was determined using the MDL method as specified in the EPA Federal Register 821-R-16-006, titled Definition and Procedure for the Determination of the Method Detection Limit, Revision 2.
- 1.3 The reporting limit for DOC is equal to the lowest standard used, which is 0.50 mg/L C. The quantitation limit is set at 5 times the MDL.
- 1.4 This procedure should be used by analysts experienced in the theory and application of organic carbon analysis. A three month training period with an analyst experienced in the analysis using the organic carbon analyzer is required.
- 1.5 This method can be used for all programs that require analysis of dissolved and total organic carbon.
- 1.6 This procedure references SM5310B.

2. SUMMARY

- 2.1 The Shimadzu TOC-L uses a high temperature combustion method to analyze aqueous samples for total carbon (TC), total organic carbon (TOC) and dissolved organic carbon (DOC), also known as non-purgeable organic carbon (NPOC). The terms DOC and NPOC are used interchangeably. TOC and TC concentrations are derived from whole unfiltered water. NPOC concentrations are derived from water that has been filtered through a 0.7 um (nominal pore size) GF/F glass fiber filter, or equivalent.
- 2.2 TOC and NPOC samples are acidified and sparged with ultra pure carrier grade air to drive off inorganic carbon. TC samples are injected directly onto the catalyst bed with no pretreatment and all sources of carbon, inorganic as well as organic carbon, are measured. High temperature combustion (680°C) on a catalyst bed of platinum-coated alumina balls breaks down all carbon compounds into carbon dioxide (CO₂). The CO₂ is carried by ultra pure air to a non-dispersive infrared detector (NDIR) where CO₂ is detected.

3. DEFINITIONS

- 3.1 Acceptance Criteria – Specified limits placed on characteristics of an item, process, or service defined in a requirement document. (ASQC)

- 3.2 Accuracy – The degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations; a data quality indicator. (QAMS)
- 3.3 Aliquot – A discrete, measured, representative portion of a sample taken for analysis. (EPA QAD Glossary)
- 3.4 Analytical Range – The instrument range is 100 ppb - 3000 ppm using a 10 - 150 µl injection volume, using regular sensitivity catalyst.
- 3.5 Batch – Environmental samples, which are prepared and /or analyzed together with the same process and personnel, using the same lot(s) of reagents. An **analytical batch** is composed of prepared environmental samples (extracts, digestates, or concentrates) and/or those samples not requiring preparation, which are analyzed together as a group using the same calibration curve or factor. An analytical batch can include samples originating from various environmental matrices and can exceed 20 samples. (NELAC/EPA)
- 3.6 Blank- A sample that has not been exposed to the analyzed sample stream in order to monitor contamination during sampling, transport, storage or analysis. The blank is subjected to the usual analytical and measurement process to establish a zero baseline or background value and is sometimes used to adjust or correct routine analytical results. (ASQC)
- 3.7 Calibrate- To determine, by measurement or comparison with a standard, the correct value of each scale reading on a meter or other device, or the correct value for each setting of a control knob. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)
- 3.8 Calibration – The set of operations which establish, under specified conditions, the relationship between values indicated by a measuring device. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)
- 3.9 Calibration Curve – The graphical relationship between known values, such as concentrations, or a series of calibration standards and their analytical response. (NELAC)
- 3.10 Calibration Method – A defined technical procedure for performing a calibration. (NELAC)
- 3.11 Calibration Standard – A substance or reference material used to calibrate an instrument. (QAMS)
 - 3.11.1 Initial Calibration Standard (STD) – A series of standard solutions used to initially establish instrument calibration responses and develop calibration curves for individual target analytes.
 - 3.11.2 Initial Calibration Verification (ICV) – An individual standard, which may be the same compound used as the calibrating standard, but not from the same vendor unless confirmed as different lots, analyzed initially, prior to any sample analysis,

which verifies acceptability of the calibration curve or previously established calibration curve.

- 3.11.3 Continuing Calibration Verification (CCV) – An individual standard which may be the same as the calibrating standard and is analyzed after every 10 field sample analysis.
- 3.12 Certified Reference Material (CRM) - A reference material one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body. (ISO 17025)
- 3.13 Combustion tube – Quartz tube filled with platinum catalyst, heated to 680° C, into which the sample aliquot is injected.
- 3.14 Conditioning Blank – Reagent water (ASTM Type I) analyzed before the calibration curve to decrease the instrument blank and stabilize the column conditions.
- 3.15 Corrective Action – Action taken to eliminate the causes of an existing nonconformity, defect or other undesirable situation in order to prevent recurrence. (ISO 8402)
- 3.16 Deficiency – An unauthorized deviation from acceptable procedures or practices. (ASQC)
- 3.17 Demonstration of Capability – A procedure to establish the ability of the analyst to generate acceptable accuracy. (NELAC)
- 3.18 Detection Limit – The lowest concentration or amount of the target analyte that can be determined to be different from zero by a single measurement at a stated degree of confidence.
- 3.19 Duplicate Analysis – The analyses of measurements of the variable of interest performed identically on two sub samples (aliquots) of the same sample. The results from duplicate analyses are used to evaluate analytical or measurement precision but not the precision of sampling, preservation or storage internal to the laboratory. (EPA-QAD)
- 3.20 External Standard (ES) – A pure analyte (potassium hydrogen phthalate (KHP)) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard is used to calibrate the instrument response for the corresponding analyte(s). The instrument response is used to calculate the concentrations of the analyte(s) in the unknown sample.
- 3.21 Field Duplicates (FD1 and FD2) – Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 provide a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.
- 3.22 Field Reagent Blank (FRB) – A aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to the sampling site conditions, storage, preservation, and all

analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

- 3.23 Furnace – Heats the combustion tube to the operating temperature of 680° C.
- 3.24 Holding time – The maximum time that samples may be held prior to analysis and still be considered valid. (40 CFR Part 136) The time elapsed from the time of sampling to the time of extraction or analysis, as appropriate.
- 3.25 Injection – The sample aliquot that is drawn into the syringe and injected into the combustion tube.
- 3.26 Instrument Detection Limit (IDL) – The minimum quantity of analyte of the concentration equivalent which gives an analyte signal equal to three times the standard deviation of the background signal at the selected wavelength, mass, retention time absorbance line, etc.
- 3.27 Laboratory Duplicates (LD1 and LD2) – Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.
- 3.28 Laboratory Reagent Blank (LRB) – A matrix blank (i.e., Reagent water) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the instrument.
- 3.29 Laboratory Control Sample (LCS) – A sample matrix, free from the analytes of interest, spiked with verified known amounts of analytes from a source independent of the calibration standard or a material containing known and verified amounts of analytes. The LCS is generally used to establish intra-laboratory or analyst-specific precision and bias or to assess the performance of all or a portion of the measurement system. (NELAC)
- 3.30 Limit of Detection (LOD) – The lowest concentration level that can be determined by a single analysis and with a defined level of confidence to be statistically different from a blank, (ACS) also known as the MDL.
- 3.31 Limit of Quantitation (LOQ) – The minimum levels, concentrations, or quantities of a target variable (target analyte) that can be reported with a specified degree of confidence. The LOQ is set at 3 to 10 times the LOD such that it is greater than or equal to the reporting limit, depending on the degree of confidence desired. Also known as the Quantitation Limit.
- 3.32 Linear Dynamic Range (LDR) – The absolute quantity over which the instrument response to an analyte is linear. This specification is also referred to as the Linear Calibration Range (LCR).
- 3.33 Material Safety Data Sheets (MSDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical

properties, fire, and reactivity data including storage, spill, and handling precautions.

- 3.34 May – Denotes permitted action, but not required action. (NELAC)
- 3.35 Method Detection Limit (MDL) – The minimum concentration of an analyte that can be identified, measured, and reported within the 99th percentile that the analyte concentration is greater than zero.
- 3.36 Must – Denotes a requirement that must be met. (Random House College Dictionary)
- 3.37 Non-Dispersive Infrared Detector (NDIR) – The detector found in the Shimadzu TOC-L analyzer. Carbon dioxide is detected.
- 3.38 Precision – The degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves; a data quality indicator. Precision is usually expressed as standard deviation, variance or range, in either absolute or relative terms. (NELAC)
- 3.39 Preservation – Refrigeration, freezing, and/or reagents added at the time of sample collection (or later) to maintain the chemical and or biological integrity of the sample.
- 3.40 Quality Control Sample (QCS) – A sample of analytes of known and certified concentrations. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials. Also known as CRM.
- 3.41 Run – One sample analysis from start to finish, including printout.
- 3.42 Run Cycle – Typically a day of operation – the entire analytical batch of runs from the first run to the last run.
- 3.43 Sample Volume – Amount of sample injected into the combustion tube.
- 3.44 Sensitivity – The capability of a test method or instrument to discriminate between measurement responses representing different levels (concentrations) of a variable of interest.
- 3.45 Shall – Denotes a requirement that is mandatory whenever the criterion for conformance with the specification requires that there be no deviation. (ANSI)
- 3.46 Should – Denotes a guideline or recommendation whenever noncompliance with the specification is permissible. (ANSI)
- 3.47 Sparge Time – The time required to aerate an acidified sample with ultra pure air to remove inorganic carbon to determine the concentration of organic carbon.
- 3.48 Standard Reference Material (SRM) – Material which has been certified for specific analytes by a variety of analytical techniques and/or by numerous laboratories using similar analytical techniques. These may consist of pure chemicals, buffers, or compositional standards. The materials are used as an indication of the accuracy of a specific analytical technique. Also known as CRM.






4. INTERFERENCES



- 4.1 Carbonates and bicarbonates may interfere with the determination of organic carbon by increasing the concentration of CO₂ detected. These are removed by adding enough acid to the sample to bring the pH to 2 or below, then sparging with ultra-pure air for a predetermined time.

5. SAFETY

- 5.1 Safety precautions must be taken when handling reagents, samples and equipment in the laboratory. Protective clothing including lab coats, safety glasses and enclosed shoes should be worn. In certain situations, it will be necessary to also use gloves and/or a face shield. If solutions come in contact with eyes, flush with water continuously for 15 minutes. If solutions come in contact with skin, wash thoroughly with soap and water. Contact Solomons Rescue Squad (911) if emergency treatment is needed and also inform the Chesapeake Biological Laboratory (CBL) Associate Director of Administration and Facilities Maintenance of the incident. Contact the CBL Associate Director of Administration and Facilities Maintenance if additional treatment is required.
- 5.2 The toxicity or carcinogenicity of each reagent used in this procedure may not have been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known hazardous materials and procedures.
- 5.3 Do not wear jewelry when troubleshooting electrical components. Even low voltage points are dangerous and can injure if allowed to short circuit.
- 5.4 The following hazard classifications are listed for the chemicals used in this procedure. Detailed information is provided on Material Safety Data Sheets (MSDS).

Table 1:

Chemical	Health Hazard	Fire Hazard	Instability Hazard	Specific Hazard	
Potassium Hydrogen	1	1	0	Irritant	
Sodium Carbonate, Anhydrous	2	0	1	Irritant	
Sodium Bicarbonate	1	0	0		
Hydrochloric Acid	3	0	2	ACID, COR	 
Chemical	Health Hazard	Fire Hazard	Instability Hazard	Specific Hazard	
Platinum Catalyst on Alumina Beads	1	0	0		

Soda Lime	3	0	0	COR	
Sulfuric Acid	4	0	2	ACID, COR	

On a scale of 0 to 4 the substance is rated on four hazard categories: health, flammability, reactivity, and contact. (0 is non-hazardous and 4 is extremely hazardous)

HAZARD RATING

Health Hazard - Blue: 4 – deadly, 3 – extreme danger, 2 – hazardous, 1 – slightly hazardous, 0 – normal material

Fire Hazard - Red: Flash Points: 4 – below 73° F, 3 – below 100° F, 2 – below 200° F, 1 – above 200° F, 0 – will not burn

Instability Hazard - Yellow: 4 – may detonate, 3 – Shock and heat may detonate, 2 – violent chemical change, 1 – unstable is heated, 0 - stable

Specific Hazard - White: Acid = ACID, Alkali = ALK, Corrosive = COR, Oxidizer = OXY

6. EQUIPMENT AND SUPPLIES

6.1 A Total Organic Carbon Analyzer capable of maintaining a combustion temperature of 680° C and analyzing for organic and inorganic carbon. The Shimadzu TOC-L is used in this laboratory.

6.2 Freezer, capable of maintaining $-20 \pm 5^{\circ} \text{C}$.

6.3 Lab ware – All reusable lab ware (glass, Teflon, plastic, etc) should be sufficiently clean for the task objectives. This laboratory soaks all lab ware related to this method in a 10% HCl (v/v) acid bath overnight, and the lab ware is rinsed copiously with ASTM Type I water. Then the glassware is baked at 400° C for at least 1 hour. Clean check bottle blanks are analyzed with every run to determine effectiveness or necessity of cleaning.

7. REAGENTS AND STANDARDS

7.1 Purity of Water – Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to ASTM Specification D 1193, Type I. Freshly prepared water should be used for making the standards intended for calibration. The detection limits of this method will be limited by the purity of the water and reagents used to make the standards.

7.2 Purity of Reagents – Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades

may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without compromising the accuracy of the determination.

- 7.3 Potassium Hydrogen Phthalate (KHP) $C_6H_4(COOK)(COOH)$ – primary standard for organic carbon.
- 7.4 Sodium Hydrogen Carbonate ($NaHCO_3$) and Sodium Carbonate (Na_2CO_3) – primary standard for inorganic carbon; may also be used to determine sparging efficiency for the NPOC method.

- 7.5 Sulfuric Acid, 9 N –
- | | |
|--|---------|
| Sulfuric acid (H_2SO_4), concentrated, | 250 ml |
| Reagent water, q.s. | 1000 ml |

In a 1000 ml volumetric flask, add 250 ml of concentrated sulfuric acid to ~600 ml of reagent water. Dilute to 1000 ml with reagent water. Allow solution to cool to near room temperature before filling completely to the graduated mark on the flask

- 7.6 Organic Carbon Stock Standard: Potassium Hydrogen Phthalate (KHP) Standard, 1000 mg/l
- Potassium hydrogen phthalate ($HOCOC_6H_4COOK$),
- | | |
|-----------------------------|----------|
| Dried at 45° C, min. 1 hour | 1.0625 g |
| Reagent water | 500 ml |

In a 500 ml volumetric flask, dissolve 1.0625 g of potassium hydrogen phthalate in ~300 ml of reagent water. Dilute to 500 ml with reagent water. Make fresh within 6 months. Store at 4° C.

- 7.7 Inorganic Carbon Stock Standard: Sodium Hydrogen Carbonate/ Sodium Carbonate ($NaHCO_3/Na_2CO_3$) Standard, 1000 mg/l
- | | |
|--|----------|
| Sodium Hydrogen Carbonate ($NaHCO_3$) | 0.875 g |
| Sodium Carbonate, Anhydrous (Na_2CO_3) | 1.1025 g |
| Reagent water | 250 ml |

In a 250 ml volumetric flask, dissolve 0.875 g $NaHCO_3$ and 1.1025 g Na_2CO_3 in ~150 ml reagent water. Dilute to 250 ml with reagent water. Make fresh within 4 months. Store at 4° C.

- 7.8 Hydrochloric Acid, 0.05 N –
- | | |
|------------------------|---------|
| Hydrochloric Acid, 1 N | 1.0 ml |
| Reagent water | 40.0 ml |

Combine 40.0 ml reagent water with 1.0 ml 1 N HCl in any appropriate storage container. This solution is used in the Type B Halogen scrubber. Make fresh as needed.

7.9 Blanks – ASTM D1193, Type I water is used for the Laboratory Reagent Blank.

7.10 Quality Control Sample (QCS) – For this procedure, the QCS can be any certified dissolved sample which is obtained from an external source. If a certified sample is not available, then use the standard material (KHP).

8 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Water collected for TOC is not filtered.

8.2 Water collected for DOC should be filtered through a Whatman GF/F glass fiber filter (nominal pore size 0.7 μm), or equivalent.

8.2 Water collected for TOC/DOC should be frozen at $\leq -20^{\circ}\text{C}$, or acidified with 9N H_2SO_4 to a pH of ≤ 2 . The sample container should be borosilicate glass, Teflon or low leaching plastic such as PETG. Other plastic containers may be used if well cleaned and aged. Freshwater samples should be frozen in Teflon or plastic to prevent breakage.

8.3 The holding time for frozen TOC/DOC samples is 28 days. It has been shown that frozen QCS samples up to a year old still fall well within the control limits, therefore if the frozen sample is stored longer than the holding time, there is minimal degradation.

8.4 Acidified TOC/DOC samples may be frozen, as above, or refrigerated at $\leq 6^{\circ}\text{C}$ for no longer than 28 days.

9 QUALITY CONTROL

9.1 The laboratory is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the continued analysis of laboratory instrument blanks and calibration standard material, analyzed as samples, as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data generated.

9.2 Initial Demonstration of Capability

9.2.1 The initial demonstration of capability (iDOC) – is used to characterize instrument performance (MDLs) and laboratory performance (analysis of QC samples) prior to the analyses conducted by this procedure.

9.2.2 Linear Dynamic Range (LDR) – The linear dynamic range for TOC/DOC should be established by using a blank and a minimum of five appropriate standards for the calibration curve.

9.2.3 Quality Control Sample (QCS/SRM) – When using this procedure, a quality control sample is required to be

analyzed at the beginning and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within $\pm 3\sigma$ of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with analyses.

- 9.2.4 Method Detection Limits (MDLs) – MDLs should be established for TOC/DOC using a spiked water sample, typically two to 10 times the estimated MDL and no more than ten times higher than the estimated MDL. Process a minimum of seven spiked samples and seven blank samples through all steps of the method. The samples used for the MDL must be prepared and analyzed in at least three batches on three separate calendar dates. If multiple instruments are used for this analysis, MDLs must include data/calculations from all instruments.
- 9.2.4.1 Make all computations as specified in the analytical method and express the final results in the method-specified reporting units.
- 9.2.4.2 Calculate the sample standard deviation (S) of the replicate spiked sample measurements and the sample standard deviation of the replicate method blank measurements from all instruments to which the MDL will be applied.
- 9.2.4.3 Compute the MDLs (the MDL based on spiked samples) as follows:

$$MDL_S = t_{(n-1, 1-\alpha=0.99)} S_S$$

where:

MDLs = the method detection limit based on spiked samples

$t_{(n-1, 1-\alpha=0.99)}$ = the Student's t-value appropriate for a single-tailed 99th percentile
t statistic and a standard deviation estimate with n-1 degrees of freedom.

S_S = sample standard deviation of the replicate spiked sample analyses.

- 9.2.4.4 Compute the MDL_b (the MDL based on method blanks) as follows:

If none of the method blanks give numerical results for an individual analyte, the MDL_b does not apply. A numerical

result includes both positive and negative results, including results below the current MDL, but not results of “ND” (not detected) commonly observed when a peak is not present in chromatographic analysis.

If some (but not all) of the method blanks for an individual analyte give numerical results, set the MDL_b equal to the highest method blank result. If more than 100 method blanks are available, set MDL_b to the level that is no less than the 99th percentile of the method blank results. For “n” method blanks where $n \geq 100$, sort the method blanks in rank order. The $(n * 0.99)$ ranked method blank result (round to the nearest whole number) is the MDL_b. For example, to find MDL_b from a set of 164 method blanks where the highest ranked method blank results are ... 1.5, 1.7, 1.9, 5.0, and 10, then $164 \times 0.99 = 162.36$ which rounds to the 162nd method blank result.

Therefore, MDL_b is 1.9 for $n=164$ (10 is the 164th result, 5.0 is the 163rd result, and 1.9 is the 162nd result). Alternatively, you may use spreadsheet algorithms to calculate the 99th percentile to interpolate between the ranks more precisely.

If all of the method blanks for an individual analyte give numerical results, then calculate the MDL_b as:

$$MDL_b = \bar{X} + t_{(n-1, 1-\alpha=0.99)} S_b$$

where:

MDL_b = the MDL based on method blanks

\bar{X} = mean of the method blank results (use zero in place of the mean if the mean is negative)

$t_{(n-1, 1-\alpha=0.99)}$ = the Student’s t-value appropriate for the single-tailed 99th percentile

t statistic and a standard deviation estimate with $n-1$ degrees of freedom.

S_b = sample standard deviation of the replicate method blank sample analyses.

- 9.2.4.5 The verified MDL is the greater of the MDLs or MDL_b. If the verified MDL is within 0.5 to 2.0 times the existing MDL, and fewer than 3% of the method blank results (for the individual analyte) have numerical results above the

existing MDL, then the existing MDL may optionally be left unchanged. Otherwise, adjust the MDL to the new verification MDL. (The range of 0.5 to 2.0 approximates the 95th percentile confidence interval for the Initial MDL determination with six degrees of freedom.)

- 9.2.4.6 MDLs should be determined annually, whenever there is a significant change in instrumental response or a significant change in instrument configuration. Data for annual MDL calculation and verification is analyzed at least quarterly, throughout the year.

9.3 Assessing Laboratory Performance

- 9.3.1 Laboratory Reagent Blank (LRB) – The laboratory must analyze at least one LRB with each batch of samples. The LRB consists of reagent water treated the same as the samples. LRB data are used to assess contamination from the laboratory environment.
- 9.3.2 Quality Control Sample (QCS)/ Certified Reference Material (CRM) – When using this procedure, a quality control sample is required to be analyzed at the beginning of the run and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within $\pm 3\sigma$ of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with the analyses. The results of these samples shall be used to determine batch acceptance.
- 9.3.3 The QCS will be obtained from a source external to the laboratory and different from the source of calibration standards.
- 9.3.4 Control Charts – The CRM data are tracked, and the slope, y-intercept, and correlation coefficient data are compiled and tracked.
- . The CRM concentrations should fall within $\pm 3\sigma$ of the expected value. The Accuracy Control Chart for QCS/CRM samples is constructed from the average and standard deviation of each batch grouping by date of QCS/CRM measurements. The accuracy chart includes upper and lower control levels ($CL=\pm 3\sigma$). These values are derived from stated values of the QCS/CRM. The standard deviation (σ) is specified relative to statistical confidence

levels of 99% for CLs. Enter QCS/CRM results on the chart each time the sample is analyzed

9.3.5 Calibration Verification – Initial Calibration Verification (ICV) - Immediately following the calibration curve, all standards are analyzed to confirm the calibration. The ICVs are several standards not used in the curve and falling within the middle of the curve, and are made from KHP which is purchased from a separate vendor or is a confirmed separate lot of the same vendor as the calibration standard. Following every 10 samples, a blank, a Continuing Calibration Verification standard, and a laboratory control sample are analyzed to assess instrument performance. The CCVs are made from the same material as calibration standards (KHP), and are to be within $\pm 3\sigma$ of the expected value. The laboratory control sample is prepared from a source of material other than the calibration standards, and is to be within $\pm 3\sigma$ of the expected value. Failure to meet the criteria constitutes correcting the problem and reanalyzing the samples. If not enough sample exists, the data must be qualified if reported.

9.4 Assessing Analyte Recovery

9.4.1 Matrix spikes are analyzed every 10 samples.

9.4.2 1.0 ml of the highest KHP standard in the curve is added to 10.0 ml of sample for a total volume of 11.0 ml.

9.4.3 1.0 ml standard $1.0/11.0 = 0.09$

9.4.4 0.09 X STD conc.

9.4.5 10.0 ml sample $10.0/11.0 = 0.91$

9.4.6 (original sample conc. X 0.91) + (0.09 x std conc.) =
(expected conc.) mg/L

9.4.7 Percent Recovery for each spiked sample should fall within $\pm 10\%$. Where:

$$\%SR = (\text{Actual/Expected}) \times 100$$

9.4.8 Relative Percent Difference (RPD) of duplicated samples should be within 10%. Where:

$$RPD = \frac{\|\text{difference of duplicates}\|}{\text{Average of duplicates}} \times 100$$

Assess whether the analytical result for the CRM/QCS sample confirms the calibration when calculated as follows

$$\% \text{ Recovery} = \text{AMC/CRM} \times 100$$

Where:

AMC = Average measured concentration of the CRM sample

CRM = Certified value of the CRM

The analytical result must fall with the range of 90-110%

9.5 Data Assessment and Acceptance Criteria for Quality Control Measures

9.5.1 The Acceptance Criteria for DOC is 0.995. If the correlation coefficient is less than acceptable, all blanks and standards analyzed during the run may be averaged into the curve.

9.6 Corrective Actions for Out of Control Data

9.6.1 If the acceptance criteria are still not met, the samples are to be reanalyzed.

Table 2:

QC Indicator	Acceptance/ Action Limits	Action	Frequency (Batch)
Correlation Coefficient	≥ 0.995	If <0.995 , rerun curve.	1 per batch if acceptable.
Quality Control Sample (QCS)/ Certified Reference Material (CRM)	$\pm 10\%$	If the QCS value falls between $\pm 10\%$ and $\pm 20\%$, assess the blanks and standards. If QCS value is outside $\pm 20\%$ of the target value reject the run, correct the problem and rerun samples.	Beginning of run following the ICV, after every 10 unknown samples, and at end of run bracketed within final CCVs.
Initial Calibration Verification (ICV)	$\pm 10\%$	Recalibrate if outside acceptance limits.	Beginning of run following standard curve.
Continuing Calibration Verification (CCV)	$\pm 10\%$	If outside 10%, correct the problem. Rerun all samples following the last in-control CCV.	After every 10 samples and at end of batch.
Method Blank/Laboratory Reagent Blank (LRB)	\leq Method Quantitation Limit	If the LRB exceeds the quantitation limit, results are suspect. Rerun the LRB. If the concentration still exceeds the quantitation limit, reject or qualify the data, or raise the quantitation	Following the ICV, after every 10 samples preceding the CCV and at the end of the run.

Method Quantitation Limit (MQL): The concentration of the lowest standard.		When the value is outside the predetermined limit and the ICV is acceptable, reanalyze the sample. If the reanalysis is unacceptable, increase the concentration and reanalyze. If this higher concentration meets the acceptance criteria, raise the reporting limit for the analyte.	Beginning of run following the LRB.
Laboratory Fortified Sample Matrix Spike	± 10%	If the recovery of any analyte falls outside the designated acceptance limits and the QCS is in control, the recovery problem is judged matrix induced. Repeat the LFM and if the sample results are again outside the acceptable recovery range, the sample should be reported with a “matrix induced bias”	1/10
Laboratory Duplicate	10%	If the RPD fails to meet the acceptance limits, the samples should be reanalyzed. If the RPD again fails to meet the acceptance limits, the sample must be reported with a qualifier identifying the sample analysis result as not having acceptable RPD for duplicate	1/20

10 CALIBRATION AND STANDARDIZATION

10.1 Calibration – Daily calibration must be performed before sample analysis may begin.

10.1.1 Reagent water is used as the “zero point” in the calibration. The standards are calculated by the following equation:

$$\text{mg DOC/L} = (A_{\text{STD}}) / m$$

Where: A_{STD} = Area of the standard
m = slope of the regression line

10.1.2 DOC sample concentration is calculated using the following equation:

$$\text{mg DOC/L} = (A_s - \|y\|) / m$$

Where: A_s = area of the sample
 $\|y\|$ = absolute value of the y intercept
 m = slope of the regression line

11. References:

- 11.1 EPA Method 415.1. Determination of Total Organic Carbon in Water using Combustion or Oxidation.
- 11.2 Standard Methods for the Examination of Water and Wastewater. Approval date 2000. Method 5310B: High Temperature Combustion Method.
- 11.3 Sugimura, Y. and Y. Suzuki. 1988. A high temperature catalytic oxidation method for the determination of non-volatile dissolved organic carbon in seawater by direct injection of a liquid sample. Mar. Chem. 24:105-131.

Appendix

I

PROCEDU

RE

Daily Operations

Make sure the 2nd stage of the regulator on the air tank (Ultra Zero Grade Air, size A) is set at no higher than 30 psi. Replace the tank when the tank pressure falls below 500 psi.

To turn on instrument, push the on/off switch on right side of instrument to on, and then push button located on front of instrument. The front indicator light will cycle through the colors ending with orange, which means the instrument is in a not-ready state. The indicator light turns green when the instrument is up to temperature and all parameters are OK. The light will be blue while the instrument is running samples. If the indicator light is red, refer to the software and the manual to determine the problem. If necessary, call Shimadzu (1-800-477-1227) for tech support.

Open software by clicking on the TOC-L sample table icon. There is no password. Just hit enter when password screen appears.

Open a new sample table by clicking on NEW in the toolbar. Click OK. Then hit CONNECT located in the tool bar. A sample table must be open to connect the instrument. The furnace automatically turns on.

At this time, refill the dilution, reagent blank, and rinse water bottles. The reagent blank water is in the 500 ml Teflon bottle or brown glass TOC reagent water bottle beside the instrument. The rinse water bottle is located behind the autosampler. The dilution bottle is located on the left side of the instrument along with the 9 N H₂SO₄ bottle, 1 N HCl bottle, and the drain bottle. Sulfuric acid is used in the NPOC analysis, and the HCl is used in the TIC analysis. Check the volume of the acid bottle in use, and the drain bottle. The liquid level of the drain bottle should be just below the arm. 250 mls of 9 N H₂SO₄ or 1 N HCl is plenty for several weeks of analysis. Unless the dilution water is being used in serial dilutions of the standard curve, it is not necessary to change daily. Replace weekly regardless.

Open the front door of the instrument and check the liquid level of the humidifier located on the right hand side. The level should be between the high and low marks. Add reagent water as needed by removing cap at top.

Check the level of liquid in the Type B Halogen Scrubber (the long tube next to the syringe which contains the rolled stainless mesh). Add 0.05 N HCl (40 ml reagent H₂O + 1 ml 1N HCl) so that the level is an inch or so above the level of the mesh screen. There is a small drain line attached to the 8-port valve at port 6 which is frequently pulled out of the drain when removing the cap of the Type B Halogen Scrubber. When recapping the

scrubber, ALWAYS check that the small tubing from port 6 on the 8 port valve is in the black capped drain port behind the scrubber. Replace the 0.05 N HCl each time the column is changed.

It is recommended that these next two steps should be performed before each run. An explanation of the Maintenance Menus can be found in the User's Manual, Chapter 7.6 p.302-308.

Before running blanks or beginning a sample run, from the program, select Instrument and Maintenance. Click on Residue Removal, then click start. Close when finished.

Next, under Instrument Maintenance, select Replace Flowline Content, and then click start. Close when finished.

If the instrument has been sitting unused, or if several runs of high salt samples have been analyzed, perform a TC Regeneration. Again, under Instrument Maintenance, select Regeneration of TC Catalyst, and then click start. This takes several minutes. Close when finished.

Loading samples: Read Section in Full before proceeding.

The volume of the sample vial is 24 mls. If the samples are contained in Teflon bottles, the volume of the bottles is 30 mls, which means, in most cases, the analysis is volume limited. Other types of containers (glass or PETG) have a larger volume. Fill the sample vial approximately half full. The absolute minimum volume to use in the sample vial is 10 mls. Choose a sample with maximum volume in the Teflon bottle ahead of time to be the QA sample for duplicates or to make a spike. Cover each sample vial with a septum (or foil square if not available) and secure with an open septum cap.

Standard Curve:

The reagent blank water is in the bottle beside the instrument. This bottle is considered Position 0 on the sample wheel.

Load the other standards in the curve in the first several slots of the wheel. There are 2 stock solutions for standards. One is marked for the calibration standards and CCVs, and the other is marked for the initial calibration verification (ICV) standards. The ICVs are not used in the curve, but are positioned within the middle of the curve and are analyzed before any samples are run.

QA/QC

Analyze a certified reference control sample (CRM) after every 10 samples along with a blank and CCV. With each batch of control samples, a method is created in the control sample folder. To insert a control sample, highlight the line in the sample table. Click on INSERT on the tool bar, and then click on Control Sample. Once the folder is open, click on the appropriate file. The control CRM will be inserted above the highlighted line.

Analyze a blank, the lowest standard, and a CRM (or a mid-range standard) every 10 samples. The CRM's are frozen in 30-ml or 60-ml bottles. Fill 2 sample vials if analyzing more than 20 samples. Fill a 24 ml sample vial to the shoulder with CRM, cover with septum and cap. There is enough volume to sample the vial three times. When inserting the control sample in the sample table, assign the same vial position for each time. The autosampler is capable of returning to a particular vial site.

After the initial CRM, load a reagent blank in a vial. This is considered a cleanliness check of each batch of vials. Using the same source of water, make a blank spike using the 10.0 ppm standard. This is the laboratory fortified blank and is considered a control sample. Load the LFB after the clean check blank.

For the sample chosen to duplicate, fill the vial to the shoulder and cover. Indicate on the bench sheet at the appropriate location that the duplicate is to be inserted at that spot. If sample volume is not an issue, two sample vials can be used instead.

For the sample chosen to be spiked, withdraw 10.0 mls of sample using a volumetric pipet and add it to a sample vial. Then add 1.0 ml of the 10.0 ppm or the 20.0 ppm standard curve to the vial. Cover and cap, then gently shake to mix. Put the spiked sample in the proper location in the sample wheel. With the leftover sample, pour into another sample vial as the original sample. There is usually not enough volume to sample rinse the vial used for the spike or original sample.

Analyze spikes every 10 samples and duplicates every 20 samples.

End the run with blanks and CCVs, with the last control sample inserted between the bracketing standards.

Sample Table:

To create a new calibration file, refer to the User's Manual, Chapter 4.1 pp. 89-93, and follow the Calibration Curve Wizard Setup. Several curve templates are set up and are overwritten with new curve data each time they are used.

Create a method by clicking on File/New/Method and follow the Method Wizard Setup. Refer to the User's Manual Chapter 4.1 pp.94-96. A new method is created with each run.

Use drop down box to select type of analysis (i.e.: NPOC). Leave default Sample Name and Default Sample ID empty.

Enter the file name, and then click Next. (Example: dnr st martins041213)

The calibration curve is chosen on the next screen. Click Next again. Confirm the injection parameters to match the calibration curve. Confirm that Multiple Injections is checked. Click Next again.

Use default settings on the next page, and None for Pharmaceutical water testing on the last page.

Click Finish. The method is complete.

Editing the Sample Table:

Highlight the first line of the sample table to insert information. From the toolbar at the top, click on Insert.

Insert 3-4 conditioning blanks by clicking on Multiple Samples. Follow the wizard prompts. The water for conditioning blanks is the same as the reagent water in position 0.

Highlight the next available line to insert the calibration curve. Click on Insert/calibration curve. Choose the proper calibration file.

Highlight the next available line to insert multiple samples. Follow the wizard prompts. Leave the Sample Name and Sample ID blank.

Once the sample table has been set up, enter the sample names and IDs.

It is easiest to insert Control samples after the sample names and IDs are in place. Highlight the line below where the control sample is to be inserted. Click on Insert and select Control Sample. Choose the proper file.

When all sample and control information is entered into the table, enter the vial position numbers. Click on the carousel icon (looks like a birthday cake) in the sample table toolbar. The vial positions correspond to the numbered positions on the bench sheet. Be sure duplicate samples are numbered to match the original if sampling from the same vial. Click OK when finished.

Proof all entries and save the sample table. Click File/Save As to name the file.

Example: 2013_05_09_dnr st martins 042213

Highlight the first line of the sample table.

Click START. The Measurement Start Window is displayed. Click on the procedure to be performed when the analysis is complete. The instrument is kept running except over weekends. If no samples are to be run the next day, select Keep Running in case samples go off scale and need to be rerun. They can be inserted at the end of the sample table and run. Insert sample information and vial positions, then SAVE the file.

To open the Sample Window, click on the graph icon on the sample table to view peak information.

Accessing the data:

When the run has finished:

To save the file to another source (i.e. the shared network drive), (TOC-L-1) click File/Save As. TOC-L-2 does not communicate with the shared network drive in this capacity. With TOC-L-2, to save the standard curve, highlight the standard curve in the sample table. Select Print: Print highlighted. Print to XPS file. Name file as a curve file. The file is saved to the shared network drive. Open the file in the Microsoft XPS Viewer. Print to printer and save to the desktop.

To export data, click File/ASCII Export. Save the file in each form, Normal and Detailed. The Normal file contains only concentration information. The Detailed file includes all injection data. The ASCII files can now be opened in Excel. This works with both instruments.

Open TOC-L Sample Table on the desktop. Open the file from the shared network drive. Save the file to the desktop using the same file name.

To print the calibration curve information, highlight the calibration curve line in the sample table. Select Print on the toolbar, and Highlighted.

Below is an example of the TOC bench sheet.

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SHIMADZU TOC-L DATA SHEET			Column Run Counter			
TODAY'S DATE:		TOC-L 1 2	CRUISE :			
ANALYST: NLK OTHER:						
FILE NAME:						
SPIKE CONC.: 10 mg/L...20 mg/L			INJECTION VOLUME: 50 uL		WORKING STDS MADE:	
VIAL#	SAMPLE ID		VIAL#	SAMPLE ID		VIAL#
0	DHOH		31			62
1			32			63
2			33			64
3			34			65
4			35			66
5			36			67
6			37			68
7			38			69
8			39			70
9			40			71
10			41			72
11			42			73
12			43			74
13			44			75
14			45			76
15			46			77
16			47			78
17			48			79
18			49			80
19			50			81
20			51			82
21			52			83
22			53			84
23			54			85
24			55			Pre Run Checks
25			56			Mark All That Apply
26			57			Residue Removal (Daily) []
27			58			Flowline Wash (Daily) []
28			59			Regenerate Catalyst (as needed) []
29			60			New Column (as needed) []
30			61			LFB SPIKED WITH 10.0 mg/L STD

APPENDIX E: UMCES CBL NASL CHAIN OF CUSTODY

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UMCES CHESAPEAKE BIOLOGICAL LABORATORY NUTRIENT ANALYTICAL SERVICES						PO Box 38 1 Williams St. Solomons, MD 20688 (410) 326-7252 (410) 326-7209 fax				Contract#/Billing Reference		Page _____ of _____		
Project:						# of Containers								
Client:						Container type								
Send Results to:						Parameters to be Analyzed/ Volumes Filtered								Client Comments
Address:														
Phone:														
CBL ID #	Sample ID#	Date Sampled	Time Sampled	Sample Matrix	Sampler's Initials									
Relinquished by:		Date/Time	Received by:		Relinquished by:		Received for Laboratory by:		Date/Time					
Relinquished by:		Date/Time	Received by:		Date/Time	Shipper:		Airbill No.:						
Relinquished by:		Date/Time	Received by:		Laboratory Comments:								Temp.	

APPENDIX F:
PREPARATION OF HYPOXIA MAPS
AND SUMMARIES BASED ON POINT
DATA IN ARCMAP 10.5

Standard Operating Procedure

Title: Preparation of Hypoxia Maps and Summaries Based on Point Data in ArcMap 10.5

Date: 5/17/18

Prepared by: Jessica Bonamusa

BACKGROUND INFORMATION:

Interstate Environmental Commission (IEC) staff collects dissolved oxygen data in Long Island Sound throughout the summer at established sampling locations. This SOP establishes standard procedures for using interpolation to create hypoxia maps and summaries based on IEC's data from the Long Island Sound sampling points.

Inverse Distance Weighting (IDW) is a type of interpolation that assigns values to unknown points based on their proximity to known points. In calculating unknown points, the 'weight' or importance of known points decreases with distance. For example, in calculating the value of an unknown point B, an IDW interpolation will use B's proximity to a known point A. If B is very far from A, then A's value will have little effect on the estimated value of B. However, if B is close to A, then A's value will have more 'weight' in the calculation of the estimated value of B. This type of interpolation is useful for calculating parameters like dissolved oxygen, because one can assume that areas near each other will have fairly similar dissolved oxygen levels.

SCOPE AND APPLICATION:

This SOP will provide detailed guidelines on how to calculate hypoxic areas both manually and with a tool created by IEC staff. It should be noted that the automated tool is meant to analyze LIS data from all 12 runs (or more) at once.

For individual runs, analysis is done manually.

This SOP will detail how to import Long Island Sound data into ArcMap, and how to interpolate dissolved oxygen data to create a surface. It will then detail how to create a final map that will depict the extent of low dissolved oxygen in Long Island Sound for display. Finally, it will describe how to make an estimate of hypoxic areas based on cell counts. While the variable discussed here is dissolved oxygen, other variables, such as salinity and pH, can be used to create similar displays with these methods.

SUMMARY:

This SOP will describe how to create hypoxia maps and summaries using Inverse Distance Weighting (IDW) interpolation on ArcMap 10.5 or higher.

Dissolved oxygen data and station information (geographic coordinates- latitude/longitude) are imported into an ArcMap document, and interpolated across a grid using the Inverse Distance Weighted function of the Spatial Analyst package to estimate dissolved oxygen concentrations in the bottom waters of Long Island Sound. The map is then exported for future use.

EQUIPMENT:

No hardware is required except for a GIS capable computer. Required software includes Microsoft Excel, ArcMap 10.5 or higher, as well as the ESRI Desktop Spatial Analyst package.

PROCEDURE:

Please note: There are two procedures shown here. The first is the automated process. The second is the manual process. The automated process is the same as the manual process, except that it is simplified into a single tool, and designed to analyze multiple runs at the same time rather than a single run at once. To analyze a single run, please use the manual method.

Process:

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AUTOMATED PROCESS:

Step 1. Save data in the correct format.

Data must be saved in Excel as a CSV file. The tool can only read the data if it is in the proper format. Longitude and Latitude must be in NAD 1983. Be sure the headers read as following:

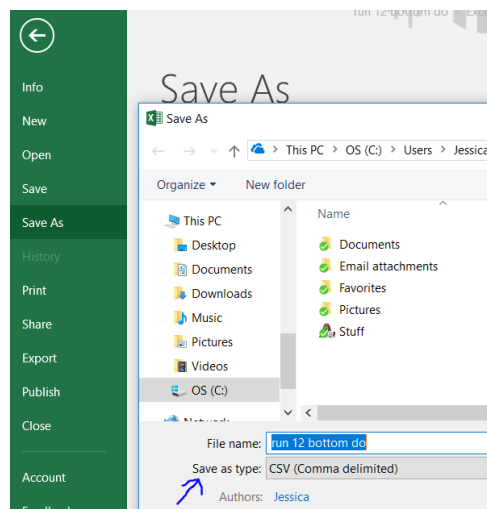
STATION	WATER COLUMN DEPTH	LATITUDE	LONGITUDE	DESCRIPTION	RUN1	RUN2	RUN3	RUN4	RUN5
---------	--------------------------	----------	-----------	-------------	------	------	------	------	------

It must be in CAPITAL. The headers must go through RUN12, even though only RUN5 is shown above. If a section has no data, leave it blank. For example:

STATION	WATER COLUMN DEPTH	LATITUDE	LONGITUDE	DESCRIPTION	RUN1	RUN2	RUN3	RUN4	RUN5
E-12	4	40.8487	-73.8045	Eastchester Bay mid-channel at N 6	6.16		6.03	3.82	
A1	26	40.8013	-73.8268	East of Whitestone Bridge	6.03	4.9	4.36	3.82	3.06
A2M	35	40.7992	-73.7913	East of Throgs Neck Bridge	4.86	4.92	3.49	2.94	2.5
8-403	3	40.7778	-73.7608	Little Neck Bay - ~0.2 nm W of yellow nun "B"	6.27		4.08	4.86	2.56
8-405	3	40.7888	-73.7582	Little Neck Bay - ~0.15 nm North of LNB mid- channel buoy	6.46	6.57	4.8	6.31	3.61

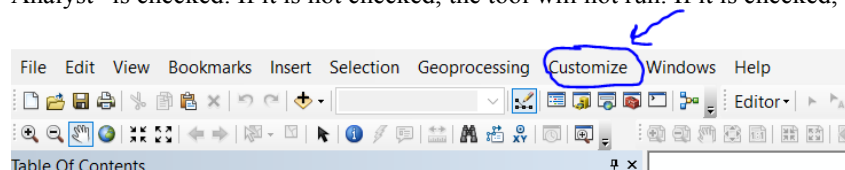
Step 2. Save excel data as a .csv file.

Open the excel document. Go to “file” and click “Save as.” Under “Save as type,” scroll down to CSV (Comma delimited). This will save the data as a comma delimited (.csv) file, which ArcMap can read.



Step 3. Check Spatial Analyst.

Open ArcMap. Go to “Customize” on the main toolbar at the top. Select extensions to make sure that “Spatial Analyst” is checked. If it is not checked, the tool will not run. If it is checked, close the dialog box.

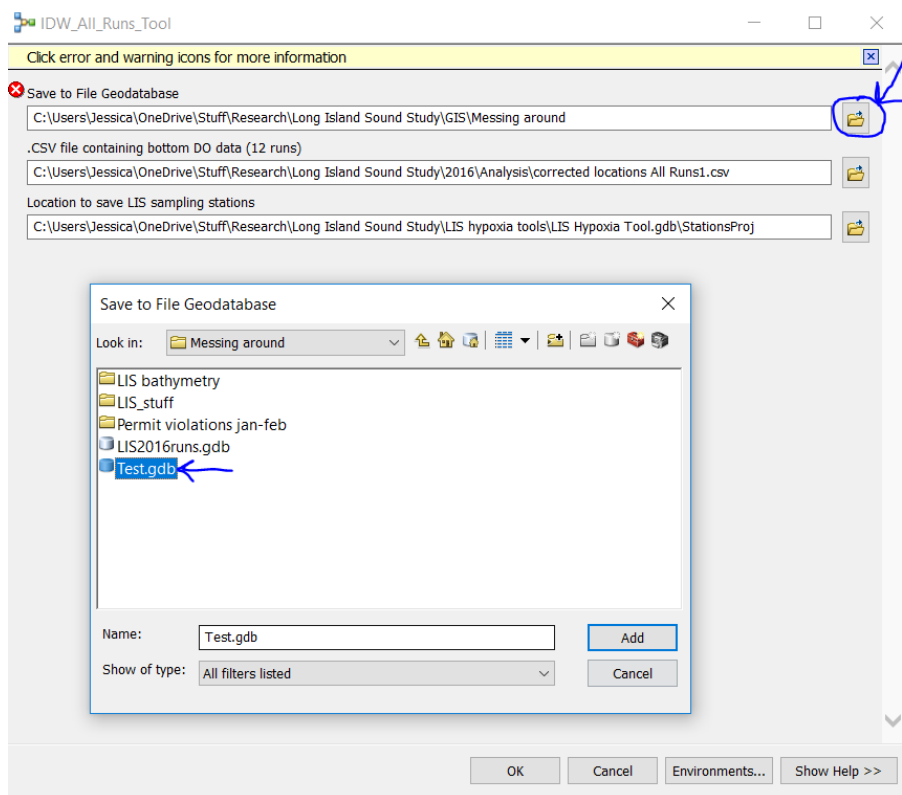


Step 4. Open the tool and run the IDW.

Open ArcMap and navigate to the LIS Hypoxia Tool Geodatabase. Double click on it, and then double click on the IDW_RUNS toolbox. Double click on the IDW_All_Runs_Tool. This will open the dialogue box.

The tool presents three menus. “Save file to geodatabase” is where the IDW calculations will be saved. It must be saved to a geodatabase rather than a folder¹. In the tool, click on the folder icon next to “Save file to geodatabase,” and then navigate to the geodatabase in which the data will be saved. Highlight it, and then click “Add.”

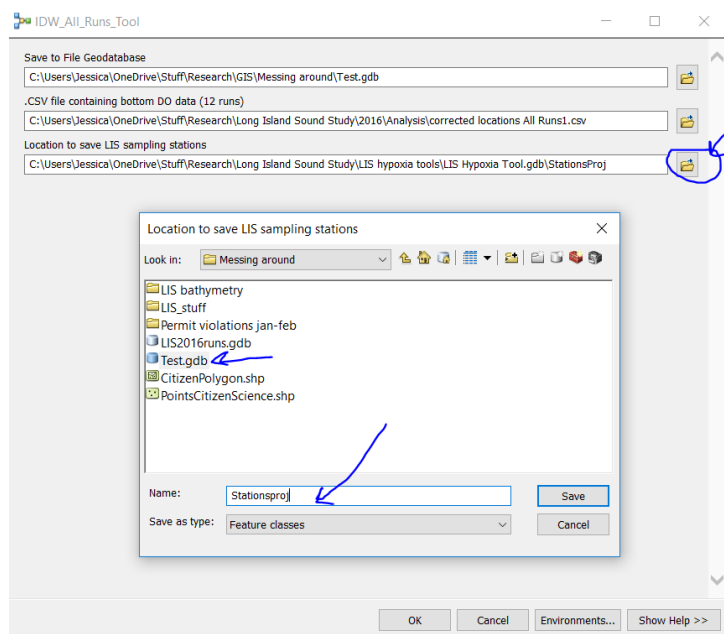
Click the folder icon next to the “.CSV file containing bottom DO data (12 runs).” Navigate to the .CSV file with the



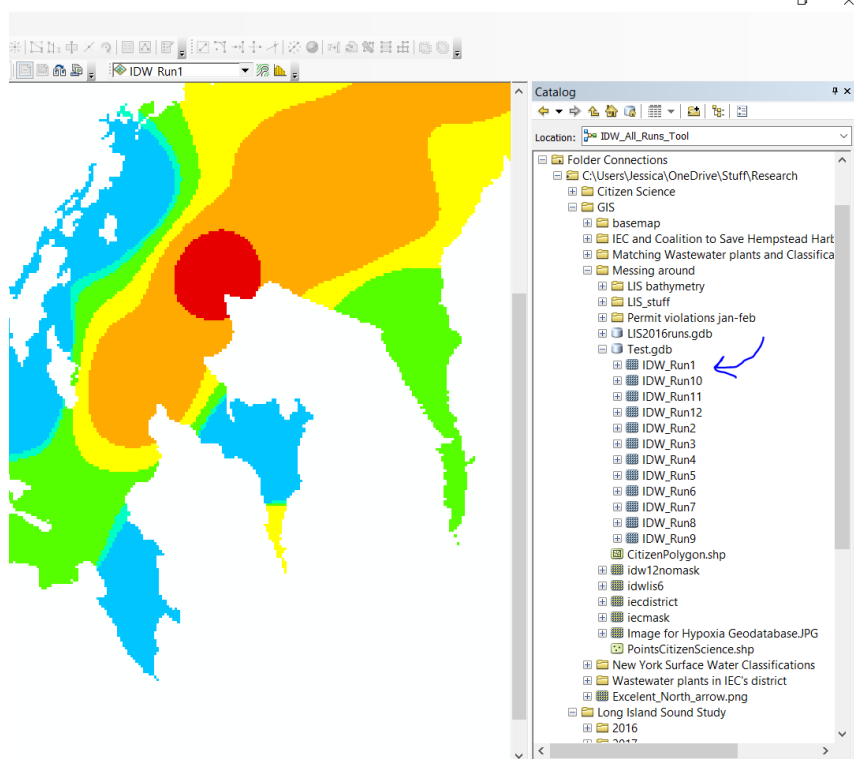
LIS run data in the proper format and click “add.”

The tool will automatically produce a shapefile of LIS sampling stations. “Location to save LIS sampling stations” can be filled with the file in which to save this shapefile. It is recommended to save it to the same geodatabase as the IDWs. If saving it somewhere else, be sure to save it to a geodatabase and not as a feature class to a regular folder. Geodatabases permit “null” values, whereas feature classes do not, so saving it as a feature class will create errors in the IDW calculation.

¹ To create a geodatabase, right click on a folder in Arc Catalog and select “new.” From the drop down menu, select “File Geodatabase.” Name the geodatabase.



Click “Save.” Click “Okay.” The tool will run.
Navigate to where the IDWs are saved, and drag them into the map to see them.



Skip to “CREATING THE MAP.”

MANUAL PROCESS

All data must begin in an excel spreadsheet, which contains, at the very least, Longitude, Latitude, Station ID and DO

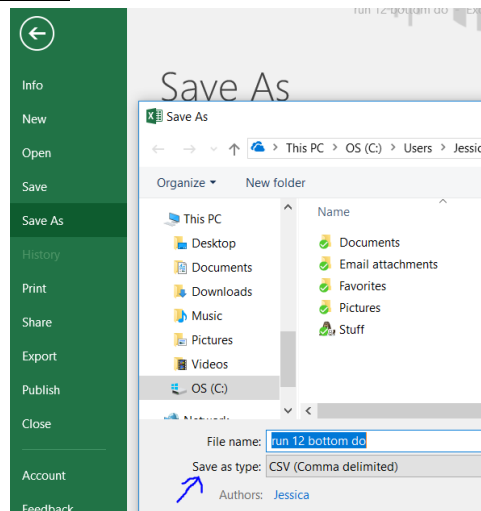
values. Longitude and Latitude MUST be in NAD 1983.

Importing Data into ArcMap 10.5

Step 1. Save excel data as a .csv file.

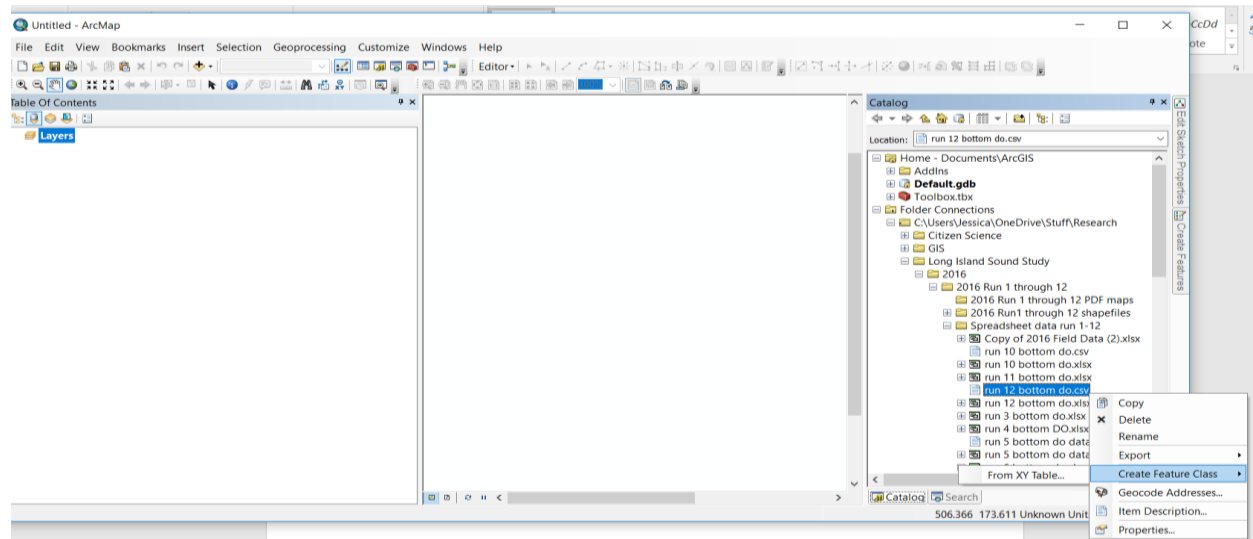
Open the excel document. Go to file and click “Save as.” Under “Save as type,” scroll down to CSV (Comma delimited). This will save the data as a comma delimited (.csv) file, which ArcMap can read.

Step 2. Import data into ArcMap 10.5.



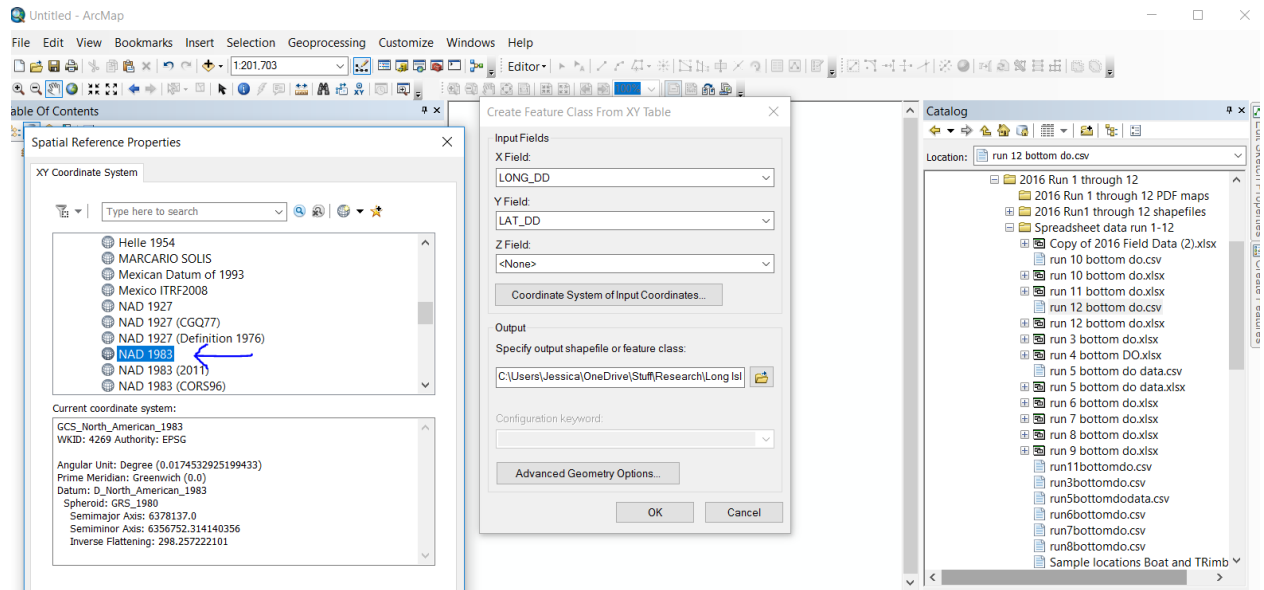
Open ArcMap. Click the “Catalog” tab on the right. If there is no existing geodatabase, one must be created. To create a file geodatabase, right click on the folder in which to save the geodatabase. Scroll to “new” and then “File geodatabase.” Name the file geodatabase.

Navigate to the folder where the CSV is, as shown in the figure below. Right click on the CSV, scroll to “Create Feature Class” and select “From XY Table.”

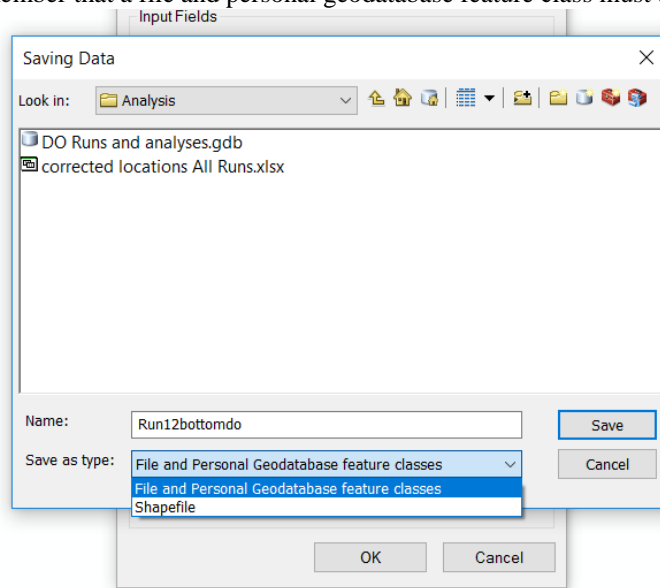


A dialog box will open. Type Longitude in the X field and Latitude into the Y field. The Z field is for parameters such as altitude: leave it blank.

Click “Coordinate system of input coordinates.” Select “Geographic Coordinate Systems” and then scroll to “North America.” Scroll down and select “NAD 1983.” Click “Okay.”



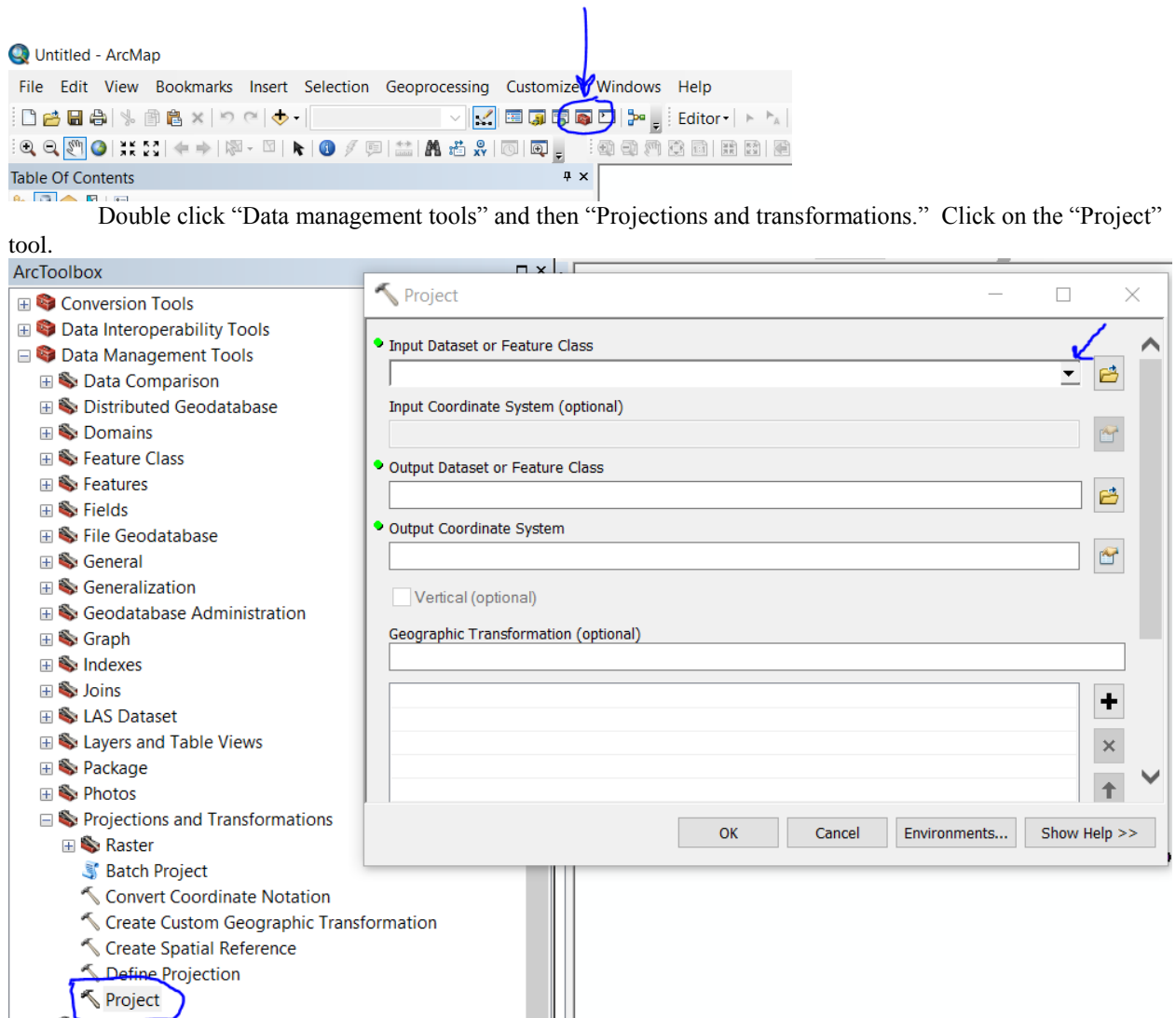
Click the folder icon next to “Specify output shapefile or feature class” and select where to save the shapefile. “Save as type” as a “File and personal geodatabase” and then save it to a geodatabase. This will permit “null” values, if no data was collected. Remember that a file and personal geodatabase feature class must be saved to a geodatabase.



Click save and then okay.
The points should show up in the image.

Step 3. Project data.

Projecting data will correct for the spherical shape of the earth.
Click on the “tools” icon.



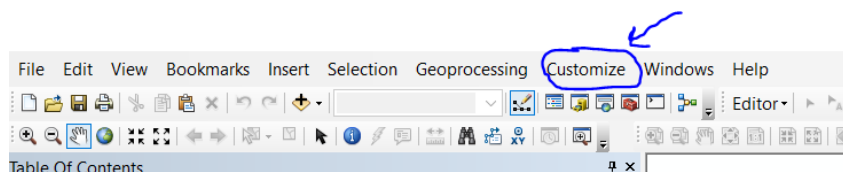
Click the drop-down menu on “Input Dataset or Feature Class.” Select the XY event layer. The box will automatically fill “Input coordinate System” and “output dataset.” Remember that “Output dataset” should be saved to a geodatabase.

Click the icon next to “Output coordinate system.” Double click “Projected Coordinate Systems” and scroll down to “UTM.” Click “NAD 1983.” Scroll down and select “UTM Zone 18N.” Click “Okay” and then click “Okay.” The data has now been imported and projected.

Interpolation

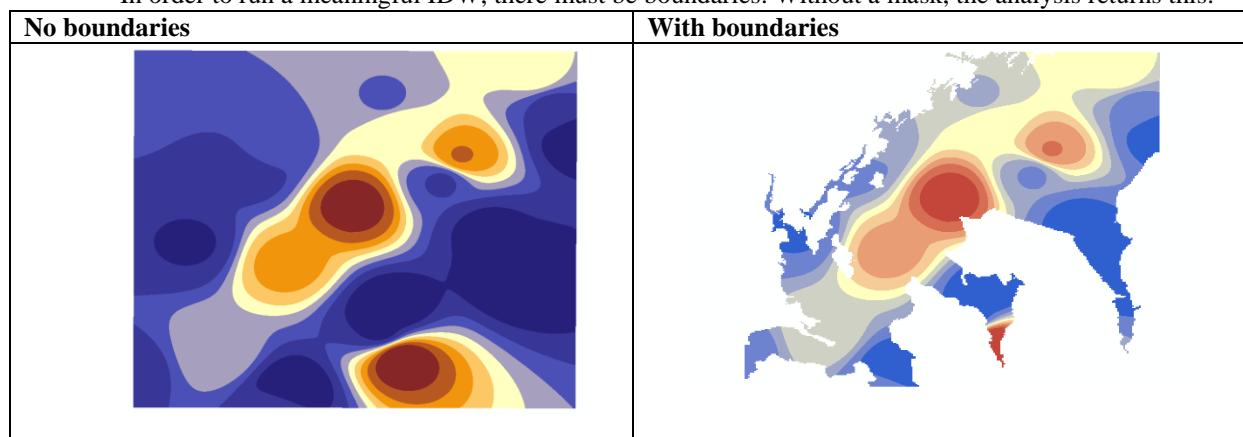
Step 1. Make sure Spatial Analyst is turned on.

Go to “Customize” on the main toolbar at the top. Select extensions to make sure that “Spatial Analyst” is checked. If it is not checked, the tool will not run. If it is checked, close the dialog box.



Step 2. Create a mask or boundaries

In order to run a meaningful IDW, there must be boundaries. Without a mask, the analysis returns this:



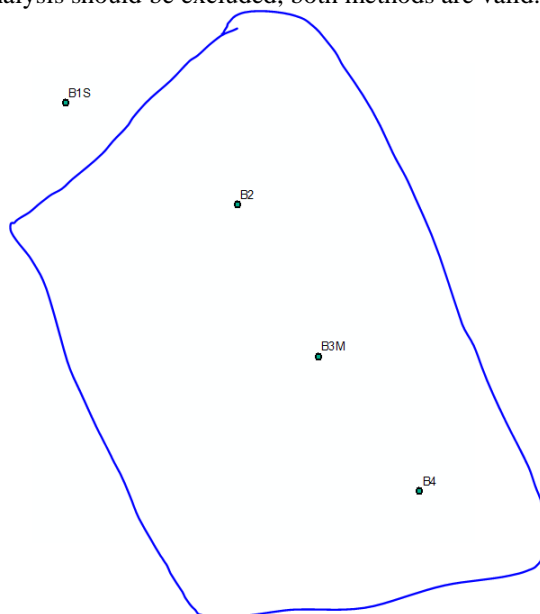
There are two options for creating boundaries in the IDW tool. If there is a file that is a polyline boundary of Long Island Sound, simply select it in “Input barrier polyline features.”

The other option is to use a mask.

The polyline barrier option uses the barrier in the analysis. If the blue line in the image below is a polyline barrier, point B1S will be excluded from the IDW.

If the image below is a mask instead of a barrier, then all points will be analyzed in the IDW, and after the analysis, the final output will be clipped to be the shape of the square.

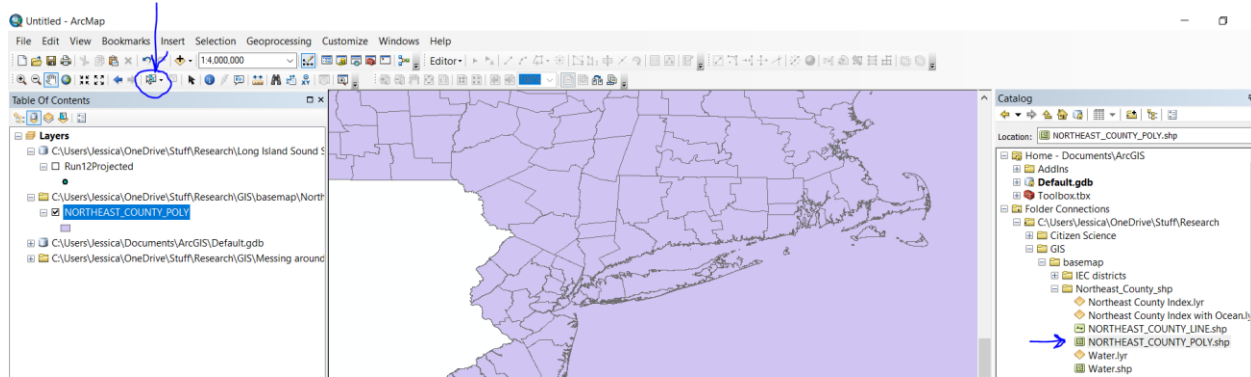
Since no points in this analysis should be excluded, both methods are valid.



To create a mask, retrieve state data. If a mask has already been created from a reliable source, that mask can be used as well. Skip to Step 3, if a mask already exists.

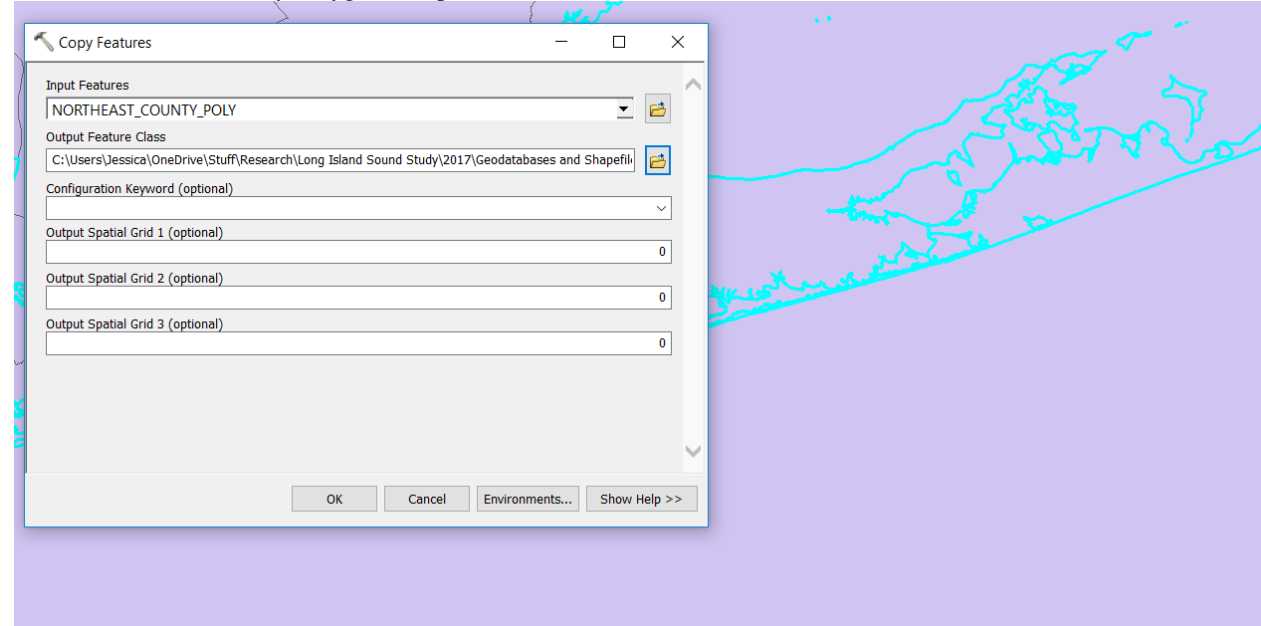
http://www.ct.gov/deep/cwp/view.asp?a=2698&q=322898&deepNav_GID=1707%20 is a clearinghouse for Connecticut GIS data. Scroll down **Northeast County Boundary Line**. Download the data. Save to a folder that can be accessed from ArcMap 10.5.

Input the data into ArcMap10.5. It can be dragged in from the catalog.
Click the select tool.



Select Long Island Sound by clicking on the Long Island Sound part of the map. The lines will turn blue.

Open the “Copy features” tool. It can be searched for in the “search” tab on the right. Input features are the selected Northeast County Polygon. Output feature class is where the result is saved. Click OK.



All that remains should be the water, which can be used as a mask.

Step 3. Run the IDW.

Click on the “tools” icon.

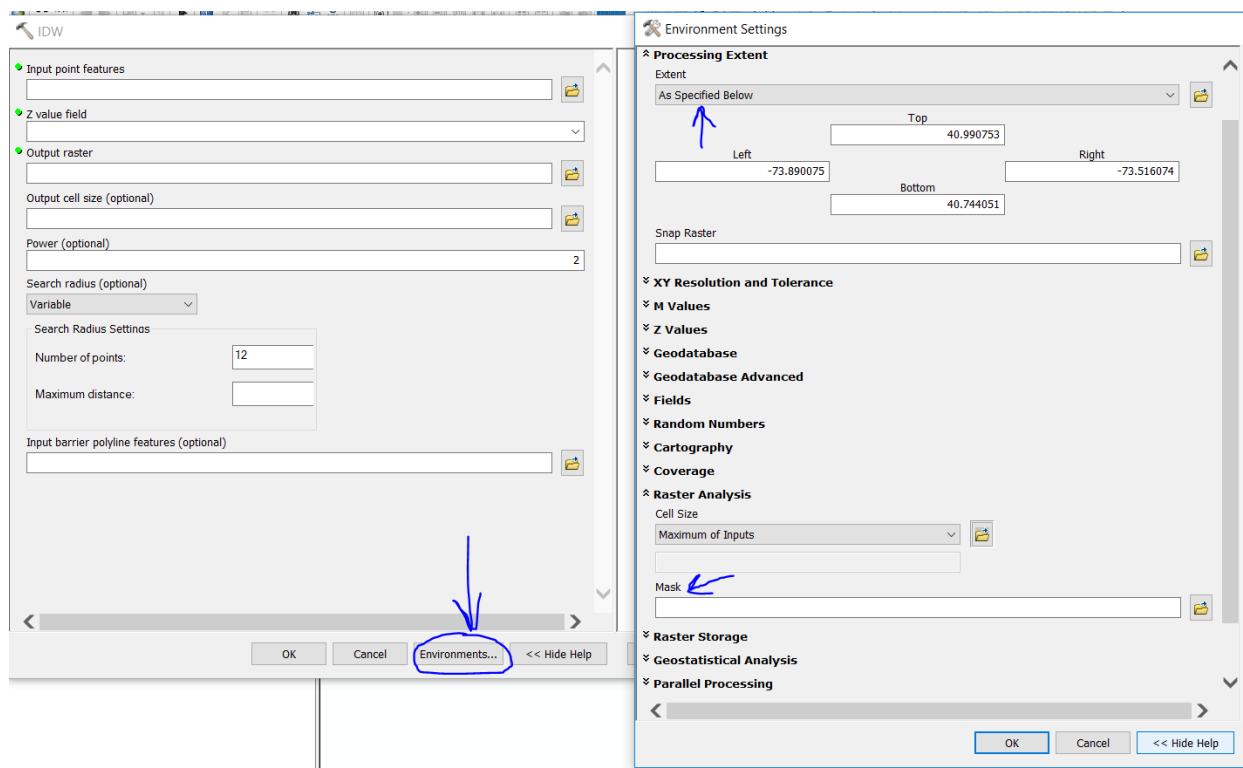
Click “Spatial analyst tools” and then “Interpolation.” Then select the tool called “IDW.” Alternatively, the IDW tool can be accessed by searching. Be sure to select the “IDW (Spatial analysis)” option when searching.

Under “input point features,” insert the point features to be interpolated. The “Z value field” is the dissolved oxygen, or any other parameter that is to be interpolated. Output Raster should be named and saved in the appropriate file.

Output cell size: Use 250 to standardize.

Power: 4

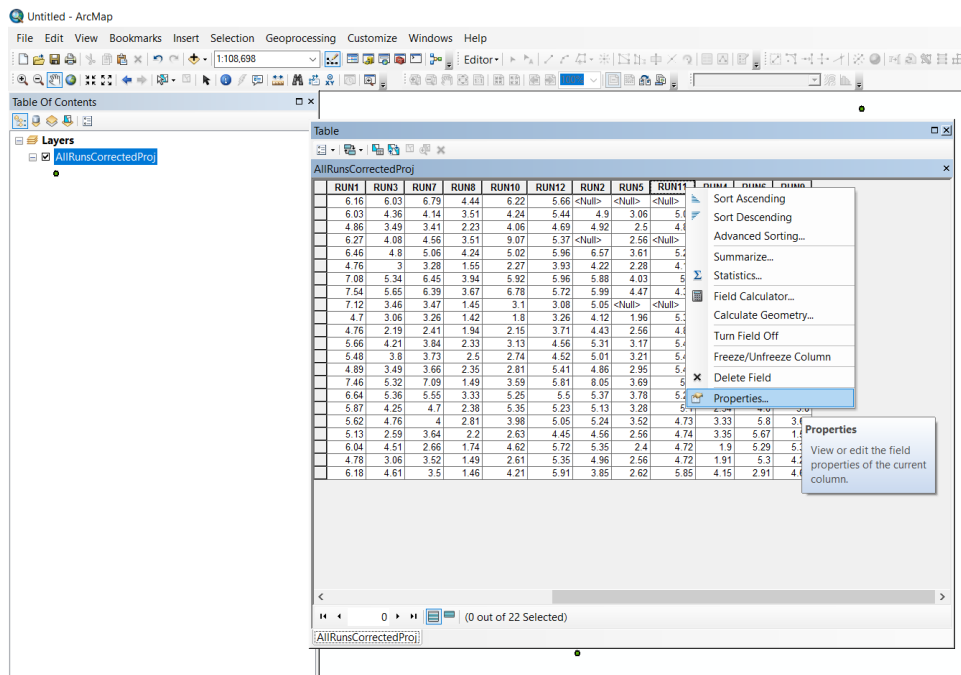
Next, click on “Environments.”



Under “Processing Extent,” fill the following: Top: 40.990753; Left: -73.890075; Bottom: 40.744051; Right: -73.516074. Under “Raster Analysis,” open the drop-down menu and select the mask. Then click “OK” in the settings, then “OK” in the IDW to run the tool.

Step 4. Troubleshooting.

If the IDW does not work or returns an error, the field type of the feature class may be incorrect. Right-click on the LIS sample stations file and click “attributes.” The attribute table should appear. Right click each of the columns and scroll down to “Properties.”



A dialog box will appear.

Field Properties

Name: RUN11

Alias: RUN11

Type: Double

Display

☐ Turn field off

☐ Make field read only

☐ Highlight field

Number Format: Numeric ...

Data

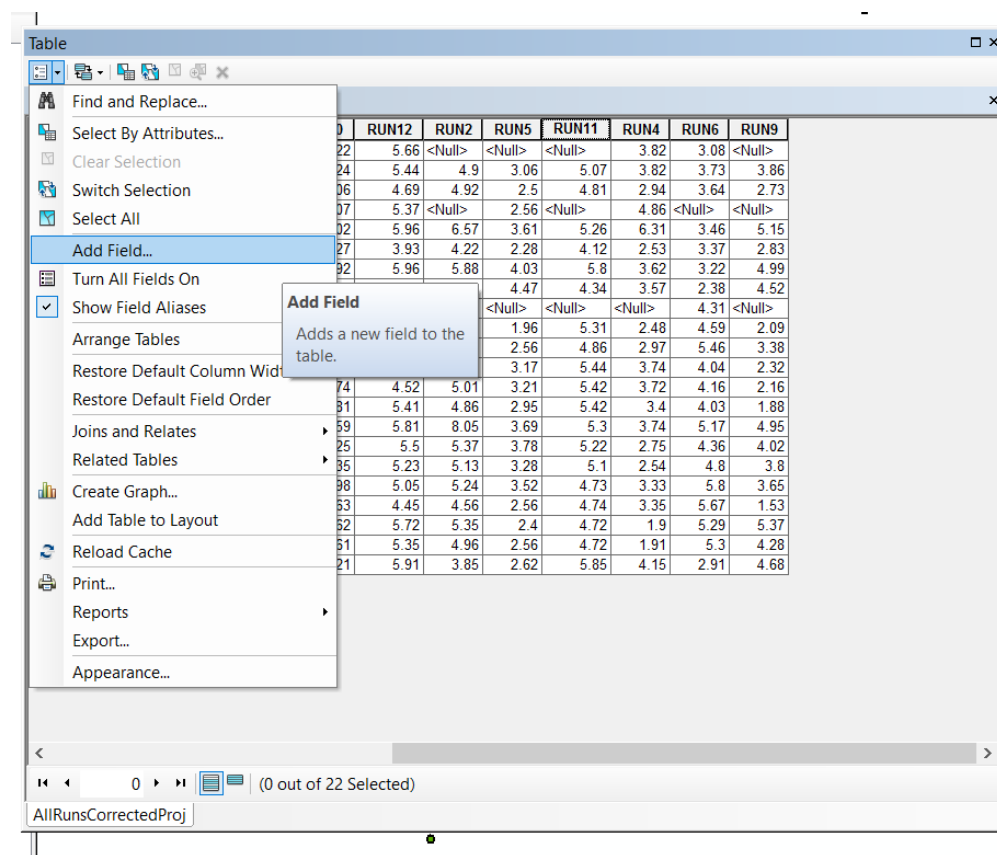
Allow NULL Values	Yes
Default Value	

OK Cancel Apply

The circled portion of the above image should read “Double.” If it reads “String” or “Text,” the IDW will not run.

Sometimes, ArcMap will import fields into an incorrect default. To fix this error, go to the top of the attribute table and

click on the “Table options icon.” Scroll down to “Add field.”



The dialog box below will appear. Re-name the new field and be sure to change the type to DOUBLE.

Add Field

Name:

Type: **Short Integer**

Field Properties	
Alias	
Allow NULL Values	Yes
Default Value	

Change to double

OK Cancel

An empty field will appear. Select it by clicking on the header, and then right click and scroll to field calculator.

Field Calculator

Parser
☒ VB Script ☐ Python

Fields:

- RUN8
- RUN10
- RUN12
- RUN2
- RUN5
- RUN11
- RUN4
- RUN6
- RUN9

Type:
☒ Number
☐ String
☐ Date

Functions:

- Abs ()
- Atn ()
- Cos ()
- Exp ()
- Fix ()
- Int ()
- Log ()
- Sin ()
- Sqr ()
- Tan ()

☐ Show Codeblock

RUN9 =
[RUN9]

About calculating fields Clear Load... Save... OK Cancel

The circled portion is the new field. Scroll up or down in the “fields” section to the old field, in the incorrect format.

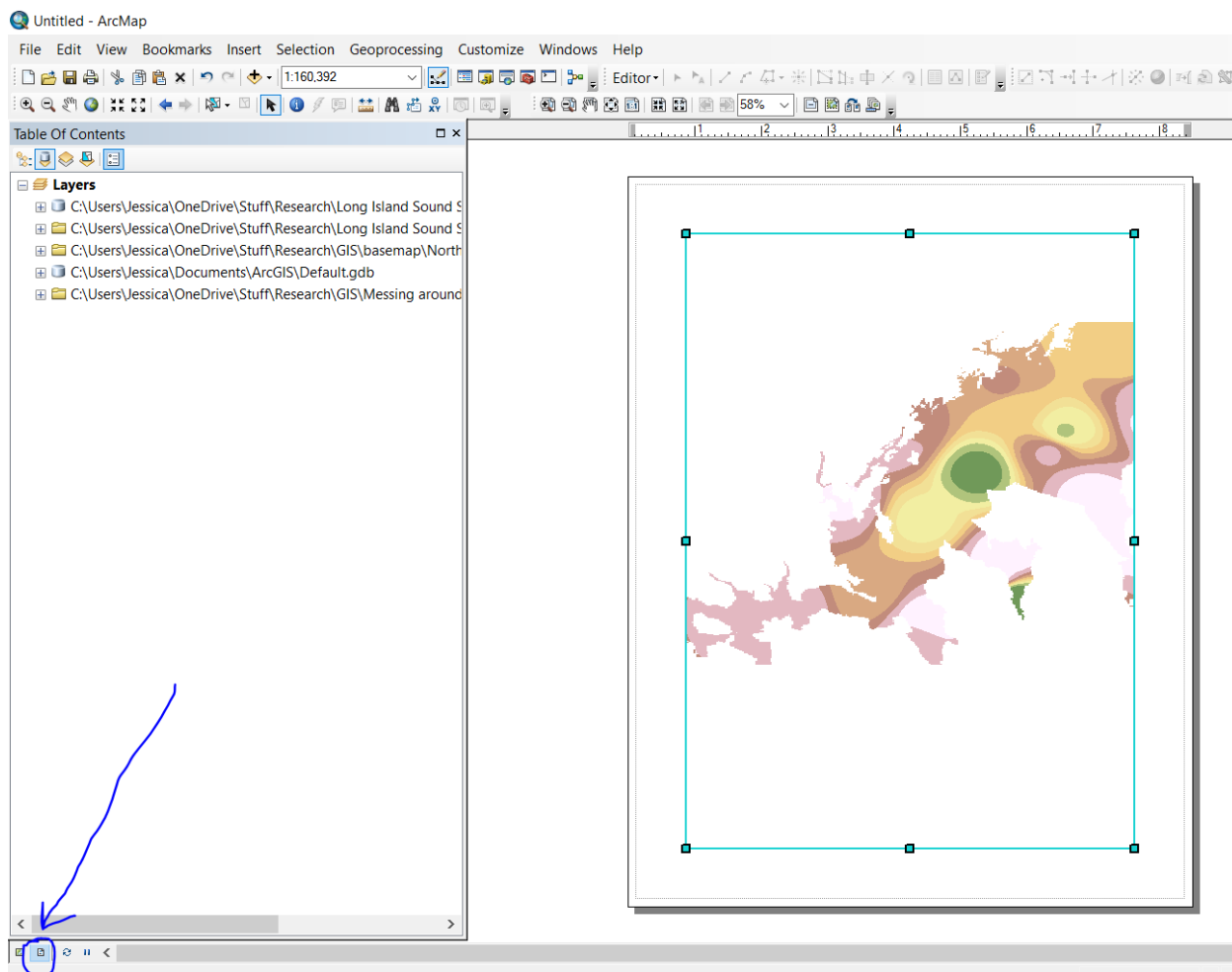
Double click it. Click okay. The new field should be populated with the old values, but in the correct format. Run the IDW again, using the new field instead.

PLEASE NOTE: The automated tool uses this method to change all fields into doubles to troubleshoot this error.

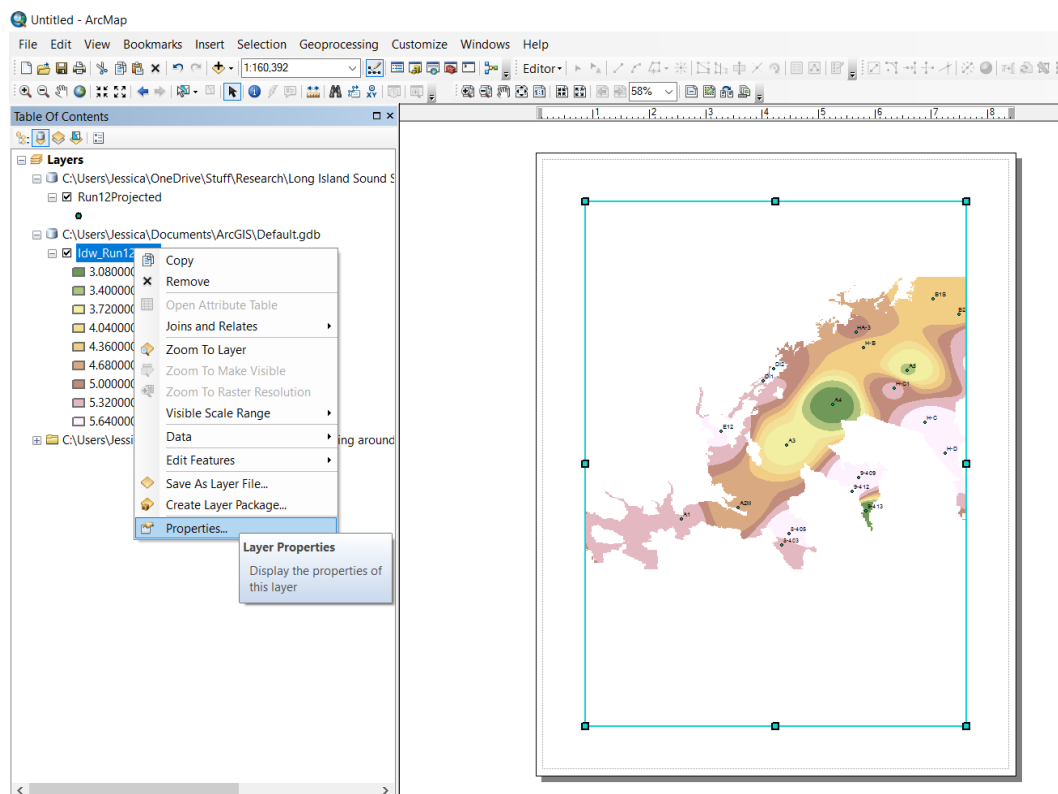
CREATING THE MAP

Step 1. Laying out the map

Click on the “Layout view” icon at the bottom of the map to plan a printable version of the map.



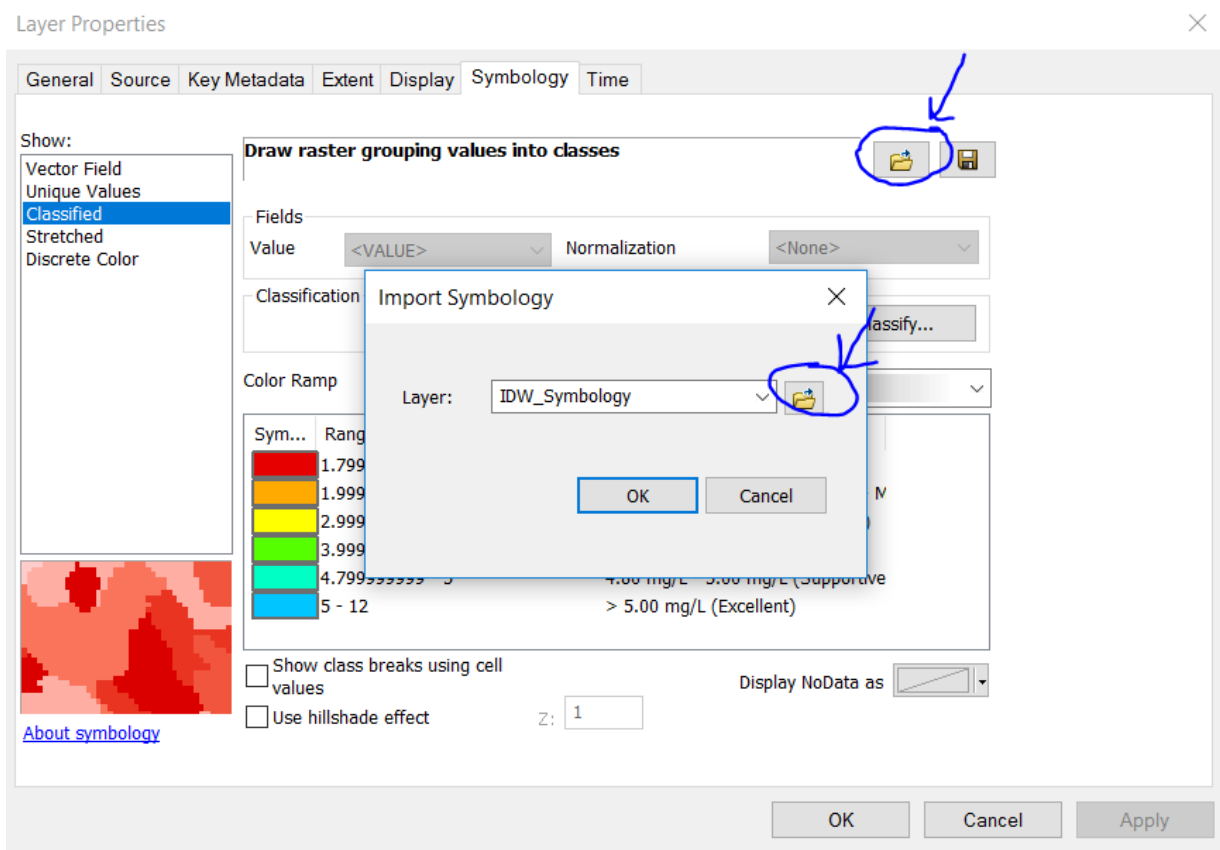
Right click on the IDW layer file in the table of contents and select “Properties.”



In the properties dialog box, click the symbology tab to open the symbology section.

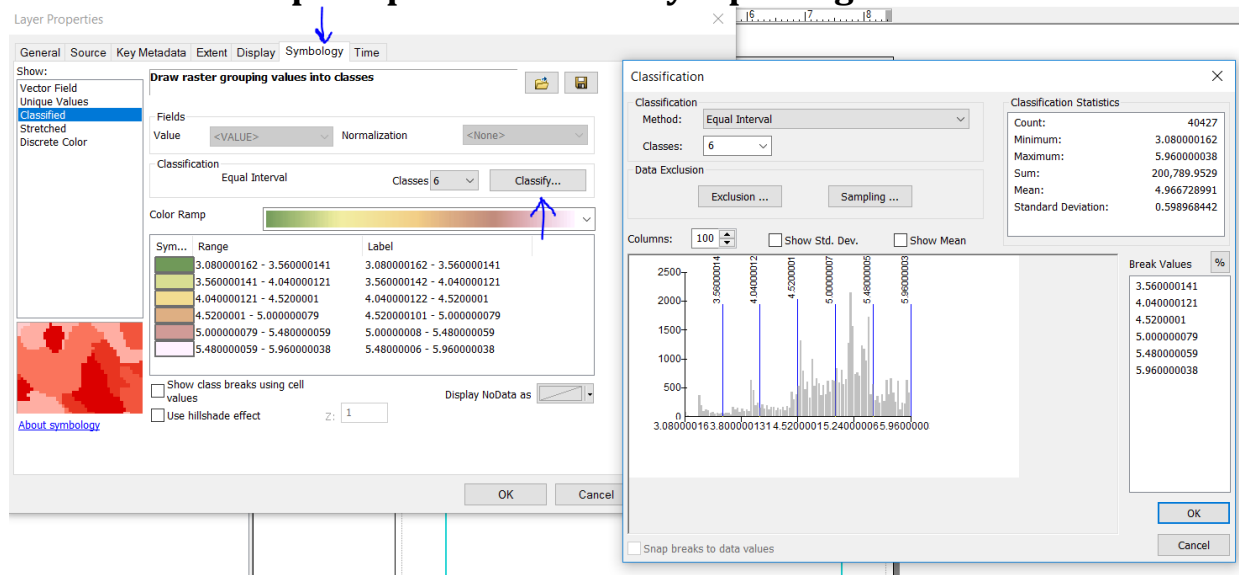
Step 2. Option a: Automatically inputting the classes:

In the properties dialog box, click the symbology tab to open the symbology section.



Click on the file icon next to “draw raster grouping values into classes.” An “Import symbology” dialog box will appear. Click on the file icon next to that, and navigate to the “IDW_Symbology” layer file that is saved in the LIS Hypoxia Tools Folder. Click “Okay” in both dialog boxes, and it will automatically classify into the correct symbology.

Step 2. Option b: Manually inputting the classes:



In the box next to the word Classes, specify the number of intervals to classify. This will depend on your range of DO values, up to a maximum of 6 classes. Those classes are:

1.99 mg/L

2.99 mg/L

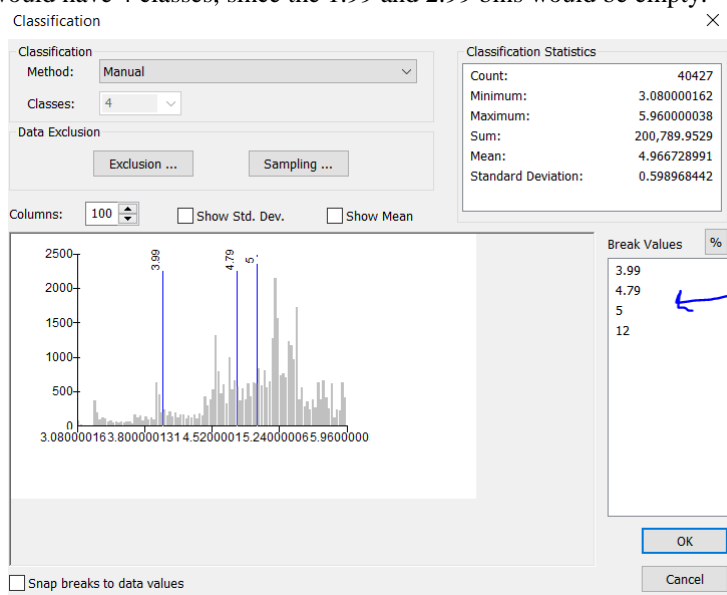
3.99 mg/L

4.79 mg/L

5 mg/L

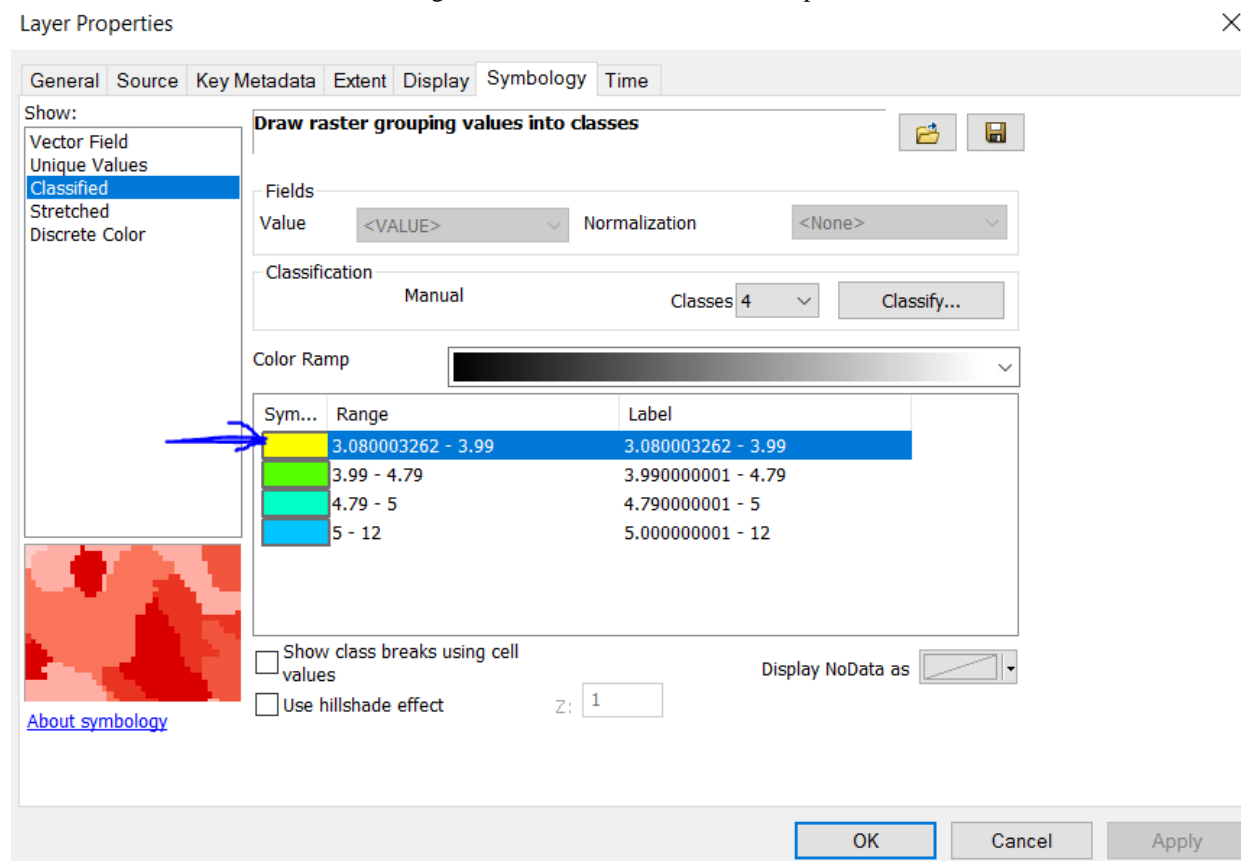
12 mg/L (which represents >5 mg/L)

In the column marked “Break values,” fill in the DO range values. If a class is empty, do not include it. For example, the map shown above would have 4 classes, since the 1.99 and 2.99 bins would be empty.



Click “OK”

Next, click on the colors next to the ranges to choose bin colors for the map.

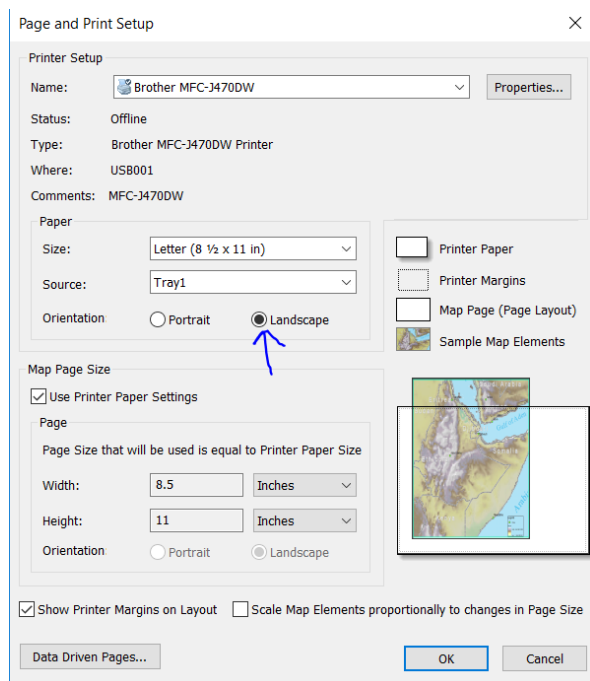


For standardization purposes, the bin colors are:

- 1.99 mg/L – Poinsettia Red
 - 2.99 mg/L – Electron Gold
 - 3.99 mg/L – Solar Yellow
 - 4.79 mg/L – Quetzal Green
 - 5 mg/L – Tourmaline Green
 - 12 mg/L (which represents >5 mg/L) – Big Sky Blue
- Click “OK.”

Step 3. Formatting the image.

Next go to “File” and “Print and page setup.” Turn the map to “Landscape.”



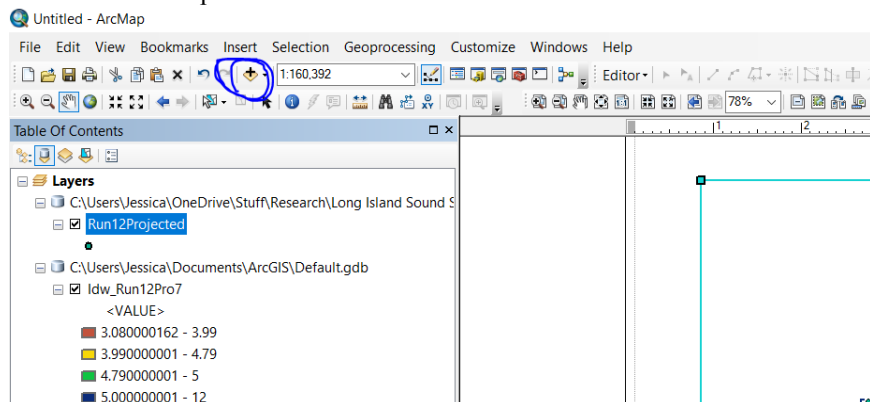
Click “OK.”

Then drag the map so that it fits evenly on the page.

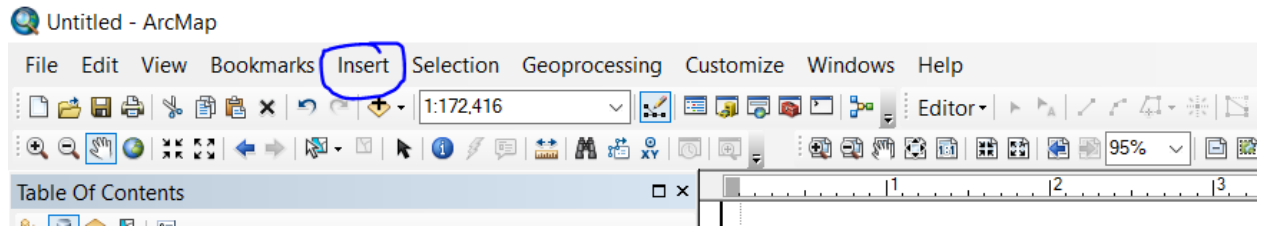
Step 4. Finishing the Map.

Be sure the original point file is checked, so it shows in the map. Right click on the point file, go to properties. Click the “Labels” tab, and then check “Label features in this layer.” Change the font to size 10, and make it bold. Make sure the label field is “Station.” Click “OK”.

Next click the drop down menu next to the “Add data” button and select “Add basemap.”



Select the “Oceans” basemap. It may take some time to upload. Then click the “Insert” tab.

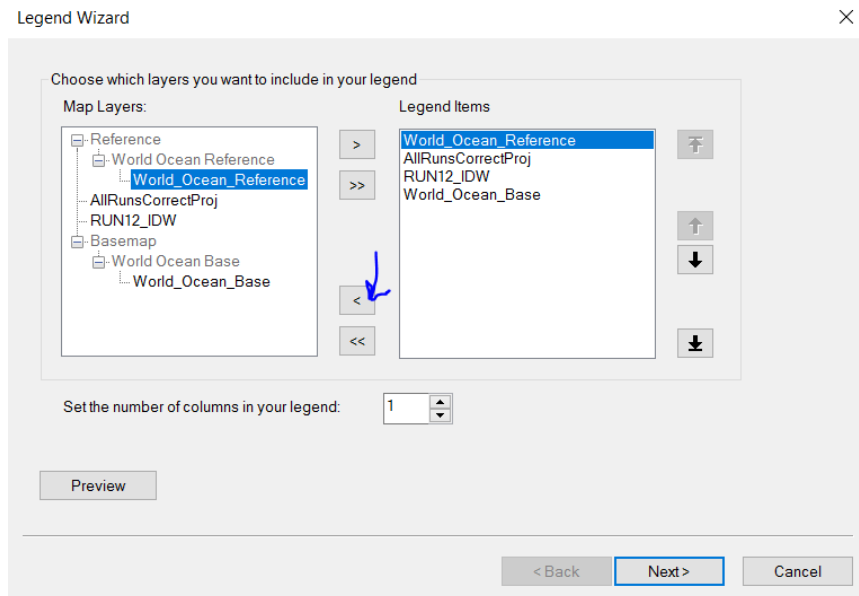


Select “North Arrow” and pick “ESRI North 72.” Drag it to the upper left corner of the map. Right click it and then select “Properties.” Increase size to 100.

Go back to “Insert” and select “Scale bar.” Select “Scale Line 1” and drag it to the lower left corner of the map.

Go back to “Insert” and add text. In the text box, record the date the map was created and by whom at IEC. Include any necessary notes about the analysis, such as whether there were any errors. Drag the text below the scale bar.

Last, go back to “Insert” and select Legend. The Legend Wizard will appear.



Select “World_Ocean_Reference” and hit the < button to remove it from the legend. Do the same with “World_Ocean_Base.” Click “Next.”

The legend title is

INTERSTATE ENVIRONMENTAL COMMISSION

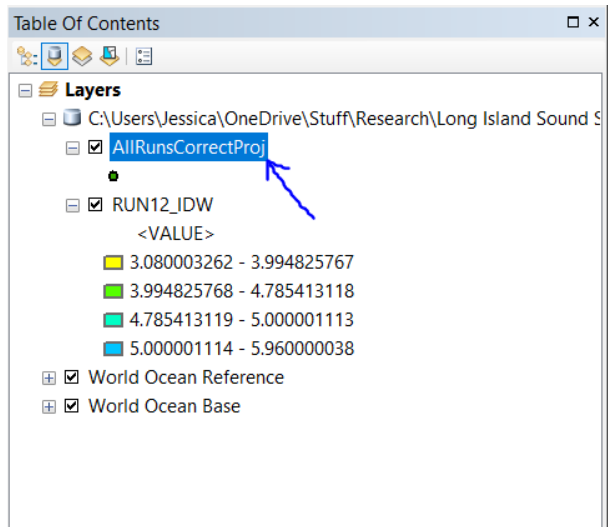
[YEAR] WLIS Bottom Dissolved Oxygen, Run [x]

Click “Next”

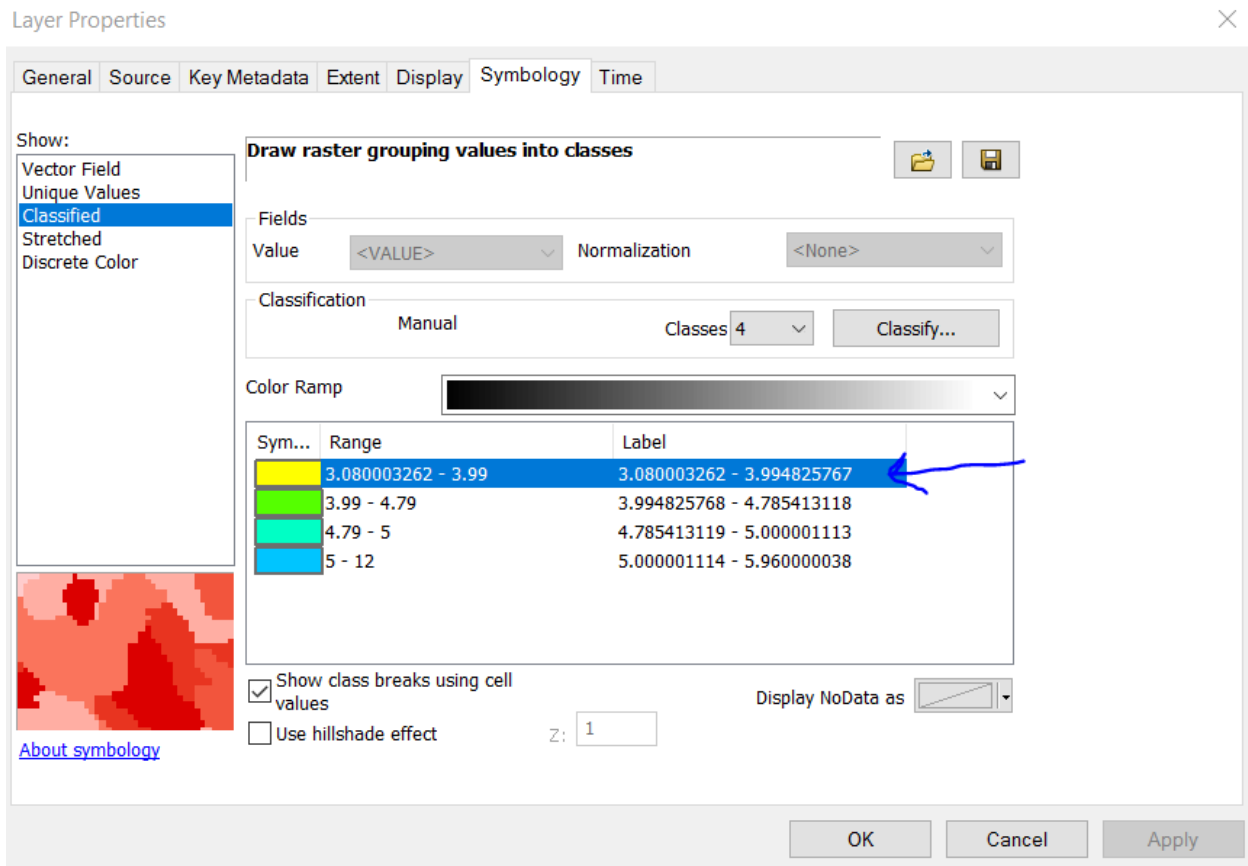
There is no frame, border or background, so click “Next.” There are no patches, so click “Next.” The default for title spacing is acceptable, so click “Finish.”

Resize the legend by dragging, and then drop it into the lower right-hand corner.

In order to re-name layers in the legend, click the bottom right corner of their labels in the table of contents.



To change the Bottom DO value in the table of contents, right click the IDW layer in the Table of Contents and go to properties. Go to the symbology tab and click on the values underneath “Label.”



Change them to read
< 1.99 mg/L (Severe)

2.00 mg/L - 2.99 mg/L (Severe - Moderately Severe)

3.00 mg/L - 3.99 mg/L (Marginal)

4.00 mg/L - 4.79 mg/L

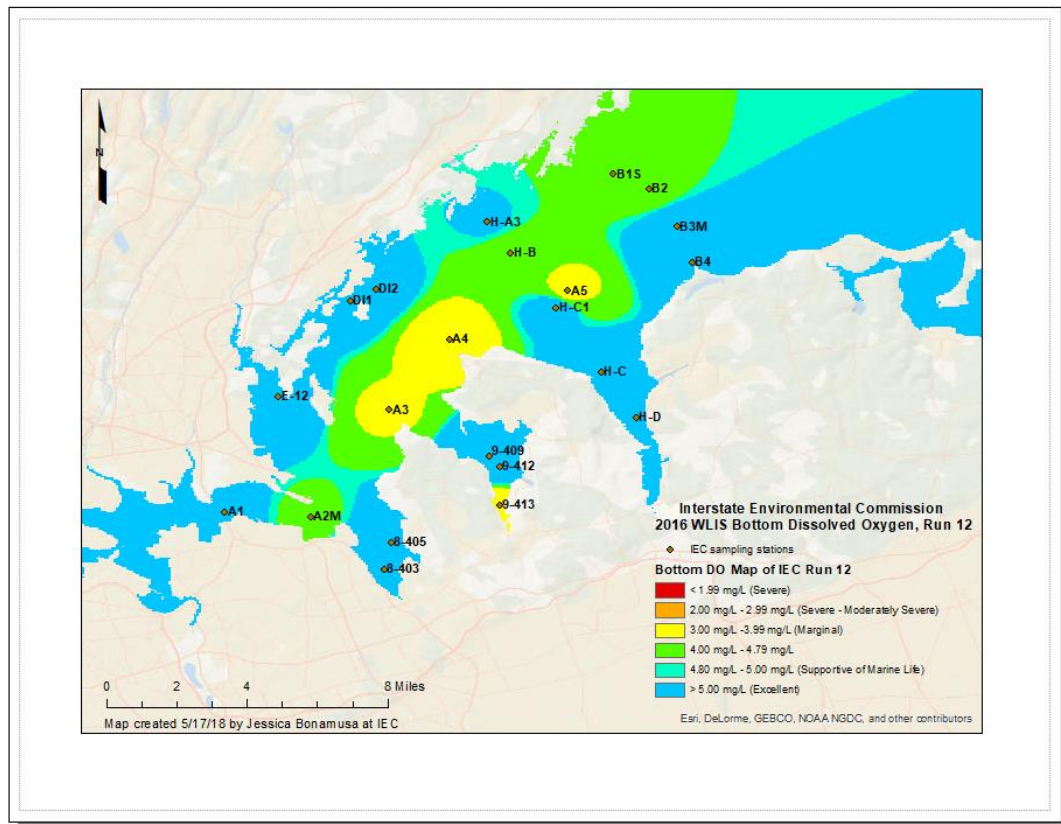
4.80 mg/L - 5 mg/L (Supportive of Marine Life)

> 5 mg/L (Excellent)

Click “OK”

Go to “file” and save the map. Then go back to “file” and click “Export map.” Export the map as a PDF and as a JPEG, so that it can be e-mailed and easily imported into documents.

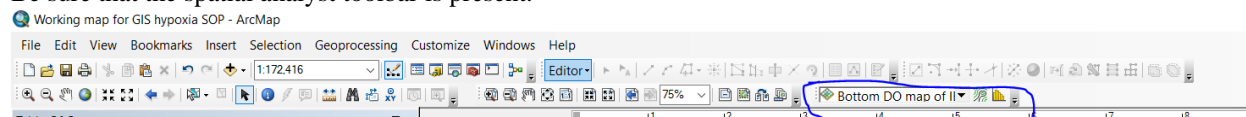
The map should look like the image below.



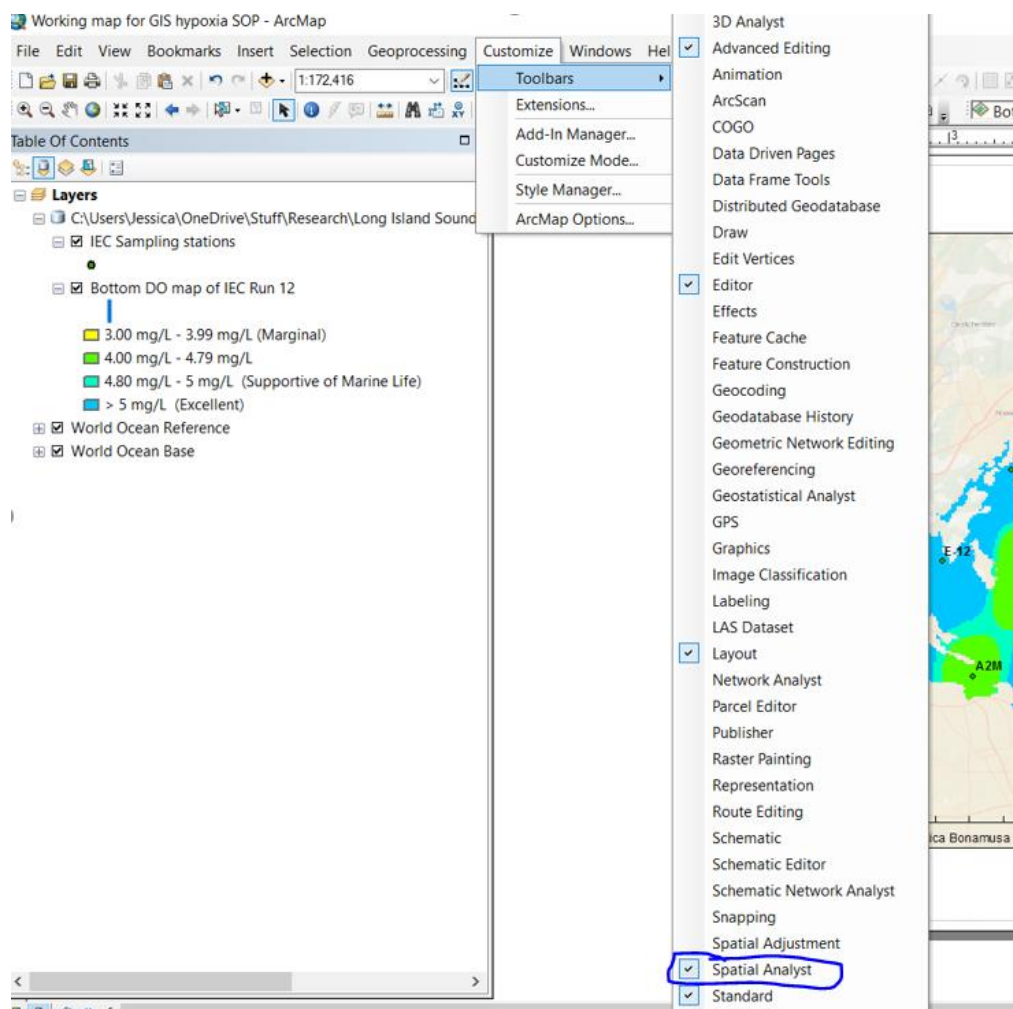
Calculating area

Step 1. Check the spatial analyst toolbar.

Be sure that the spatial analyst toolbar is present.

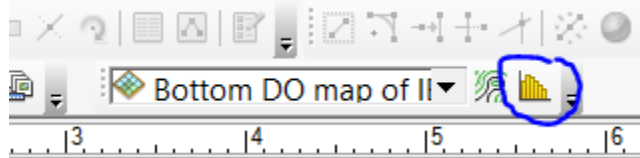


If it is not there, go to “Customize” then “Toolbars” then select “Spatial analyst”

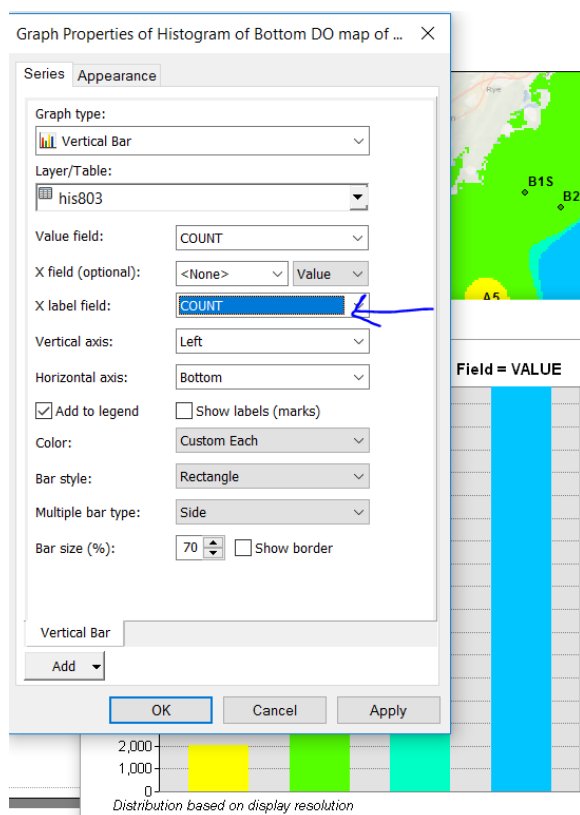


Step 2. Create a Histogram.

Go to the spatial analyst toolbar and click the histogram icon.

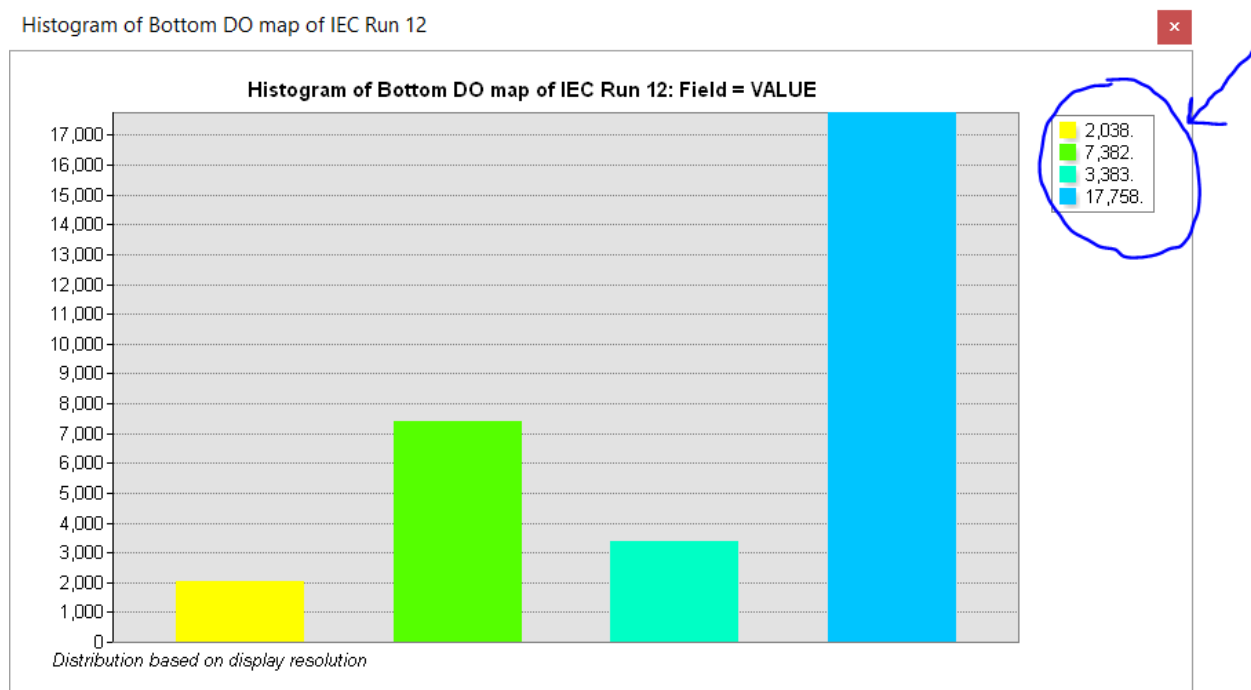


Right click the histogram and select properties. Under the “X label” field, select “COUNT.” Click OK.



Step 3. Calculate area.

The color in the graph will correspond to the color of the DO ranges on the map, and the bins created in earlier steps. Divide the number in the cell count cells by 10 for Area in Km².



For example, in the image above, there were 203.8 km² of water that had a DO value of 3-3.99 mg/L on Run 12 (2038/10).

Convert Km² to Mi² using 1km²= 0.3861022 mi². This is the area estimate to be used for summary reports and graphs. Save the map again.

QUALITY CONTROL

Prior to distributing summary and hypoxia maps, data should be checked by a senior environmental analyst or the QC officer.

CORRECTIVE ACTIONS

If maps and/or summaries are found to be disseminated to stakeholders containing incorrect/inaccurate data, corrected versions are issued via email and accompanied by a statement indicating the reason for the discrepancy.